CLXXIII. THE SURFACE TENSION OF PROTEIN SOLUTIONS. PART III.

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THE surface tension of protein solutions has been given a definite meaning by the work of du Noüy [1926] who has shown strikingly the adsorption of the protein molecule at the surface, forming there an organised unimolecular layer similar in every respect to the orientated unimolecular films of fatty acids and other long chain organic compounds of Langmuir and Adam. Du Noüy's calculations of the dimensions of the egg-albumin molecule from surface tension measurements, which give a value for molecular weight in close agreement with those of other observers, are further evidence that the colloidal behaviour of protein solutions is due to the size of the single molecule, although agglomeration into micellae may occur under certain conditions. Thus, for a given concentration, volume of solution and area of surface, any alteration, chemical or physical, in the molecule will affect the surface tension by the alteration of the surface layer.

In previous communications [1925, 1926] the Donnan equilibrium was considered to affect the surface tension of a protein solution according to the Lippmann effect of the charge on the particle, which Loeb [1922, 1] has proved to be due to the potential difference between the particle and the solution. In view of du Noüy's researches establishing the existence of an organised surface layer in protein solutions, it can be seen that the greater the charge on the molecules of protein, the greater the repulsive forces between adjacent molecules, hence the lower the cohesive forces and the tensile strength of the surface layer.

The earlier work on surface tension of gelatin solutions showed, however, that this is not the only factor affecting the surface tension of protein solutions, the solubility, hydration and denaturation of the protein molecule also being important influences independent of a membrane equilibrium which will alter the manner of the packing of the surface layer.

In the present communication the effects on the surface tension of an alteration in the protein molecule due to solubility, change of reaction, presence of neutral salts, and denaturation were studied for caseinogen and egg-albumin in order to extend the previous work of St Johnston and Peard [1925, 1926].

EXPERIMENTAL.

The method used both in the present and previous work for the determination of the surface tension was the maximum bubble pressure method of Sugden [1922, 1924]. This method, together with hanging-drop methods of which it is the inverse, is open to the criticism that it is comparative only. Du Noüy [1926] has also shown the importance of the time factor in the determination of the surface tension of colloidal solutions due to the slow rate of adsorption of the particles or molecules at the surface; he states, "It is thus essential when giving the surface tension of a colloidal solution to state the time which has elapsed since the solution was violently agitated, and, unless the method is a true static one, for results to be even comparable amongst themselves, everything being otherwise equal, the time required for the formation of a drop or bubble must be about the same for a large number of drops." Wolf [1923] has shown for bacteriological media that the surface tension as determined by a modification of du Noüy's static ring method is about 10 dynes lower than that determined by the drop method with a fast rate of dropping, whereas when the time of dropping was much prolonged agreement between the two methods was close.

The bubble method employed in this investigation was carried out under standard conditions with an approximately constant bubble rate, and the mean of several readings taken for each determination. Since the solutions employed were relatively concentrated compared to those of du Noüy, equilibrium at the surface would be attained much more rapidly owing to the more rapid diffusion towards the surface layer. Again, the pressure difference between two jets is observed; hence the effect of small variations in the bubble rate is partly compensated.

A further objection to Sugden's method when applied to colloidal solutions has been made by King [1925] on account of the frothing due to the bursting of the bubbles. Peard and St Johnston [1925], however, have shown for brewery worts that the amount of protein removed in this manner is negligible in the course of a determination of the surface tension, whilst frothing at the surface of the solution may be very much reduced or entirely prevented by the presence of a trace of toluene there without affecting the surface tension at the fresh bubble surface formed within the bulk of the liquid.

The determinations were carried out at 25° and the solutions were prepared 24 hours beforehand and kept at 25° to ensure chemical equilibrium being attained, a drop of toluene being added to ensure sterility and to prevent frothing. The bubble rate on the small jet was timed for each reading and was at a rate of one bubble in 1-3 seconds, but no appreciable constant difference was found experimentally between the pressure-difference readings for one bubble in one second and one in five seconds. The mean of five or six readings at various bubble rates within the above limits was taken as the value of the surface tension required. All the instruments used had jets of

approximately the same size, were calibrated with pure benzene and checked with water; the pressure difference readings for water were from 7 to 11 cm. on the alcohol gauge for various instruments, and all gave values for the surface tension of water within 0.5 %. They also agreed with each other to that extent when checked with the same protein solution. All the containers used were cleaned with chromic acid and washed and dried. Corks or rubber bungs were covered with tinfoil. The surface tension instruments were cleaned with nitric acid and well washed between each determination. In the preparation of solutions only boiled distilled water kept sterile and CO_2 -free was used, and for alkaline solutions a small soda-lime tube was attached to the bubbler of the surface tension instrument to prevent any access of CO_2 from the air. 5 cc. of solution were required for a determination.

The p_{H} determinations below p_{H} 7.5 were made at 20° with the quinhydrone electrode, using a gold electrode according to the technique of Corran and Lewis [1924] and 0.03 g. ground quinhydrone crystals per 20 cc. of solution. Above p_{H} 7.5 the determinations were made with the hydrogen electrode.

The caseinogen used was a commercial pure preparation which was fat-free and contained 9.58 % moisture, 0.11 % ash and 14 % N. It was therefore deemed suitable for use without further purification, being ash-free except for that organically combined, and, taking 6.47 as the protein factor, it contained 90.6 % caseinogen. In all experiments 0.56 g. of this caseinogen was dissolved in 50 cc. of solution containing the requisite quantity of $N/10$ acid or alkali to give a 1 % solution.

The crystallised egg-albumin was prepared from new-laid eggs according to the method of Sørensen [1915], recrystallised twice and dialysed for 3 weeks at 5°. Owing to the somewhat prolonged dialysis and slight leaks, the resulting albumin solutions were only about 0.6 %, so that when diluted with the requisite amount of $N/10$ acid or alkali they were reduced to 0.3 %. This was, however, sufficient to give a marked lowering of surface tension. Solution A contained 0.92 mg. protein-N and 0.016 mg. ammonium sulphate-N per cc. and had p_{H} 5.6; it was faintly turbid. Solution B contained 0.96 mg. protein-N and 0.036 mg. ammonium sulphate-N per cc. and had p_{H} 6.76; it was quite clear.

The effect of time on the surface tension of caseinogen solutions.

Owing to the low solubility of caseinogen, a considerable time was required for solution to take place, even when the conditions were favourable for complete solution. It was therefore necessary to determine the effect of the age of the solution on the surface tension, in order that future determinations of surface tension should be made when equilibrium had been attained. Caseinogen will only give completely dissolved 1 % solutions with hydrochloric and phosphoric acids between p_{H} 3.0 and 1.6, and with sodium hydroxide of p_{H} greater than 7.5. The effect of time on the surface tension of 1 % caseinogen solutions in HCl at p_{H} 2.46 is shown in Table I. Solution in

acids occurs with swelling and there is a rapid fall of surface tension in the first few minutes followed by a slower fall during about 24 hours, after which there is little change. The solutions were opalescent at first and did not clear for about 12 hours. Similarly with a partial solution in sodium hydroxide, p_H 6.8, equilibrium was not reached until nearly 24 hours had elapsed, although in alkali no swelling occurs.

Henceforth all determinations of surface tension of 1 % caseinogen solutions or suspensions were made 24 hours after preparation.

Table I. *Effect of age of solution of caseinogen on the surface tension.*

1 % caseinogen in <i>N</i> /100 HCl at 25° (2 experiments)			1 % caseinogen in <i>N</i> /250 NaOH at 25°	
Time	Surface tension		Time	Surface tension Dynes per cm.
	(a) Dynes per cm.	(b) Dynes per cm.		
2 mins.	71.5	64.9	2 mins.	70.8
4 "	70.8	62.6	5 "	60.1
6 "	70.2	61.5	8 "	59.6
10 "	69.5	60.5	12 "	59.0
15 "	63.1	60.5	15 "	58.6
20 "	61.9	—	30 "	58.1
50 "	61.5	—	45 "	58.0
60 "	61.0	59.7	60 "	58.0
2 hours	60.5	59.2	2 hours	58.0
6 "	59.5	—	19 "	57.0
18 "	59.0	58.5	26 "	56.9
24 "	58.7	58.6	43 "	56.3
43 "	—	58.5		

The effect of p_H on the surface tension of caseinogen solutions.

The titration curves of caseinogen in presence of HCl and H_3PO_4 were identical with those of Loeb [1922, 1], but the curve obtained for caseinogen in presence of *N*/10 NaOH differed from Loeb's, being always less alkaline for a given amount of NaOH added, particularly on the part of the curve where the change of p_H with increased addition of alkali is most rapid, *i.e.* between p_H 6 and 10. A regular curve was however obtained, and values of p_H determined with the hydrogen electrode agreed well with those determined by the quinhydrone electrode up to the limit where the use of the latter is possible. A clear 1 % solution of caseinogen in NaOH was not obtained below p_H 7.5. The rapid hydrolysis of caseinogen by alkali when the p_H is greater than 10 where a change in direction of the titration curve is apparent has been pointed out by Zoller [1921]. It was noted that solution took place very rapidly in liquids alkaline to p_H 10 and that all power of "head" retention was lost, foam formed on shaking disappearing immediately. The results of the variation of the surface tension with p_H are shown in Fig. 1. It will be seen that the general form of the curve is similar to that obtained by St Johnston and Peard [1925, 1926] for gelatin, the maximum surface tension being at the iso-electric point, p_H 4.62, as expected from Donnan's theory, though for caseinogen the value did not rise to that of pure water as in the case of gelatin, being 67.8 dynes per cm. Robertson [1918] and Cohn and Hendry [1923] have,

however, shown that caseinogen is slightly soluble in water when pure, even at the isoelectric point. The fall of surface tension is very rapid on either side of the isoelectric point and there is a minimum of surface tension on the acid side at p_H 3.8–3.9 followed by a well-marked secondary maximum at p_H 3.4, such as was obtained for gelatin at p_H 2.0–2.2. This maximum at p_H 3.4 is just before the caseinogen becomes completely soluble (about p_H 3.0). In presence of H_3PO_4 the surface tension proceeds parallel to that in presence of HCl but is uniformly a dyne lower. The maximal values of the osmotic pressure and viscosity of caseinogen in presence of HCl are at p_H 3.0, and Zoller [1921] states that “acid hydrolysis of caseinogen must of necessity commence somewhere in the neighbourhood of this drop or flattening of the curve.” St Johnston and Peard [1925], however, were unable to find in the case of gelatin solutions acid to p_H 2.2 any evidence of hydrolysis as evinced by formaldehyde titration commencing suddenly below p_H 2.2. This point is dealt with later. The minimum value p_H 8.5 agrees fairly well with Zoller’s value of p_H 9.0 for the maximum of viscosity of caseinogen in alkaline solution. Owing to the

Fig. 1. Effect of p_H on the surface tension of caseinogen and egg-albumin solutions.

- 1 % caseinogen in presence of HCl and NaOH
 □ " " " " H_3PO_4
 Δ 0.3 % egg-albumin " " HCl and NaOH

limited solubility above p_H 3.0 it would not be expected that the maxima of viscosity and osmotic pressure of caseinogen solutions would agree with the minimum surface tension, since the degree of solubility greatly influences the properties, in fact Loeb [1922, 1] states that the maximum for the influence of caseinogen hydrochloride on viscosity lies at p_H equal to or greater than 3.0. The surface tension curve seems to indicate that the Donnan equilibrium gives a maximum potential difference at a higher p_H than 3.0, since the minimum surface tension is at p_H 3.8–3.9. If solubility were the only factor affecting the surface tension no minimum would be observed.

On the alkaline side the fall of surface tension was much greater, the minimal value being at p_H 8.5, followed by a rise in surface tension with increasing alkalinity with a possible secondary maximum at about p_H 11.5.

The effect of p_H on the surface tension of egg-albumin solutions.

A similar series of determinations of surface tension at various hydrogen ion concentrations within the range of the quinhydrone electrode was carried out with crystallised egg-albumin solutions. The stock solution of albumin purified as described above was mixed with the requisite quantity of HCl or NaOH to give a final concentration of 0.3 % albumin. The solutions were kept for 24 hours at 25° before determining the surface tension at 25°. The results are also shown in Fig. 1, where it will be seen that the maximum surface tension of egg-albumin solutions is also at the isoelectric point, p_H 4.8. Like caseinogen, it does not attain the value for pure water as flocculated isoelectric gelatin does, which is as expected from the solubility of isoelectric albumin. Loeb [1922, 2; 1923] has also shown that neutral salts even in very low concentrations may impart a surface potential to isoelectric denatured albumin, and experiments on the effect of neutral salts on protein solutions to be described later also show that in low concentrations they decrease the surface tension; hence the presence of ammonium sulphate even in concentration of $M/3000$ will tend to lower the surface tension of isoelectric albumin solution, accounting for the value of 68.5 dynes/cm. instead of the theoretical value 72.2 dynes/cm. of pure water.

A sharp secondary maximum of surface tension on the acid side of the isoelectric point occurs at p_H 3.0. The minimum between these maxima is at p_H 3.4–3.5; thus the maximum lowering of surface tension agrees well with Loeb's values of p_H 3.4 for the maximum osmotic pressure and p_H 3.6 for the maximum membrane potential.

So far as the alkaline side has been investigated there is a minimum surface tension at p_H 6.0. Loeb found the potential difference of albumin to be still increasing at p_H 5.8 from the minimum value at the isoelectric point.

As discussed in an earlier communication [St Johnston and Peard, 1925], the surface tension of a protein solution should be a maximum when the potential difference is a minimum and *vice versa*, thus, as in the cases of gelatin and caseinogen, the fall of surface tension of egg-albumin solutions is explicable up to a point by Donnan's membrane equilibrium theory.

The probable cause of the further fall in surface tension in more acid solutions, observed in all the proteins investigated, giving rise to the second maximum of surface tension, which is not accountable by the Donnan equilibrium will be dealt with in a succeeding section.

The effect of neutral salts on the surface tension of caseinogen solutions.

Since caseinogen is only completely soluble in HCl between about p_H 1.6 and 3.0, a solution of 1 % caseinogen in $N/100$ HCl of original p_H 2.46 was used to study the effect of neutral salts on the surface tension in acid solution. Since on the acid side of the isoelectric point the influence of the anion is greatest, all the salts investigated had the same cation, sodium, to eliminate any possible effect due to the cation. The salt to be added was weighed out and dissolved in the solution at the same time as the caseinogen, keeping the solution for 24 hours at 25° as usual. The effect of NaNO_3 , NaCl and Na_2SO_4 and also of a non-electrolyte, glucose, is shown in Fig. 2. All the salts, in spite of a difference in valency of the anion, produce the same lowering of surface tension below that of the original caseinogen solution up to a concentration of $M/256$, and in greater concentrations produce a rise in surface tension, which is greater for Na_2SO_4 than for NaNO_3 and NaCl . The effect of glucose on the surface tension was practically negligible.

In experiments on the influence of neutral salts on protein solutions, the effect of the neutral salts on the p_H must always be considered. In all cases the p_H as determined electrically was increased slightly by the addition of a salt, in proportion to the concentration added, as was observed by Chick and Martin [1911]. NaCl and NaNO_3 at the same concentration raised the p_H to approximately the same extent, whilst Na_2SO_4 caused about double the rise due to NaCl or NaNO_3 at the same concentration. The rises of p_H were, however, not enough to account for the changes in surface tension observed. At p_H 2.46 it will be seen from Fig. 1 that an increase in p_H should raise the surface tension; up to $M/256$ for all three salts a fall of 2 dynes was observed, whereas the p_H changed from 2.46 to 2.52 for NaCl and NaNO_3 and to 2.66 for Na_2SO_4 , which should have raised the tension by 0.2 and 0.6 dyne respectively.

At $M/32$ the surface tension in presence of NaCl and NaNO_3 had risen again so that it was only 1 dyne below the original value, whilst the p_H had increased to 2.64, a rise of p_H 0.18, which should increase the surface tension by 0.5 dyne. Na_2SO_4 at $M/32$ raised the surface tension 3 dynes above the original value, with an increase of p_H 0.36, which alone would only increase the surface tension by 1 dyne. Thus the changes in surface tension observed in presence of neutral salts are far greater than can be accounted for by the changes in p_H produced.

If the surface tension of protein solutions depended only on the Donnan effect at the p_H of the above experiment, addition of neutral salts even in lower concentrations than $M/1024$ should depress the potential difference, as shown by Loeb [1922, 1], and therefore raise the surface tension, and the effect should be in proportion to the valency of the anion added. Evidently this is not what takes place in concentrations below $M/256$ since the sulphate ion has exactly the same effect as the chloride and nitrate ions in lowering

the surface tension. A possible cause of the fall of surface tension in presence of neutral salts below a concentration of $M/256$ is that denaturation of the protein is increased by their presence. Mastin and Schryver [1926] have shown that denaturation of egg-albumin occurs on standing in presence of acid and neutral salt, and in a succeeding section it will be shown that denaturation produces a lowering of surface tension and that denaturation is occurring during the 24 hours at 25° in solutions of caseinogen at p_H 2.46 or of albumin at p_H 2.6. This may be increased by the presence of salts thus lowering the surface tension, though in greater concentration than $M/256$ the Donnan effect and the lyotropic effect of removal of associated water predominate and raise the surface tension, so that when the salt concentration is sufficiently great the protein is salted out completely and the surface tension becomes that of the salt solution.

The effect of a neutral salt on the surface tension of albumin solutions.

The effect of Na_2SO_4 upon 0.3 % albumin solution in presence of HCl was followed in the same manner as for caseinogen, and found to produce the same results. The p_H of the solutions was originally 2.6, which is at a similar point to a caseinogen solution of p_H 2.46 in respect to the surface tension- p_H curves of Fig. 1. The p_H rose with the addition of Na_2SO_4 up to a value of p_H 3.0 when in presence of M Na_2SO_4 .

No sign of precipitation occurred up to a concentration of $M/16$ Na_2SO_4 , where there was slight turbidity. With $M/4$ Na_2SO_4 there was much precipitation and with M Na_2SO_4 (nearly saturated solution) the albumin was salted out almost entirely.

The effect on the surface tension is also shown in Fig. 2, where it will be seen that there is a similar fall of surface tension to that of caseinogen solutions to a minimum at $M/256$, followed by a rise. After $M/16$, however, the rise is much increased, when the albumin is almost entirely salted out and the surface tension is nearly that of water. Evidently in the stronger solutions the lyotropic effect of the salt in withdrawing associated water from the protein is acting as well as the effect due to the salt on the Donnan equilibrium.

The denaturation of egg-albumin solutions in presence of acids.

In a previous communication [St Johnston and Peard, 1926] it was suggested that the fall in surface tension of gelatin solutions acid to the maximum of surface tension at p_H 2.0-2.2 might be due to hydrolysis or denaturation of the protein. No evidence of immediate hydrolysis as shown by formaldehyde titration was found however, though on standing for some months the most acid solutions had increased in formaldehyde titre and the surface tension had fallen further. Harris [1923], also from formaldehyde titrations on fresh and denatured egg-albumin, came to the conclusion that no detectable change occurred on denaturation, although there is a change

in the sulphur linkages, and Lewis [1927] found no detectable change in titratable acidic or basic groups of haemoglobin on denaturation. Young [1922] found that light-denatured albumin had a lower surface tension than before exposure.

Since caseinogen and egg-albumin behave similarly to gelatin in respect to the effect of p_H on the surface tension, experiments were undertaken with egg-albumin to ascertain if the fall in surface tension in the more acid solutions, which is not in accordance with the membrane equilibrium theory, could be ascribed definitely to denaturation. Whatever the nature of the change in the protein on denaturation, it is possible that it might lower the surface tension.

Fig. 2. Effect of neutral salts on the surface tension of caseinogen and egg-albumin solutions.

○	1 % caseinogen in presence of	NaNO ₃	and HCl originally	p_H 2.46
+	"	NaCl	"	p_H 2.46
×	"	Na ₂ SO ₄	"	p_H 2.46
□	"	glucose	"	p_H 2.46
Δ	0.3 % egg-albumin	Na ₂ SO ₄	"	p_H 2.60

Egg-albumin, free from salts, is not coagulated by heating, whilst denaturation is greatly accelerated by heating; thus a dialysed albumin solution is very suitable for studying the effect of denaturation on surface tension, since if coagulation occurred there would inevitably be a rise of surface tension to that of pure water on complete coagulation. The solutions employed were of such a salt concentration that only those between the isoelectric point and neutrality were coagulable. As will be seen from Fig. 1, the surface tension of albumin solutions varies with the p_H in a manner explicable by Donnan's membrane equilibrium theory between p_H 3.0 and neutrality, but below p_H 3.0 the surface tension again falls sharply with increasing acidity. If this further fall is due to denaturation occurring while standing for 24 hours at 25° in presence of acid giving a p_H less than 3.0, the effect should become more marked on accelerating the denaturation by heating.

Sørensen [1925] has shown that coagulation proceeds most rapidly near the isoelectric point, whilst the preceding process of denaturation occurs more rapidly the greater the hydrogen ion concentration. Somewhat similar experiments were therefore carried out and the surface tensions observed. A recently dialysed, clear solution of albumin (B) was used, and 0.3 % solutions of various p_H were kept at 25° for 24 hours. The salt content was about $M/1500$ $(NH_4)_2SO_4$ which was insufficient to cause coagulation of the denatured protein on heating except in the region of the isoelectric point. The surface tension was determined at 25°, then the solution, still in the surface tension bubbler, was put in a water-bath at 60° for exactly 15 minutes, quickly cooled under the tap and the surface tension redetermined at 25°.

Chick and Martin [1911, 1912] have shown a fixation of acid on denaturation of egg-albumin. It was therefore necessary to determine the changes in p_H occurring under the conditions of the experiment. Another preparation of dialysed albumin was used for this purpose and the p_H of the 0.3 % solutions determined after they had stood 24 hours at 25° and again after heating for 15 minutes at 60°. It will be seen from Table II that the only appreciable changes in p_H occur between p_H 3.8 and p_H 2.5, which show a maximum increase of p_H 0.15; this would have some effect on the surface tension, but it will be seen from Table III that over the range of p_H 3.0–4.0 there is practically no change of surface tension. From Fig. 1 it will be seen that a rise of p_H between 2.5 and 3.0 would tend to raise the surface tension, whereas from Table III at p_H 2.85 it falls. Between p_H 3.0 and 3.5 increase of p_H would lower the surface tension and between 3.5 and the isoelectric point raise it, only at p_H 4.0 does this begin to occur, when coagulation is also beginning. Thus the change of p_H on denaturation by no means accounts for the changes of surface tension observed, and where the maximum fall of surface tension is observed no change of p_H occurs. That no change occurs on further heating the most acid solutions seems to be due probably to the fact that a certain amount of denaturation and consequent fixation of acid has taken place while standing for 24 hours at 25°.

Table II.

Original p_H	p_H after heating 15 mins. at 60°	Change of p_H
6.63	6.64	+ .01
3.78	3.93	+ .15
3.33	3.47	+ .14
2.78	2.91	+ .13
2.54	2.62	+ .08
2.38	2.39	+ .01
2.24	2.25	+ .01
1.87	1.87	0
1.70	1.71	+ .01

The surface tension results are recorded in Table III.

Thus in solutions alkaline to and including the isoelectric point a heavy coagulation accompanied by a rise in surface tension occurred, and at the isoelectric point the surface tension rose to that of water.

Table III.

p_H	Surface tension		Change of surface tension (dynes per cm.)	Appearance after heating
	Before heating (dynes per cm.)	After 15 mins. at 60° (dynes per cm.)		
6.76	67.9	70.2	+2.3	Coagulation
6.28	65.5	68.6	+3.1	"
5.32	68.0	71.3	+3.3	"
4.77	68.5	72.1	+3.6	"
4.00	65.9	68.0	+2.1	Slight turbidity
3.66	64.0	64.0	0	Clear
3.22	63.8	63.9	+0.1	"
3.10	64.7	64.6	-0.1	"
2.85	63.7	63.1	-0.6	"
2.03	63.3	62.5	-0.8	"
1.79	63.3	62.2	-1.1	"

The solution of p_H 4.0 showed a marked rise in surface tension with only a very slight turbidity, whilst solutions between p_H 3.0 and 4.0 gave no change of surface tension or of appearance.

From the point at which the solutions became acid to the maximum of surface tension at p_H 3.0 a marked fall in surface tension occurred on heating, the fall increasing with increasing hydrogen ion concentration.

The experiment was carried a stage further by heating the solutions to 60° for 15 minutes, then bringing to the isoelectric point by the addition of a buffer solution and again heating. In this case an albumin solution (A) which had been prepared about 6 weeks previously was used. This solution, again of 0.6 % concentration but with only about $M/3000$ $(\text{NH}_4)_2\text{SO}_4$ and saturated with toluene, had been kept at about 4° in the dark and had become opalescent with a slight deposit, agreeing with the observation of Sørensen [1926] who found a similar change under the same conditions, namely, a slight deposit of denatured albumin, whilst the filtered solution could be recrystallised, giving material with normal properties, except that it contained less phosphorus than fresh albumin. The solution was filtered and solutions of various p_H prepared and kept at 25° for 24 hours as before. The surface tensions were found to be lower than the values previously determined for the same p_H ; thus some change had occurred, but this was immaterial to the experiment.

The results are given in Table IV.

Table IV.

(a) Experiments as in Table III.

p_H	Surface tension		Change of surface tension (dynes per cm.)	Appearance after heating
	Before heating (dynes per cm.)	After 15 mins. at 60° (dynes per cm.)		
4.9	64.5	69.4	+4.9	Coagulation
4.0	62.2	65.2	+3.0	Slight turbidity
2.6	62.1	61.0	-1.1	Clear
1.43	61.4	60.1	-1.3	"

(b) Equal volume 0.2 *N* acetate buffer, p_H 4.87, added to above heated solutions.

Original p_H	p_H after buffer added	Surface tension after		Change of surface tension (dynes per cm.)	Appearance after heating
		Buffer added (dynes per cm.)	Heating 15 mins. at 60° + buffer (dynes per cm.)		
4.9	4.81	69.5	69.8	+0.3	Coagulation still present
4.00	4.88	67.5	67.6	+0.1	Slightly increased turbidity
2.60	4.81	64.0	67.8	+3.8	Ditto.
1.43	4.56	64.8	66.4	+1.6	Ditto.

(c) Control experiment: 0.2 *N* acetate buffer, p_H 4.87, added to solutions as above not previously heated at 60°.

Original p_H	p_H after buffer added	Surface tension after		Change of surface tension (dynes per cm.)	Appearance after heating
		Buffer added (dynes per cm.)	Heating 15 mins. at 60° (dynes per cm.)		
4.9	4.81	66.5	66.5	0	No coagulation
4.00	4.88	66.4	66.9	+0.5	"
2.60	4.81	64.8	65.4	+0.6	"
1.43	4.56	65.2	65.6	+0.4	"

The effect of heating for 15 minutes at 60° was the same as before, and on the addition of an equal volume of an 0.2 *N* acetate buffer, p_H 4.9, and again heating for 15 minutes at 60°, rises of surface tension were now observed in those solutions which had previously been acid to p_H 3.0 but not in the others. There was no coagulation after adding the buffer, though for surface tension determinations this was an advantage. Control experiments were made by adding the buffer to equal volumes of solutions which had not been heated at 60°; these were thus only heated at that temperature in the neighbourhood of the isoelectric point. It was observed that the presence of the buffer prevented the coagulation of the nearly isoelectric albumin, which coagulated readily when heated alone. Lepeschkin [1922] found that some salts have an accelerating and others a retarding effect on the coagulation-velocity of denatured albumin, whilst Lewis [1926] found the denaturation-velocity accelerated or retarded according to the salt and its concentration. Apparently with the relative concentrations present here, namely 0.15 % albumin and 0.1 *N* acetate, there is a retardation sufficient to prevent coagulation during 15 minutes at 60°.

The control experiment, Table IV (c), shows that a slight amount of denaturation during the 24 hours at 25° has occurred in all but the isoelectric solution, as, although no visible coagulation occurred, the surface tension rose slightly on heating in presence of the buffer. The solution originally most acid was not brought quite to the isoelectric point by the addition of the buffer, hence the rise of surface tension was not as great as for the other two acid solutions, both in the control experiment and in experiment IV (b), although in experiment IV (a) it showed the greatest fall of surface tension after heating without buffer. Since denaturation is increased by heating, the falls of surface tension in acid solutions after heating recorded in Tables III

and IV (a) clearly indicate that denaturation is accompanied by a fall of surface tension. Coagulation is accompanied by a rise in surface tension as would be anticipated and is well shown by those solutions in Table III giving visible coagulation; thus in those solutions heated with buffer near the isoelectric point the rises of surface tension are due to coagulation of the previously denatured protein although it is insufficient to be visible. With the above-mentioned exception of the most acid solution, p_H 1.43, the greatest rises of surface tension on heating with buffer occur in those acid solutions which showed the greatest falls on previous heating without buffer. This further proves the falls in surface tension to be due to denaturation, since Sørensen [1925] has shown that the greatest amount of coagulation when brought to the isoelectric point occurs in those solutions which had previously been most denatured, namely, those with the greatest hydrogen ion concentrations originally.

The experiments bring out most clearly the marked increase in denaturation after p_H 3.0 which is the point of inflexion of the surface tension- p_H curve of albumin, hence the previous assumption that this was due to denaturation seems to be correct. Recent work by Mastin and Schryver [1926] has shown that denaturation of egg-albumin is brought about by long standing in the presence of acid and salt, and Chick and Martin [1911, 1912] and Lewis [1926] have proved the denaturation velocity to increase rapidly with the H or OH ion concentration from a minimum at neutrality. Hence there will be a certain amount of denaturation occurring in an egg-albumin solution during 24 hours at 25°, increasing with the acidity, and the above experiments show that a sudden great increase occurs after p_H 3.0. Since gelatin and caseinogen have similar surface tension- p_H curves to egg-albumin, with acid maxima at p_H 2.0 and p_H 3.4 respectively, it is reasonable to assume for these proteins also that denaturation is the cause of the fall of surface tension in solutions acid to these points.

Thus denaturation also accounts for the fall in surface tension of gelatin solutions on keeping over a long period, as reported previously [St Johnston and Peard, 1926], and for the low values obtained with albumin solution A given in Table IV compared with the values in Fig. 1 obtained some weeks previously.

CONCLUSION.

Although du Noüy has shown the surface tension phenomena of protein solutions to be due to the single molecules of proteins, the surface tension may be brought into line with other properties of colloidal behaviour as being dependent upon the Donnan equilibrium except where denaturation occurs or where adsorption or lyotropic effects of neutral salts predominate over the membrane equilibrium effect. As was remarked by Michaelis [1926], and would be expected since the charge on the particle is a minimum, the maximum surface tension is at the isoelectric point for the three proteins investigated, gelatin, caseinogen and egg-albumin, whilst the hydrogen ion concentrations

of the maximum lowerings of surface tension on either side of the isoelectric point agree fairly well with those of the maxima of osmotic pressure, viscosity and potential difference found by Loeb [1922, 1] for the same proteins.

The published results with other proteins are somewhat at variance, particularly in respect to the surface tension at the isoelectric point. Buglia [1908], Bottazzi [1912, 1913], Bottazzi and d'Agostino [1912, 1913] found the surface tension of proteins, in particular those of the body fluids, to vary with the acidity or alkalinity much as described above, though of course the p_H was not considered. Whereas in some cases suspensions of a serum-protein in water did not appreciably lower the surface tension, with other serum-proteins a minimum surface tension at the isoelectric point was apparently observed, and Bottazzi's inference that the undissociated protein produces the greatest lowering of surface tension has been generally quoted. Although Quagliariello [1921] found the same for haemoglobin, he considers it anomalous. Bottazzi's view was apparently confirmed for gelatin by de Caro [1925] but this was shown not to be so in a previous communication [St Johnston and Peard, 1926] and the maximum surface tensions of caseinogen and egg-albumin at their isoelectric points are a further confirmation that this is not the case.

The sensitiveness of surface tension determinations of protein solutions to slight differences of p_H , to denaturation and to small amounts of neutral salt seems a likely explanation of the variable results with other proteins, particularly as in most cases no account was taken of p_H .

Hence it appears that surface tension lowering is a general property of the proteins which varies with the hydrogen ion concentration, as do other properties dependent upon the Donnan equilibrium, but the influence of denaturation appears in more acid solution and in presence of neutral salts.

SUMMARY.

1. The surface tension of caseinogen and egg-albumin solutions varies with the p_H similarly to that of gelatin solutions, being maximum at the isoelectric point and minimum at points corresponding to the maxima of other properties, whilst a secondary maximum occurs in more acid solutions.
2. Neutral salts in low concentration lower the surface tension of caseinogen and egg-albumin in acid solution, but raise it in a greater concentration.
3. Denaturation of egg-albumin lowers the surface tension, which accounts for the fall of surface tension in solutions acid to the secondary maximum in the surface tension- p_H curve, for the initial fall in presence of low concentrations of neutral salts, and for the fall in surface tension on long standing.
4. The surface tension of protein solutions appears to be a general property which can be brought into line with the other properties of proteins as being governed by the Donnan equilibrium when due allowance is made for denaturation and the lyotropic effect of neutral salts.

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