LIX. THE DENATURATION OF PROTEINS.
VIII. THE EFFECT OF DENATURATION ON THE
VISCOSITY OF THE SOLUTIONS OF
CERTAIN PROTEINS.

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ALTHOUGH a considerable amount of work has been carried out on the measurement of the viscosities of various protein solutions, little attention has been paid to the determination of whether any change in viscosity occurs on denaturation.

Thus, it is only just possible to infer from the results of Pauli [1928] on various solutions, and those of Ettisch and Sachsse [1931] for egg-albumin, that an increase in viscosity occurs on denaturation. The present work was therefore undertaken to obtain more precise information, measurements being made separately on solutions of undenatured protein, and on solutions of deliberately denatured but unflocculated protein.

Measurements on undenatured solutions of egg-albumin have been made by Loeb [1924], who showed that the viscosity- p_H curve was a straight line parallel to the p_H axis, *i.e.* that the viscosity was independent of p_H . This has been confirmed in the present work, and furthermore it is shown that the same is true for solutions of oxyhaemoglobin.

Since the completion of the work described in the present paper an account has appeared of an investigation by Anson and Mirsky [1932] in which it is shown that denaturation is accompanied by an increase in viscosity. This is referred to later.

EXPERIMENTAL.

The proteins used were oxyhaemoglobin and crystalline egg-albumin, the former being prepared by a modification of the method of Schulz [1898], and the latter by the method of Sørensen [1917]. In both cases the proteins were freed from salt by prolonged dialysis¹. The concentration of protein in the stock solutions was estimated by the method of Devoto as modified by Hopkins [1900].

¹ The dialysis was allowed to proceed for several months until two litres of the dialysate when boiled down to a volume of 2 cc. gave no precipitate with barium chloride solution.

The viscosities were measured by the times of outflow through the capillary tube of an Ostwald quartz viscosimeter. In all cases the "relative viscosity" given is the ratio of the time of outflow of a certain volume of protein solution to the time of outflow of the same volume of pure water through the same viscosimeter at the same temperature.

It was shown by Poiseuille that the flow of a liquid through a capillary is expressed accurately by the formula

$$V = \frac{KPr^4t}{l},$$

where V is the volume passing through the tube in time t , K is a constant characteristic of the liquid, P is the pressure, r is the radius of the tube, and l its length.

In terms of viscosity this equation becomes

$$V = \frac{\pi Pr^4t}{8l\eta},$$

where η is the viscosity of the liquid.

If therefore we wish to use relative viscosities, we must be satisfied that the liquids in question, *viz.* the protein solutions and water, follow Poiseuille's law, as otherwise the value obtained would not be independent of the instrument used. According to Grüneisen [1905] the simplest method for testing whether a liquid follows this law is by making observations of the times of flow at a given temperature when the pressure is varied. The product of pressure and time of flow will then be constant so long as the liquid follows Poiseuille's law.

The apparatus used for the test was that of Applebey as described by Hatschek [1928]. The liquids tested were water and a 3 % oxyhaemoglobin solution. In both cases a range of pressure was used which gave times of flow from 100 to 200 secs., *i.e.* a much greater range than that required for the subsequent readings. In both cases the product of pressure and time of flow was constant, as shown by Table I.

Table I.

Water		
Pressure (P) (cm. of water)	Time of flow (t) (secs.)	$P \times t \times 10^{-2}$
15.77	102.3	16.1
13.27	121.7	16.2
12.27	131.6	16.1
10.57	153.0	16.2
10.07	160.2	16.1
9.07	184.2	16.7
Mean value of $P \times t = 16.2 \times 10^2$		
Haemoglobin solution		
14.76	127.7	18.8
13.51	141.7	19.1
12.76	149.5	19.1
11.86	163.7	19.4
11.26	170.0	19.1
10.66	180.6	19.2
10.16	189.1	19.2
Mean value of $P \times t = 19.1 \times 10^2$		

Thus the flow of water and of protein solution up to a concentration of 3 % through the given viscosimeter follows Poiseuille's law. This means that the viscosities measured are true viscosities and may be regarded as having a definite significance from which deductions can be drawn with regard to the changes occurring during denaturation.

The viscosimeter was cleaned and dried by the method recommended by Barr [1931]. Constancy of temperature was obtained by using a thermostat at 25° regulated to $\pm 0.05^\circ$. The front of the thermostat was transparent, and the viscosimeter was clamped vertically in such a position that the liquid in it was always completely immersed below the level of the water in the thermostat, and also so that the marks on the instrument could be viewed directly through the window.

After the liquid had been introduced into the viscosimeter, the instrument was left in the thermostat for 10–15 minutes for the attainment of temperature equilibrium.

The times of flow were taken with a stop-watch correct to 0.1 sec. In all cases five readings were taken and a mean value obtained. For solutions containing 3 % protein the max. error amounted to ± 0.3 %; in the case of 0.2 % solutions the error did not exceed ± 0.2 %.

The method used for the p_H determinations was that of Harrison [1930] using the glass electrode. The temperature of the determinations was 18°. Harrison claims an accuracy of 0.02 of a p_H unit between the p_H values 1 and 12.

The viscosity of undenatured and denatured egg-albumin.

The volume of stock egg-albumin solution (prepared as described previously) required to give 15 cc. of a 3 % solution was measured out, and to it was added sufficient dilute sulphuric acid to make the volume up to 15 cc., the concentration of the acid being such as to bring the p_H of the solution to the required value. For measurements on undenatured egg-albumin, 10 cc. of this mixture were immediately pipetted into the viscosimeter which was then fixed in the thermostat, and after 10–15 minutes the mean time of flow was obtained as previously described.

In the case of the denatured egg-albumin, the 15 cc. of 3 % solution was heated on a water-bath to a temperature at which the process of denaturation was complete in about a minute at the p_H of the solution. This temperature was obtained by a study of the results of Lewis [1926] and Cubin [1929] and was in most cases between 80 and 90°. The solution was then cooled down rapidly and 10 cc. measured out for the viscosity determination as before. The mean of at least five readings was taken. The p_H of the solution was subsequently determined.

If the viscosity readings did not agree within the limits of experimental error, but showed a tendency to rise as time went on (owing to incipient flocculation), they were rejected. If no rise occurred, a sample of the solution was kept in the ice-chest for several days. If no visible flocculation occurred in this time it was assumed that the aggregation of molecules subsequent to denaturation had not occurred during the time taken for the viscosity measurements. If any flocculation became apparent in the solution even after 4 days, the readings were rejected.

As an added precaution, in case flocculation had occurred, but to an extent insufficient to be visible to the naked eye, one solution, after being kept in the ice-chest for 24 hours, was removed and its viscosity redetermined. The mean of six readings was 0.6 sec. higher than the previous mean, *i.e.* an increase of only 0.4 %. As the viscosity measurements are very sensitive to flocculation, this was conclusive evidence that flocculation had not even begun during the time taken for the determination of the viscosity. Thus we are justified in assuming that any change in viscosity can be attributed solely to the effect of denaturation.

Owing to the comparatively slow rate of denaturation at ordinary temperatures, it was possible to obtain the viscosities of undenatured solutions

from high p_H values down to p_H 2.0. Measurements for denatured solutions were made at $p_H < 2.9$, but between this p_H and the isoelectric point flocculation occurred and the results were accordingly rejected.

Viscosity of 3 % salt-free solutions of egg-albumin at 25°.

Mean time of flow for water = 133.3 secs. In all cases the mean time given below is the mean value of six readings.

Egg-albumin.

Undenatured solutions

p_H	Mean time of flow (secs.)	Relative viscosity
1.97	154.9	1.162
2.19	155.6	1.167
2.67	156.6	1.175
3.77	155.7	1.168
4.25	156.5	1.174
5.08	154.8	1.161
5.67	155.0	1.163
6.67	154.9	1.162
7.61	156.7	1.175

Denatured solutions

2.12	174.3	1.308
2.26	173.5	1.302
2.26	172.2	1.292
2.82	175.1	1.314
2.90	173.5	1.301

Inspection of the foregoing results indicates that the viscosity of solutions of undenatured crystalline egg-albumin is independent of p_H , the variations being due to experimental error only. This confirms the results of Loeb [1924]. Furthermore, the viscosity of denatured solutions of egg-albumin is similarly independent of p_H provided that incipient flocculation has not occurred.

The mean value of the viscosity of a 3 % solution of undenatured egg-albumin is 1.168, and of a 3 % denatured solution 1.303. The increase in viscosity which occurs on denaturation is constant and independent of p_H . In view of the constancy of viscosity over the p_H range 2.1 to 2.9, it is reasonable to conclude that even at the isoelectric point the viscosity of a 3 % solution of denatured egg-albumin is 1.303. This conclusion receives further support from the measurements on oxyhaemoglobin solutions which are given below.

Viscosity of solutions of undenatured and denatured solutions of oxyhaemoglobin.

These measurements were carried out in a manner similar to those already described for egg-albumin, the same precautions being taken to ensure that denaturation was not accompanied by flocculation even to the slightest extent. The work on 3 % solutions was performed by N. Booth¹ and the results which he obtained are given here for the sake of comparison.

¹ Unpublished work carried out in this laboratory.

Data of Booth for 3 % salt-free solutions of oxyhaemoglobin.

The mean time of flow for water in the instrument used by Booth was 130.0 secs.

Oxyhaemoglobin.

Undenatured solutions

p_H	Mean time of flow (secs.)	Relative viscosity
4.58	165.5	1.286
5.14	163.7	1.272
5.53	166.5	1.294
5.78	161.6	1.255
6.09	162.1	1.259
6.89	163.7	1.272
7.42	163.4	1.270
7.82	164.5	1.278
8.21	161.9	1.257

Mean value of the relative viscosity = 1.272

Denatured solutions

2.38	201.2	1.564
3.83	201.6	1.567
4.50	211.6	(1.644)
5.07	204.1	1.586

Mean value of the relative viscosity = 1.572

To confirm the results of Booth, and also to see whether the increase in viscosity on denaturation was dependent on the concentration of the solution, measurements were made on a series of more dilute solutions, *viz.* 0.2 % solutions. In this case we are dealing with quite small differences in viscosity, but that the results are as significant as those already obtained for the more concentrated solutions is seen when the following facts are taken into consideration.

The mean time of flow for 0.2 % undenatured solutions was about 2 secs. greater than that for water. The mean time for the denatured solutions was about 3.5 secs. greater than that for water. For these dilute solutions the separate readings agree far better than do those for the more concentrated solutions. This is shown by the fact that the highest and lowest single readings for the undenatured solution differed by only ± 0.5 sec., and for the denatured solutions by ± 0.4 sec. Also these various readings were made with separate solutions made up independently from the stock solution. Hence a difference of 1.5 secs. between the mean values for undenatured and denatured solutions respectively must be regarded as being a significant difference. This point is emphasised if the readings are plotted in the form of a graph, which is here omitted, however, for the sake of brevity.

In both cases therefore it will be seen that the viscosity of a given concentration of undenatured oxyhaemoglobin solution is constant and independent of p_H . The effect of denaturation on the solution is to cause a definite increase in viscosity, this increase being independent of the p_H of the solution. Consequently the effect of denaturation on viscosity is similar in the cases of oxyhaemoglobin and egg-albumin.

Viscosity data for 0.2 % salt-free oxyhaemoglobin solutions.

Temperature = 25°.

Mean time of flow for water = 133.3 secs.

Oxyhaemoglobin.

Undenatured solutions

p_H	Mean time of flow (secs.)	Relative viscosity
5.24	134.9	1.012
5.35	135.1	1.014
6.49	135.0	1.013
7.21	135.2	1.014
7.26	135.5	1.017
8.42	135.6	1.017

Mean value of the relative viscosity = 1.0145

Denatured solutions

2.29	136.8	1.026
2.41	137.1	1.028
2.68	136.6	1.025
2.71	136.9	1.027
2.86	136.6	1.025
3.02	136.7	1.025
3.46	136.7	1.025
4.16	136.7	1.025

Mean value of the relative viscosity = 1.026

Thus the experimental results confirm the conclusions just published by Anson and Mirsky [1932] that the viscosity of the protein solutions under consideration is increased by denaturation. It must be pointed out, however, that in the present work care has been taken to use denatured solutions in which the process of flocculation has not even commenced, whereas in general Anson and Mirsky must have been using solutions in which flocculation had proceeded at least to some extent. This is evidenced by the fact that some of their solutions were at a p_H quite close to the isoelectric point, and the present work has shown that in this case aggregation of the particles is inevitable.

At p_H 2-3 the relative viscosity of a 4 % denatured egg-albumin solution was found by Anson and Mirsky to be only of the order 1.5. In this range their work is most nearly comparable to our own as aggregation would not have occurred, so that increase in the viscosity due to the aggregation of the particles would not be experienced. As in our own case, with very acid solutions stiff gels were formed so that measurements of the increase in viscosity due to denaturation alone were impossible.

In view of the above considerations it seems that the present work must be regarded as demonstrating more concisely that the process of denaturation itself causes an increase in the viscosity of the protein solutions, as distinct from the subsequent further increase when aggregation of the particles in the denatured solution occurs.

Considering the three sets of viscosity measurements together, we see that in each case the viscosity of the denatured solution is constant over the whole range of p_H used. In two of the three cases the p_H ranges used for the undenatured and denatured solutions overlap, and even in the overlapping range the viscosities of the two solutions remain quite constant at their respective values. Thus, although it is not possible to measure the viscosity of a denatured but unflocculated protein solution at the isoelectric point, we are justified in concluding that the viscosity at this point will be the mean value which we have obtained for a considerable range of p_H . This conclusion will be employed later in the discussion of the significance of the viscosity results.

Dilatometer measurements.

A series of experiments was also undertaken using a dilatometer of the type described by Sreenivasaya and Sastri [1929] to determine whether the net volume of the protein solutions altered on denaturation. A mark was made near the lower end of the capillary tube and it was found that 30 cc. of liquid were required to fill the instrument to this mark.

The radius of the capillary was 0.125 cm. so that the cross-section was approximately 0.05 cm.² Consequently an increase in the volume of the solution of 1 cc. would cause a rise in the capillary of 20 cm. Alternatively this means that a 1 % increase in the volume of the solution would cause a movement of the meniscus in the capillary tube of 6 cm. Consequently a small change of volume would cause a quite appreciable movement of the meniscus.

As the dilatometric measurements were to be made at 45° in order to obtain a suitable rate of denaturation, it was necessary to observe first of all what change in volume occurred when the instrument, initially filled to the mark at room temperature, was raised to a temperature of 45°. This was done by filling the instrument to the mark with distilled water at room temperature, and then placing it in the thermostat at 45°. The meniscus in the capillary tube rose for about 10 minutes, but when temperature equilibrium had been attained, the meniscus remained stationary. A mark was made on the capillary corresponding to this new position of the meniscus.

The dilatometer was emptied, dried thoroughly, and then filled to the lower mark on the capillary with a 3 % salt-free undenatured egg-albumin solution (p_H about 3.0) at room temperature, care being taken that no air bubbles were enclosed. The instrument was then clamped in the thermostat and the meniscus was observed through the window. In the first 10 minutes the meniscus rose to the upper mark on the capillary, but thereafter it remained stationary, although a control experiment showed that the protein was in process of being denatured.

This experiment was repeated at several different p_H values with the same result. There was the initial definite expansion due to the warming of the solution, but no subsequent volume change was noted during the time (one to several hours according to the p_H of the solution) required for the process of denaturation. A 3 % oxyhaemoglobin solution gave the same result.

From these experiments we conclude that no change occurs in the net volume of a solution of egg-albumin, or of oxyhaemoglobin on denaturation. The importance of this conclusion will be seen in the subsequent discussion.

DISCUSSION.

As is well known Einstein [1906] was the first to attempt a mathematical treatment of the viscosity of a disperse system. Making several simplifying assumptions he arrived at the equation:

$$\eta = \eta_0 (1 + 2.5 \phi),$$

where η is the viscosity of the disperse system, η_0 that of the medium, and ϕ the aggregate volume of spheres in unit volume of the suspension. This formula, however, as Einstein himself points out, can only be used when ϕ is very small.

Various formulae have been suggested to overcome this limitation, in nearly all cases a value larger than 2.5 being given to the coefficient of ϕ , but none of these formulae allows specifically for any possible influence of the electric charge on the particles on the viscosity. It is obvious however that if we restrict ourselves to the isoelectric point for both the undenatured and the denatured material, the effects considered by Smoluchowski [1916] do not enter, and we are justified in employing the simpler type of formula.

Further than this, certain conclusions can be drawn from the viscosity data at the isoelectric point which do not demand a precise knowledge of the numerical coefficient of ϕ in the formulae of the Einstein type.

Thus at the isoelectric point we may employ the general equation,

$$\eta = \eta_0 (1 + k\phi),$$

where k is a constant.

Let us represent the viscosity of the undenatured solution at the isoelectric point by η_1 , and of the denatured solution also at the isoelectric point by η_2 .

Then

$$\eta_1/\eta_0 = 1 + k\phi_{\text{undenatured}}$$

and

$$\eta_2/\eta_0 = 1 + k\phi_{\text{denatured}}.$$

Whence by division we obtain

$$\frac{\phi_{\text{undenatured}}}{\phi_{\text{denatured}}} = \frac{\frac{\eta_1}{\eta_0} - 1}{\frac{\eta_2}{\eta_0} - 1}.$$

Thus the ratio $\frac{\phi_{\text{undenatured}}}{\phi_{\text{denatured}}}$ is independent of the value of k .

For 3 % egg-albumin solutions the value of $\frac{\phi_{\text{undenatured}}}{\phi_{\text{denatured}}}$ is $\frac{0.168}{0.303} = \frac{1}{1.8}$.

For 3 % oxyhaemoglobin solutions it is $\frac{1}{2.1}$ and for 0.2 % oxyhaemoglobin solutions it is $\frac{1}{1.8}$.

These results indicate that on denaturation at the isoelectric point the volume of the dissolved protein unit increases, the increase being of the order 80–100 %.

The question now arises—do the protein units, in swelling, imbibe part of the continuous medium, or are they impermeable to it? In the latter case, the increase in volume would be transmitted to the total volume of the system, *i.e.* the process of denaturation would be accompanied by an increase in the net volume of the protein solution.

The increase in the value of ϕ on denaturing a 3 % egg-albumin solution is $\frac{0.303 - 0.168}{k}$ if we use the general equation. The maximum value given to k in any of the empirical formulae is 4.5, so that the increase in ϕ must be not less than

$\frac{0.135}{4.5}$ or 0.03. This means that if 1 cc. of a 3 % egg-albumin solution is denatured the volume of the protein units must increase by 0.03 cc. In the dilatometer experiments 30 cc. of the solution were used, so that the increase in volume to be expected is 0.9 cc. This would produce a movement of the meniscus of 18 cm. The dilatometric experiments however showed no appreciable expansion on denaturation. We may conclude therefore that the protein units are permeable to the continuous medium, *i.e.* as they expand on denaturation, the continuous medium flows in to such an extent that there is no change in the total volume of the solution.

Thus at the isoelectric point at least, the process of denaturation is accompanied by an increase in the particle size of the protein units, water flowing into the units during the swelling. Consequently we have an explanation of the observations of Chick and Martin [1910] that denaturation will not occur in the absence of water. This phenomenon in fact would be expected in view of the present work.

If we wish to obtain more precise knowledge of the absolute values of the size of the protein units before and after denaturation at the isoelectric point, it is necessary to employ the empirical equation proposed by Kunitz [1926] which is as follows:

$$\frac{\eta}{\eta_0} = \frac{1 + 0.5\phi}{(1 - \phi)^4},$$

which on expansion becomes

$$\eta/\eta_0 = 1 + 4.5\phi + 12\phi^2 + 25\phi^3,$$

i.e. when ϕ is very small it tends to

$$\eta/\eta_0 = 1 + 4.5\phi.$$

This corresponds to the Einstein formula with the coefficient of ϕ changed from 2.5 to 4.5; in other words it is the formula suggested by Hatschek [1910].

Kunitz has tested his formula and finds that it holds very well for as high concentrations as 50 % solutions of such substances as sugars, glycogen, caseinogen and rubber. Furthermore, for sugars and sulphur, the viscosity value agrees with the actual volume of substance in the dry state, whilst for caoutchouc in benzene ϕ agrees with the values obtained from the equation for osmotic pressure.

Applying Kunitz's formula in its simplified form to the results for 3 % undenatured egg-albumin we get

$$1.167 = 1 + 4.5\phi,$$

i.e. $\phi = 0.037$.

If we assume the egg-albumin molecules to be spherical, an assumption supported by the ultra-centrifuge experiments of Nichols [1930], for a 3 % solution we obtain

$$\phi = \frac{3}{M} \cdot N \cdot \frac{4}{3} \pi r^3 \cdot \frac{1}{100},$$

where M is the molecular weight of egg-albumin, *viz.* 34,500, r is the radius and N is the Avogadro number, 6×10^{23} .

Equating these two expressions for ϕ we find that the radius of the undenatured albumin molecule (r) is 2.57×10^{-7} cm. as compared with Nichols's [1930] value of 2.17×10^{-7} cm. In a similar manner we find that the value of ϕ for 3 % denatured solutions of egg-albumin is 0.067, from which r is 3.13×10^{-7} cm.

Thus the viscosity data obtained can be interpreted to mean that on denaturation at the isoelectric point the dissolved units of albumin increase in size corresponding with an increase in the radius from $2.57 m\mu$ to $3.13 m\mu$.

The density of solid egg-albumin according to Arrhenius [1917] is 1.36. From this we can calculate a quantity ϕ' which would be the fraction of the volume of the solution occupied by the solute if the albumin underwent no change, *i.e.* did not become hydrated on being dissolved in water to give a 3 % solution.

$$\text{Thus} \quad \phi' = \frac{3}{1.36} \cdot \frac{1}{100} = 0.022.$$

But the viscosity data for 3 % undenatured solutions showed that ϕ is 0.037. This means that in a 3 % undenatured solution 0.022 cc. of protein is associated with 0.015 cc. of water, *i.e.* 1 cc. of protein is associated with 0.7 cc. water, or 1 g. of protein is associated with 0.5 g. of water. This value 0.5 is termed the "hydration factor" of the albumin. Incidentally this compares with the value 0.7 calculated by Arrhenius [1917] using the viscosity results of Chick [1914], and Chick and Lubrzynska [1914].

In the case of 3 % denatured egg-albumin solutions, by a similar calculation we find that 1.5 g. of water is associated with 1 g. of protein.

Passing now to the results for 0.2 % solutions of oxyhaemoglobin we find that for the undenatured protein at the isoelectric point, ϕ is 0.0033. If v is the volume of an individual dissolved protein particle, then

$$\phi = \frac{0.2}{66800} \times 6 \times 10^{23} \times \frac{v}{100} = 0.0033,$$

where 66800 is the molar or "micellar" weight of oxyhaemoglobin; from which v is found to be 184×10^{-21} cc.

The value of v given by Svedberg and Nichols [1927] is 110×10^{-21} cc. so that there is fair agreement between our value obtained from viscosity measurements and the value obtained from ultra-centrifuge measurements. On denaturation, the value of ϕ is 0.0058 from which the volume of an individual denatured particle at the isoelectric point is 323×10^{-21} cc.

Svedberg and Nichols [1927], taking the density of solid haemoglobin as 1.33, calculate that the volume of an individual particle should be 83×10^{-21} cc. Thus for undenatured particles 1 g. of protein is associated with 1.62 g. of water, and for denatured particles 1 g. of protein is associated with 3.85 g. of water.

From these figures we can infer that on denaturation at the isoelectric point oxyhaemoglobin undergoes a similar change to egg-albumin, *viz.* the protein particles distend by abstracting water from the continuous phase.

It is to be emphasised that the numerical values for the sizes of the undenatured and denatured protein units calculated above are intended to hold good only at the isoelectric point, since it is only at this p_H that we can be sure that there is no effect on the viscosity due to the electric charges on the protein particles.

Probably the most striking fact in connection with the viscosity is its independence of p_H , and the point is how this is to be interpreted in terms of the size of the colloidal unit. At first sight it appears that no electroviscous effect in the sense of Smoluchowski [1916] is operating, but at the present juncture it is not possible to state whether this is actually the case.

The general conclusion, however, that the denaturation of the proteins under consideration is accompanied by an increase in the size of the units, obviously holds at all values of p_H independently of the electroviscous effect.

SUMMARY.

1. Viscosity measurements have been made on solutions of undenatured egg-albumin and oxyhaemoglobin, and on deliberately denatured but unflocculated solutions of the same proteins.

2. In both cases the viscosity of a given concentration of undenatured solution is constant and independent of p_H .

3. Similarly the viscosity of a given concentration of denatured solution is also constant and independent of p_H provided that no flocculation has occurred.

4. On denaturation, the viscosity of the solutions increases, the increase being the same at all values of p_H for any given concentration of protein.

5. Dilatometer measurements show that there is no change in the total volume of the protein solutions when these undergo denaturation.

6. It is shown that at the isoelectric point the process of denaturation is accompanied by an increase in the size of the protein unit, the volume of the denatured unit being approximately twice that of the undenatured. In the light of the dilatometer results it follows that the process of denaturation is accompanied by an increase in the amount of water associated with each individual protein particle, this conclusion being in accordance with the well-known fact that denaturation will not occur in the absence of water.

7. It is further shown that this conclusion is probably true at p_H values other than the isoelectric point, even allowing for the possible effect of the electric charge carried by the particles on the viscosity of the solution.

8. Briefly the process of denaturation from the physical standpoint consists in a change from the molecular to a micellar state (without aggregation however) on the part of each protein unit.

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