

XXXV.—*The Acid-Gelatin Equilibrium.*

By HENRY RICHARDSON PROCTER and JOHN ARTHUR WILSON.

IN an earlier paper by one of us (T., 1914, **105**, 313) it was shown that gelatin forms hydrolysable salts with acids, that swelling is due to the ionisation of these and the osmotic pressure so produced, and that an equilibrium results, in which the concentrations of anion, hydrion, and ionised gelatin salt can all be expressed as functions of the concentration of acid in the external solution, within the limits of experimental error. In this earlier paper the concentration of the ionised gelatin chloride in the equilibrium gelatin-hydrochloric acid was shown to be approximately  $Cl_g = \sqrt{0.02x + 0.0002}$ , where  $Cl_g$  was the chlorine ionised from the gelatin salt, and  $x$  the concentration, in terms of normality, of the external hydrochloric acid. (Through an error  $Cl_g$  was given

as  $\sqrt{2x+0.02}$ , but all actual calculations were made from the above formula.) It was also assumed that the numerical values in the expression were constants, as those adopted sufficiently closely represented the experimental results then quoted, but closer theoretical investigation has shown that this is not strictly the case, but that both  $Cl_g$  and  $x$  are functions of a quantity  $e$ , which is the difference in osmotic pressure between two phases of which the ionic products are equal, but in one of which the factors are unequal; and that it is this difference which causes the swelling of the jelly.

In the paper cited the hydrochloric acid and gelatin salt were for the sake of simplicity supposed to be wholly ionised, and as the ionisation in both cases is very high and the solutions were dilute, such an assumption was quite justifiable as regards experimental results. In the closer theoretical examination which we now propose, however, we must define the concentrations as referring only to the actual ions; and we shall again first consider the comparatively simple case of hydrochloric acid and gelatin, where the ionisation is in reality almost complete.

The following system of notation will be employed :

At equilibrium :

in the external solution :

$$x = [H^+] = [Cl'].$$

in the jelly phase :

$$y = [H^+].$$

$z$  = concentration of gelatin ions.

$$y + z = [Cl'].$$

$a$  = concentration of non-ionised gelatin chloride.

$g$  = sum of concentrations of gelatin, gelatin chloride, and gelatin ions.

$e$  = excess of concentration of diffusible ions of the jelly over that of the external solution.

$V$  = volume of the jelly in c.c.

All concentrations are expressed in gram-equivalents per litre. In all experiments 1 gram of dry gelatin was immersed in 100 c.c. of the acid solution and allowed to remain for forty-eight hours to reach equilibrium, the temperature being about  $20^\circ$  in each case. The concentration of acid in the external solution was determined by titration, and that in the jelly by titrating the solution expelled from the jelly by the addition of salt, and with these, knowing the initial concentration of the acid and the percentage of ionisation of acid in the external solution, the actual ionic concentrations can be calculated.

In a two-phased equilibrium, such as the present, two different equations must be fulfilled. It is necessary that the products of hydrion and chloridion should be equal in the two phases; that is:

$$x^2 = y(y + z) \quad \dots \dots \dots (1)$$

This is not only proved by the thermodynamical equation of Donnan, quoted in the earlier paper (*loc. cit.*), but also follows from the ordinary laws of ionisation, since the non-ionised portion of hydrochloric acid which, although small, must exist, takes no direct part in the equilibrium, and must be equal in both phases since the jelly is permeable to it, and

$$x^2 = [\text{H}_1^+] \times [\text{Cl}'_1] = K[\text{HCl}] = [\text{H}_2^+] \times [\text{Cl}'_2] = y(y + z).$$

It was, however, previously pointed out that the equations

$$\begin{cases} x^2 = y(y + z) \\ 2x = 2y + z \end{cases}$$

cannot simultaneously be fulfilled, since in the jelly  $[\text{H}^+]$  and  $[\text{Cl}']$  are necessarily unequal, the chloridion being greater than the hydrion by the amount  $z$ , and the sum of the sides of an unequal rectangle is necessarily greater than that of the sides of a square of equal area (see Fig. 1). In other words,  $2y + z$  is greater than  $2x$  by an amount we shall call  $e$ , and the corrected equation becomes:

$$2x + e = 2y + z \quad \dots \dots \dots (2)$$

The concentration of diffusible ions of the jelly is therefore greater by  $e$  than that of the outer solution. It is obvious, since water and hydrogen chloride can pass freely into the jelly, that there must be some force equal to and opposing the osmotic pressure produced by this excess  $e$  of concentration at equilibrium, for otherwise the jelly would tend to swell to infinity.

Before attempting to speculate about the nature of this opposing force, we must consider its mathematical relations to the other concentrations of the equilibrium as defined by equations (1) and (2). The general theory of the equilibrium as developed in earlier papers (*Koll.-chem. Beihefte*, 1911, **2**, 243, and T., *loc. cit.*) is that when gelatin is placed in dilute acid it absorbs it freely and forms a hydrolysing salt, the proportion of which to the whole gelatin-base present is determined by the hydrolysis equation. The gelatin salt, like other salts, is highly ionised into the anion and a colloid cation, which either from polymerisation or other causes peculiar to the colloid state cannot diffuse and exerts no measurable osmotic pressure, whilst its anion is retained in the jelly by the electrochemical attraction of the colloid ion, but exerts osmotic pressure which, on the one hand, causes the mass to swell with absorption of the external solution, and, on the other, expels a portion of the

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acid, both anion and hydrion, from this solution absorbed, the result in equilibrium being that the jelly is poorer in hydrion and more concentrated in anion than the external acid solution, the difference of concentration between anion and hydrion in the jelly being, of course, equal to the ionised anion of the gelatin salt, and electrically balanced by the positive gelatin ions; whilst the hydrion concentration in the jelly is less than that of the outer solution by the amount of acid expelled, which may be called  $v$  (the isotonic volume of hydrion or chloridion expelled at a concentration of  $x$ );  $v$  bears the simple relation to  $y$  that  $y + v = x$  and the concentration of ionised gelatin chloride,  $z = 2v + e$ .

By solving simultaneously equations (1) and (2) the following interesting relations are derived:

$$x = y + \sqrt{ey} = \sqrt{y^2 + yz} = \frac{z^2 - e^2}{4e}$$

$$y = \frac{-z + \sqrt{z^2 + 4x^2}}{2} = \frac{2x + e - \sqrt{4ex + e^2}}{2} = \frac{(z - e)^2}{4e}$$

$$z = \frac{x^2 - y^2}{y} = \sqrt{4ex + e^2} = e + 2\sqrt{ey}$$

$$e = \frac{(x - y)^2}{y} = z + 2y - 2\sqrt{y^2 + yz} = -2x + \sqrt{4x^2 + z^2}$$

These relations can be represented graphically for any value of  $x$ , as is shown in Fig. 1.

Any one variable can be derived in terms of any other two, but in no case, from only the two equations given, can an equation be derived containing only two variables. As was pointed out previously, however,  $z$  was found from experiment to be equal approximately to  $\sqrt{0.02x + 0.0002}$ , which bears a resemblance to one of the derived equations, namely,  $z = \sqrt{4ex + e^2}$ . Putting  $e = 0.005$ , we get  $z = \sqrt{0.02x + 0.000025}$ , which is strikingly like the one obtained empirically, but gives low values for concentrations less than  $x = 0.03$ . Theoretical considerations rendered it improbable that  $e$ , considered in the general way in which we have done, could be a real constant, but it is difficult to obtain a smooth experimental curve from the formula  $e = \frac{(x - y)^2}{y}$ , because small errors in  $y$  correspond with large errors in  $e$ , and it is not possible to determine  $y$  in the most dilute solutions with very great accuracy by volumetric methods.

It occurred to us, however, that if we could incorporate the volume of the jelly, which can be determined with great accuracy, into an equation containing only two other variables, it would at once be possible to calculate any variable from  $V$  and  $x$  only. It



By solving (a) and (b) simultaneously to remove the term in  $a$ :

$$(c) Kgy = y^2z + Kyz + yz^2 + KK'z.$$

Taking Procter's figure of 839 for the molecular weight of a unit of gelatin, 1 gram of gelatin represents 0.00119 gram-equivalent. Therefore at any volume:

$$(d) g = \frac{1.19}{V}$$

Substituting (d) in (c) and simplifying:

$$(e) V = \frac{1.19Ky}{z(y^2 + Ky + yz + KK')}.$$

Or in terms of  $x$  and  $y$ :

$$(f) V = \frac{1.19Ky^2}{(x^2 - y^2)(x^2 + Ky + KK')}$$

The value of  $K'$  has been considered to be so small that neglecting it should produce no appreciable errors in concentrations greater than  $x = 0.005$ , so that for these more concentrated solutions the equation reduces to:

$$(g) V = \frac{1.19Ky^2}{(x^2 - y^2)(x^2 + Ky)}.$$

Now  $K$  has been assumed, with good reason, to be nearly equal to the ionisation-constant of hydrochloric acid for corresponding concentrations. It will be noted from the above equation that small errors in the value of  $K$  will produce negligible errors in the calculations so long as the value of  $Ky$  is considerably greater than that of  $x^2$ , and such a condition does obtain in these more concentrated solutions so long as  $K$  is the ionisation-constant of a strong electrolyte. For the present set of calculations, then, it will be permissible to take  $K$  as the ionisation-constant of hydrochloric acid, which is known approximately for any given concentration. Moreover, since it has been shown that, in these more concentrated solutions, almost all the gelatin has been converted into the monochloride, we are justified in taking  $K$  as the ionisation-constant of hydrochloric acid at concentration  $g$ , where  $g$  is simply  $\frac{1.19}{V}$ . Now from experimental values for  $V$  and  $x$  it is possible to calculate values for  $y$ , which should not differ from the value obtained from experiment by more than would be attributed to experimental error. The results are given in table I.

TABLE I.

Determined as noted above.*	By experiment.			Calculated.
	$V$	$x$	$y$	$y$
$K$				
0.95	16.9	0.262	0.228	0.237
0.94	17.5	0.220	0.186	0.195
0.88	20.2	0.174	0.145	0.152
0.85	21.6	0.153	0.123	0.132
0.85	21.6	0.130	0.105	0.108
0.83	22.4	0.108	0.080	0.087
0.80	24.1	0.087	0.066	0.068
0.75	25.9	0.064	0.049	0.047
0.65	34.3	0.0386	0.028	0.026
0.56	45.6	0.0165	0.0084	0.0084
0.55	49.4	0.0118	0.0057	0.0050
0.50	56.4	0.0071	0.0020	0.0022

\* These calculations are based on the figures of Bray and Hunt (*J. Amer. Chem. Soc.*, 1911, **33**, 781) and those of Noyes and Falk (*ibid.*, 1912, **34**, 454).

The agreement between experimental and calculated values bears out the theory remarkably well, the differences being not greater than was to be expected, considering the difficulties in titrating small quantities of solutions containing traces of organic matter. We feel that values for  $e$  calculated from  $V$  and  $x$  by this formula will be approximately correct. Of course, it is evident that in the very dilute solutions  $K'$  ceases to be a negligible quantity. Preliminary experiments with the hydrogen electrode show that  $K'$  is of the order of 0.00015, which fully justifies our assumptions in neglecting it for the higher concentrations.

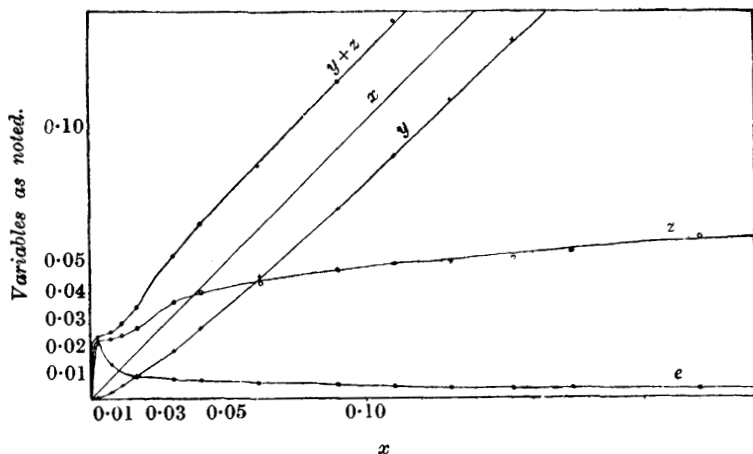
In Figs. 2 and 3 curves are given for the various variables as functions of  $x$ . The values for  $V$ ,  $x$ , and  $y$  were obtained directly from experiment, whilst those of  $z$  and  $e$  were calculated by means of the above formula. It will be noted by reference to Figs. 2 and 3 that  $e$  apparently varies directly as the volume. Such a relation, if it could be proved, would simplify all other relations to the extent that any variable could be expressed, with suitable constants, in terms of any other one, and the theory, as such, might well be said to be complete.

It was conceived that by means of the hydrogen electrode we could determine all the variables in a special experiment in which the volume was kept more nearly constant. One gram of gelatin was dissolved in such a quantity of water that at 33° the volume was 21 c.c. This high temperature was used because it is necessary to make hydrion determinations in the melted jelly. The method of using the hydrogen electrode was similar to that used and described by Sørensen (*Compt. rend. lab. Carlsberg*, 1909, **8**, 59). Acid was added at intervals and the hydrion concentration

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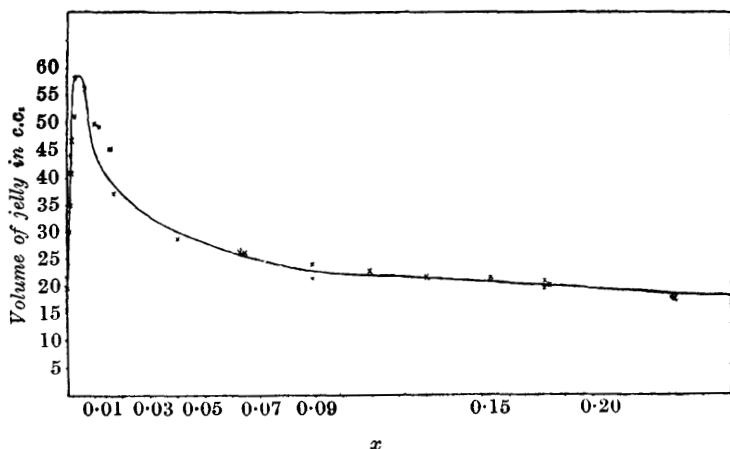
determined. From the figures obtained it was found possible to calculate all the variables desired. Knowing the quantity of

FIG. 2.



acid added, it was possible to calculate, from the hydrion concentration found, the amount which had combined with the gelatin, and consequently the total concentration of gelatin chloride, giving

FIG. 3.



values for  $y$  and  $a+z$ . Whilst actually there is no external solution, there is a theoretical one, since the products of hydrion and chlorid-



ion must be equal in both phases; that is, the product  $y(y+z)$  of the jelly is equal to the  $x^2$  of a theoretical external solution. If, as before, we regard the gelatin chloride as ionised to about the same extent as hydrochloric acid, we can take  $K$  as the ionisation-constant of hydrochloric acid at a concentration of  $a+z$ , which is known. From the equation  $z(y+z) = Ka$  it follows that:

$$z = \frac{-(K+y) + \sqrt{(K+y)^2 + 4K(a+z)}}{2}$$

in which all terms on the right-hand side are known, and consequently  $z$  can be calculated. From  $y$  and  $z$ , also, all other variables can be calculated by formulæ presented earlier in the paper. The results obtained in this way are given in table II, and are shown graphically in Fig. 4.

TABLE II.

c.c. N/30 HCl added.	Volume produced. V.	Concentration of acid produced.	Concentration hydron found. y	Concentration of acid found.	Concentration acid combined. a+z	z	x	e	y+z
0.0	21.0	—	0.000003	—	—	—	—	—	—
0.1	21.1	0.002381	0.000006	0.000006	0.002375	0.002351	0.000119	0.002128	0.002357
0.2	21.2	0.004762	0.000011	0.000011	0.004751	0.004614	0.000226	0.004202	0.004625
0.3	21.3	0.007143	0.000016	0.000016	0.007127	0.006965	0.000334	0.006320	0.006981
0.4	21.4	0.009345	0.000030	0.000030	0.009315	0.009064	0.000522	0.008069	0.009094
0.5	21.5	0.011628	0.000046	0.000046	0.011582	0.011229	0.000718	0.009817	0.011275
0.6	21.6	0.013888	0.000048	0.000048	0.013840	0.013390	0.000803	0.011875	0.013438
0.8	21.8	0.018348	0.000117	0.000117	0.018231	0.017665	0.001442	0.015005	0.017782
1.0	22.0	0.022727	0.000246	0.000247	0.022480	0.021555	0.00232	0.01741	0.021801
1.4	22.4	0.031250	0.00100	0.00101	0.03024	0.02881	0.00559	0.02107	0.02981
2.0	23.0	0.043478	0.00501	0.00511	0.03837	0.03621	0.01437	0.01748	0.04122
3.0	24.0	0.062500	0.0193	0.0201	0.0424	0.03928	0.0306	0.01059	0.0586
4.0	25.0	0.080000	0.0335	0.0350	0.0450	0.04098	0.0500	0.00813	0.0745
5.0	26.0	0.096154	0.0492	0.0521	0.0440	0.03935	0.0660	0.00573	0.0886

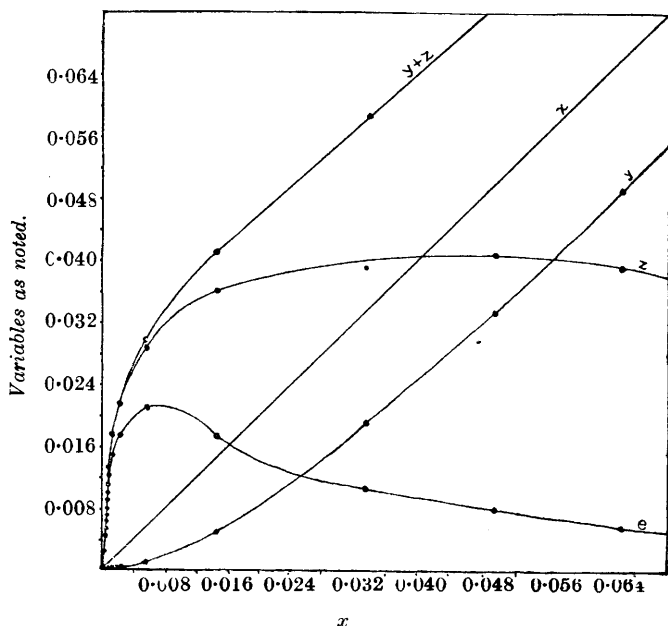
The curve of special interest is that of the variable  $e$ , which is seen to increase to a maximum at a very low concentration and then to fall in a manner similar to that of the volume curve (Fig. 2). Since  $e$  represents the measure of an outward pressure, we have, when the jelly is free to swell, an application of a special case of Hooke's Law, *ut tensio sic vis*, where stress =  $c \times$  strain; and since  $e$  is a uniform pressure, it follows that it will produce an increase in the size of the jelly, but not in its shape, and that the increase in volume will be directly proportional to the pull. If we take the volume of 1 gram of dry gelatin as 0.7 c.c., then, so long as the elastic limit is not exceeded,  $e = k(V - 0.7)$ , where the value of the constant is determined by the bulk modulus of the gelatin or particular protein under consideration. The relation is therefore dependent on the temperature, and that this is an appreciable factor is shown by the following rough experiments:

Initial concentration of acid.	Volume of jelly		
	7°.	15°.	33°.
0.200	11.3	17.6	—
0.100	13.0	19.8	—
0.050	14.6	23.2	33.0*
0.025	18.5	27.3	—
0.010	22.2	34.3	—

\* The value for the volume at 33°, a temperature well above the melting point of the jelly, is approximate only, and was obtained by gradually raising the temperature of the swollen jelly and its equilibrium acid, when the jelly, from its gravity and viscosity, does not mix with the supernatant liquid.

It is probable that the effect produced by this limited rise of temperature is not due to material changes in ionisation or chemical

FIG. 4.



activity, but almost solely to the diminution of the solid cohesion of the jelly. Many reasons convince us that the cohesive forces of the jelly opposing  $e$  are still maintained beyond the melting point.

It is evident that the volume of the jelly, at a constant temperature, is dependent for its value on the value of  $e$ , and the only remaining question is why the value of  $e$  should follow a curve of the particular type that it does. As was noted earlier in the paper,

the following equation results from a simultaneous solution of the thermodynamic and osmotic equations given :

$$e = -2x + \sqrt{4x^2 + z^2}.$$

As the concentration of acid is increased from zero to some small, but finite, value,  $z$  must necessarily increase at a very much greater rate than  $x$ . This is shown very markedly in the most dilute solutions, where almost all the acid added combines with the gelatin: but  $z$  has a limiting value, which is determined by the total concentration of gelatin with which we started. Now  $z$  must either approach this limiting value or diminish, which it would do if the ionisation of the gelatin chloride were sufficiently repressed. In either case:

$$\lim_{x \rightarrow \infty} \sqrt{4x^2 - z^2} = \sqrt{4x^2},$$

from which it follows that:

$$\lim_{x \rightarrow \infty} e = -2x + 2x = 0.$$

It is clear from this that, as  $x$  increases from zero,  $e$  must increase to a maximum and then decrease, approaching zero asymptotically, regardless of whether or not the ionisation of the gelatin salt is appreciably repressed. In Fig. 4 it will be seen that  $e$  begins to decrease at a considerable rate while  $z$  is still increasing slightly, which would be expected. It should be noted that the apparent decrease in  $z$  in the most concentrated solution, given in table II, is due chiefly to the increased volume.

An interesting point is raised here regarding the action of salt in repressing the swelling of jelly swollen with acid. Whilst the salt undoubtedly represses the ionisation of the gelatin chloride to some extent, it would scarcely be sufficient to account for the fact that salt reduces the volume of jelly almost to that of dry gelatin. The chief action is probably that the addition of salt corresponds with an increase in the value of  $x$ , and that this increase in  $x$  must, according to the equation just discussed, produce a decrease in the value of  $e$ , with a corresponding diminution of the volume of the jelly.

#### *Summary.*

When gelatin is immersed in a dilute solution of an acid, combination takes place between the gelatin molecules and the hydrogen ions, resulting in the formation of a highly ionisable salt of gelatin, the anion of which in tending to diffuse exerts on the jelly mass an outward pull, which, being uniform in all directions, produces, according to Hooke's Law, an increase in the volume of the jelly proportional to the magnitude of the pull. In the case of gelatin

immersed in a very dilute solution of a highly ionisable acid (say, 1 gram of gelatin in 100 c.c. of  $N/1000$ -hydrochloric acid) almost all the acid combines with the gelatin, and we have the simplest type of equilibrium, where, practically,  $x=0$ ,  $y=0$ ,  $y+z=z=e$ , and the concentration of the anion of the jelly is the measure of the outward pull and consequently of the increase in volume. In more concentrated acid solution (say, 1 gram of gelatin in 100 c.c. of  $N/10$ -hydrochloric acid) only a part of the acid combines with the jelly, and we have  $y+z > z > e$ , but here it is neither the total concentration of anion of the jelly nor that of the ionised gelatin salt which is the measure of the force producing swelling, but it is the excess of concentration of diffusible ions of the jelly over that of the external solution. This quantity  $e$  is a direct measure of the swelling so long as the swelling does not exceed the elastic limit, and offers a complete explanation of the peculiar swelling curve obtained by immersing gelatin in increasing concentrations of hydrochloric acid (see Fig. 2). In the most dilute solutions  $e$  will increase almost directly with the increasing initial concentration of acid, but will approach a maximum as the formation of the gelatin monochloride nears completion, and must then decrease as  $x$  becomes larger, according to the equation  $e = -2x + \sqrt{4x^2 + z^2}$ , where  $z$  has a limiting maximum value. The repression of swelling by the addition of salt is caused by the apparent increase in the value of  $x$  produced, which results in a diminution of the value of  $e$  and consequently in a repression of the swelling, this action being assisted to some extent by the repression of the ionisation of the gelatin salt.

In the case of weak acids, like acetic, a greater total concentration of the acid is required to produce nearly complete combination of the gelatin with the acid, because the degree of combination is determined by the value of  $y$ , which, even in the more concentrated solutions, will be small because of the repression of the ionisation of the acid by the highly ionisable gelatin salt. For this reason the swelling of gelatin in acetic acid increases with increasing total concentration of acid, and is not repressed by the addition of an excess; in fact, the swelling continues up to a strength of acid of  $N/1$  beyond which solution of the gelatin takes place. The somewhat stronger formic acid actually shows slight repression, whilst very weak acids, such as boric, as would be expected, produce little, if any, swelling.

In pure water, combination must take place, although probably only to a very slight extent, between the gelatin molecules and the hydrion of the slightly dissociated water, leaving in the jelly a corresponding excess of hydroxyl ions which tend to diffuse out-

ward, causing the jelly to swell. The presence of sulphites in the gelatin and carbonic acid in the water tend, of course, to produce a greater swelling than the minimum, which would result from pure gelatin and water, difficult, if possible, to obtain.

Some work has been done on the equilibrium of gelatin and alkalis, but solution of the gelatin took place at so low a concentration of the alkali (at about  $x=0.04$  for sodium hydroxide at  $20^{\circ}$ ) that the work could not be carried out to the extent desired. Work on hide has shown that the swelling is repressed either by the addition of excess of alkali or by the addition of ammonium chloride. In the former case the swelling is repressed by the increase in the value of  $x$ , according to the law derived for acids; in the latter case by bringing the solution back almost to a condition of neutrality, the gelatin compound being again decomposed. It is probable that the laws governing alkaline-swelling are the same as those governing acid-swelling.

It will be seen that the laws discussed are quite general, and that for any particular sample of gelatin at constant temperature any variable can be expressed as a direct function of  $x$ , and that for all acids the value of  $K'$  will be the same, whilst the value of  $K$  is merely dependent on the degree of ionisation of the gelatin salt formed. With suitable values for  $K$ ,  $K'$ , and  $k$ , the laws are probably applicable to any protein and any acid or alkali.

If, as the authors believe, the foregoing theory is not merely applicable to gelatin, but, with appropriate constants to the colloidal swelling of all proteins, it is obviously of far-reaching importance, not merely to the special technology in which it originated, but to many physiological and medical problems. It is only necessary to allude to the work of Loeb on the fertilisation of the *Echinus* egg by saline solutions, of Fischer on oedema, and of Pauli and others who attribute muscular energy to colloidal swelling and contraction produced by the alternate action of sarcolactic acid and the saline constituents of the blood; whilst many of the problems of plant-growth and of the semipermeability of vegetable membranes are probably due to analogous causes; and the laws which regulate the swelling of carbohydrate jellies, such as agar-agar, starch, and cellulose itself demand a similar investigation.

PROCTER INTERNATIONAL RESEARCH LABORATORY,  
UNIVERSITY OF LEEDS.

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