XIX. ON THE SWELLING OF GELATIN IN HYDROCHLORIC ACID AND CAUSTIC SODA.

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I. INTRODUCTORY.

In 1900 Hardy [1900] put forward evidence to show that gelatin gels are physically heterogeneous, *i.e.* 2-phase systems, and stated in addition that one phase was a solid solution of water in gelatin, the other a liquid solution of gelatin in water. The solid phase (unless the concentration of gelatin were very great) formed an open framework throughout the system.

Procter [1914] discarded the 2-phase theory of gel structure as gratuitous, merely postulating a "molecular network" throughout the jelly. He considered that the equilibrium in a gelatin-acid-water system was due to the chemical combination of gelatin and acid to form dissociated salts, and that the osmotic pressure of these salts determined the volume of the jelly. Procter's theory will be discussed in detail later; at this juncture it may be summarised as a theory which postulates physical homogeneity, but chemical heterogeneity.

In the following pages it is hoped to establish the thesis that both physical and chemical heterogeneity are essential to the gel state, and to show that the volume occupied by a gelatin gel is determined by the resultant of two opposing sets of forces localised respectively in the two phases of the jelly. The experimental evidence will now be considered.

II. MATERIALS AND METHODS.

Coignet's "Gold Label" gelatin was used for all experiments. An analysis of the sample used showed that it contained 20 % of water, which was removable by drying for 6-8 hours in a hot air oven at 110°. Actually, therefore, there was probably a slightly higher percentage of water, but a correction for 20 % of water only has been made in the initial weights of leaf gelatin taken for the experiments. The gelatin had an ash content of 1.0 %. This consisted mainly of calcium sulphate, with traces of phosphates and chlorides. A small fraction of the base present was combined with the gelatin.

The distilled water used was always of recent manufacture; it was made in an ordinary laboratory still and had a reaction of about $P_H = 5.5$. After boiling and cooling the reaction moved to $P_H = 7.0$. The acidity therefore was due to dissolved carbon dioxide. Solutions of the gelatin in boiled distilled water gave a reaction $P_H = 5.25$, at concentrations of 0.2 and 0.5 %. Since the iso-electric point of gelatin is at $P_H = 4.6$, this shows that the gelatin contained a small quantity of base.

In the experiments recorded below discs of leaf gelatin weighing from 0.05 to 0.08 g. were used in most cases. These were placed in Gooch crucibles where they remained at all stages of the experiment. In experiments on equilibria in liquids, both crucible and gel were dried rapidly with filter paper before each weighing. The gels were weighed in their crucibles, but a cover was always placed on the crucible during weighing to check loss of weight by evaporation. The same applies to weighings made during experiments on equilibria in saturated vapour. In drying the gels care was taken to avoid mechanical pressure. The smoothness of the experimental curves appears to justify the simplicity of the method.

The volume of liquid used was in most experiments 150 cc. The experimental liquids were placed in glass bottles with wide necks and ground-in stoppers. The crucibles were easily suspended in these by a harness of fine thread. For experiments conducted in vapour, a glass desiccator was used with distilled water at the bottom. This was partially evacuated, but the air pressure in the chamber does not appear to have an appreciable influence on the shape of the experimental curves.

In all experiments, apparatus in use was kept submerged in a water-bath at 20°. The variation of temperature was $\pm 0.25^\circ$.

In all experiments a few drops of toluene were added to the system to prevent the development of bacteria and moulds.

III. THE SWELLING OF GELATIN IN WATER, HYDROCHLORIC ACID AND CAUSTIC SODA.

The swelling of gelatin in water and the influence of acid or alkali in increasing both the rate and degree of such swelling has been described many times by many writers. The rate at which the dry gelatin absorbs water,

and the final amount taken up, depend on a variety of factors among which may be mentioned: hydrogen-ion concentration [Chiari, 1911; Procter, 1914; Loeb, 1918]; temperature [Procter and Burton, 1916]; the ratio of the area of surface of the gelatin to its volume [Spiro, 1910; Fischer, 1910; Hofmeister, 1890; Pauli, 1897]; the ratio of the mass of the gelatin in the system to the mass of dissolved electrolyte (see below), the elastic modulus of gelatin [Procter and Wilson, 1916], and finally, since in every case of absorption of

Fig. 1.

Abscissa = time.

Ordinate = weight of gel expressed as percentage of dry weight of gelatin.

water by dry leaf gelatin the initial stage must consist in the restoration of a preformed structure, a factor must be introduced involving the conditions under which the gel was originally formed [see Hardy, 1900]. This last factor is usually unknown. The importance of the mass relations in the system gelatin-hydrochloric-acid-water (other variables being kept constant) is illustrated by the curves shown in Fig. 1. These curves illustrate the progress of swelling in three systems where the ratios $\frac{\text{dry weight of gelatin}}{\text{volume of } 0.001N \text{ HCl}}$ were 0.8/100, 0.8/1000, 0.8/10,000 respectively.

The actual experimental figures for the three systems are:

	I	II	III
Weight of leaf gelatin containing 20 % moisture =	0.820 g.	0.1443 g.	0.0148 g.
Corrected weight of gelatin =	0.656 g.	0.1154 g.	0.0118 g.
Volume of $N/1000$ HCl added =	82 cc.	144 cc.	148 cc.
Weight of swollen jelly at equilibrium =	12.04 g.	8.21 g.	—
Weight at equilibrium expressed as percentage of the weight of dry gelatin } =	1840	7100	—
Initial reaction of acid, P_H =	3.0	3.0	3.0
Final reaction of acid, P_H =	4.4	3.57	(3.25)

The intermediate weights of the gel system expressed as percentages of the dry weight of gelatin, and the time relations, are shown in Fig. 1. System III ultimately went into solution.

The influence of the reaction, or hydrogen ion concentration, on both rate and degree of swelling is shown by the curves in Figs. 2 and 3. The problem of the influence of concentration of acid on the degree of swelling has been studied very thoroughly by Procter. It is to be noticed that Procter used 1 g. of moist gelatin in 100 cc. of experimental fluid. In the experiments shown below the ratio of dry gelatin to fluid varies from $1/2200$ to $1/3750$. The importance of keeping this factor constant unfortunately had not been fully realised at the time the experiment was made. The curves therefore carry a small relative error due to this cause.

	I	II	III	IV	V	VI
Concentration of HCl. Volume in } all cases = 150 cc.	0.001N	0.005N	0.01N	0.05N	0.1N	0.5N
Weight of leaf gelatin, g.	0.0640	0.0500	0.0644	0.0852	0.0838	0.0781
Weight of gelatin corrected for water content, g. }	0.0512	0.0400	0.0515	0.0682	0.0670	0.0625
Ratio, weight of gelatin/acid volume	$1/2930$	$1/3750$	$1/2950$	$1/2200$	$1/2240$	$1/2400$

The progress of swelling in the six systems can be followed in the curves in Fig. 2. In systems IV, V, and VI the gelatin dissolved in the acid in the course of a few days. System III was unfortunately attacked by mould and the end of the curve was therefore lost, but from other experiments it is known that $0.01N$ hydrochloric acid also dissolves gelatin in the proportions given. It will be shown later that this is a true case of solution and that it does not involve any break-down of the gelatin molecule.

Curves I and II appear to reach an equilibrium state. The daily increase of weight becomes imperceptible, but even after two or three months the swollen jellies still show a slow, steady increase in weight. This type of behaviour has already been recorded by Masson [1905], studying the absorption of water by silk fibres. It may therefore be characteristic of all proteins. It suggests the possibility that both gelatin and silk fibre would ultimately dissolve in water or dilute acid, given a sufficient quantity of solvent and sufficient length of time.

It is interesting to compare curve I, Fig. 2 with curve II, Fig. 1. The very different level reached in the two cases is probably due to the difference in thickness of the two sheets of gelatin from which the discs were cut in the two different experiments. This factor, *i.e.* thickness of the plate, appears in the equations of both Hofmeister [1890] and Pauli [1897] for the swelling of gelatin in water.

Fig. 2.

Abscissa = time.

Ordinate = weight of gel expressed as percentage of dry weight of gelatin.

In Fig. 3 are given the time-weight curves of the alkaline series. The isoelectric point of gelatin is at $P_H = 4.6$. All media having a value for P_H greater than 4.6 are therefore "alkaline" to gelatin. For this reason the curve for distilled water has been placed in the same series as the curves for caustic soda. The experimental quantities used in the experiment were:

	I	II	III	IV	V	VI	VII
Concentration of NaOH.							
Volume in all cases, 150 cc.	0.5N	0.1N	0.05N	0.01N	0.005N	0.001N	Water
Weight of leaf gelatin, g.	0.070	0.0627	0.0659	0.0595	0.0592	0.0586	0.0801
Weight of gelatin corrected for water content, g.	0.056	0.0502	0.0527	0.0476	0.0474	0.0469	0.0641
Ratio, weight of gelatin/alkali volume	1/2680	1/2980	1/2850	1/3150	1/3160	1/3200	1/2350

The same sheet of gelatin was used for cutting the discs used in all the experiments charted in Figs. 2 and 3. The variation between the two series due to varying thickness of the gelatin has therefore been eliminated. The two series differ in two striking ways; the first is the much greater rapidity with which the gelatin is dissolved in the more strongly alkaline solutions

than in acids of equal normality, the second is the much greater degree of swelling attained by the gels in the acid systems. The last point is well shown in Fig. 4.

In Fig. 4 the curve weight of gelatin gel against reaction of fluid has been plotted. The time has been fixed arbitrarily at 48 hours. The similarity of this curve to the corresponding curve for skeletal muscle [Jordan Lloyd, 1916] is very striking.

Fig. 3.

Abscissa = time.

Ordinate = weight of gel expressed as percentage of dry weight of gelatin.

Fig. 4.

Abscissa = hydrogen-ion concentration.

Ordinate = weight of gel expressed as percentage of dry weight of gelatin.

IV. THE STATE OF GELATIN DISSOLVED BY ACID OR ALKALI IN THE COLD.

If solutions of gelatin in hydrochloric acid or in caustic soda which have been made by allowing the gelatin to stand in contact with these fluids in the cold are examined *at the moment of solution*, there is no chemical evidence that there has been any break-down of the protein molecule.

Consider first the case of gelatin dissolved in dilute hydrochloric acid. The following experiment may be cited: 2 g. of gelatin were placed in a flask containing 200 cc. *N*/10 hydrochloric acid. The gelatin was completely dissolved after six days at 20°, 20 cc. *N* NaOH were added to the solution, which was tested and found neutral to litmus, and 220 cc. saturated ammonium sulphate. A white flocculent precipitate was formed. This was filtered off. The filtrate was tested with the biuret test and shown to be free from protein. The precipitate was insoluble in cold water, and was washed several times. It was found to be rapidly soluble in hot water. The precipitate was completely dissolved in 2 cc. of hot water and the solution set to a gel on cooling. A control experiment made by dissolving 2 g. of gelatin in 220 cc. of water with 1.12 g. of sodium chloride behaved in a similar manner.

Gelatin therefore can be recovered from an acid solution.

A similar solution of gelatin (*i.e.* 2 g. in 200 cc. *N*/10 hydrochloric acid) was poured into 3 litres of 96 % alcohol. A white gelatinous precipitate formed which settled slowly to the bottom of the flask. This was filtered off and was washed with absolute alcohol. On cold water being added to the precipitate it swelled rapidly to a clear, glassy, but very soft gel. This was melted and was found to have an acid reaction. The volume was brought to 2 cc. and the solution set to a gel on cooling.

Solutions of gelatin in acid (made in the cold) were compared against control solutions in water for the presence of free ammonia and free amino-groups. Nessler's method was used for the detection of ammonium groups, and Sørensen's formol titration method for the estimation of amino-groups. No difference could be detected between the acid solution and the water solution, *i.e.* there was no evidence of break-down of the gelatin molecule at the moment of solution. If the two solutions are left standing for a day at room temperature, it can be shown that in the acid solution a very slow hydrolysis is taking place.

These facts accord fully with Procter's theory that gelatin forms salts with hydrochloric acid. They also show that the change

is reversible.

The case of solution in alkali is more complicated. Though the gelatin goes rapidly into solution without molecular break-down, there is evidence that the formation of the solution is accompanied by some internal structural

change in the protein molecule since gelatin is not readily recovered from a caustic soda solution, *i.e.* the change

is not reversible. Brailsford Robertson [1918] has suggested that acids and bases attach themselves to protein molecules at the —COHN— linkage. He points out that this linkage may exist as an enol-linkage $\text{—}\overset{\text{OH}}{\underset{|}{\text{C}}}=\text{N—}$ or as a

keto-linkage $\text{—}\overset{\text{O}}{\underset{||}{\text{C}}}\text{—N—}$, and favours the enol-form since it offers a point of

attachment to both acids and bases. A more probable explanation seems to be that under the action of acids gelatin goes to the keto-form, and under the action of bases to the enol-form. This would conform with the observation that the free acid from sodium gelatinate differs in properties from the free base of gelatin hydrochloride. It can also be harmonised with Dakin's theory [1913] that the non-terminal groups in proteins go from the keto-form (1) to the enol-form (2)

with loss of optical activity under the action of bases at low temperatures.

The experimental evidence that gelatin dissolves in caustic soda with change of properties, but without molecular break-down, is given below.

0.2 g. gelatin was placed in a flask with 200 cc. *N*/10 NaOH. The gelatin was completely dissolved after two days at 20°. 20 cc. hydrochloric acid were added to the solution, which was tested and found neutral to litmus, and 220 cc. saturated ammonium sulphate. A white flocculent precipitate formed immediately and was filtered off. The filtrate was tested with the biuret test and found free from protein. The precipitate dissolved completely and rapidly in a few cc. of cold water. The solution was reduced to a volume of 2 cc. (= 10 % concentration of gelatin) but it did not gelatinise on cooling.

A similar solution (*i.e.* 0.2 g. of gelatin in 200 cc. *N*/10 NaOH) was poured into 3 litres of 96 % alcohol. A white gelatinous precipitate was formed which settled slowly. This was filtered off and washed with alcohol. It was strongly alkaline. Enough *N* HCl was added to the gelatinous, slimy mass to make it neutral to litmus. 2 cc. of hot water were added; the precipitate dissolved completely forming a turbid solution but this did not set to a gel on cooling.

Two solutions of gelatin were made:

(A) by dissolving 0.2 g. of gelatin in 200 cc. *N*/10 NaOH in the cold and neutralising with *N* HCl.

(B) by dissolving 0.2 g. of gelatin and 1.12 g. of sodium chloride in 200 cc. water by means of heating.

These two solutions contained identical concentrations of gelatin, sodium ions and chlorine ions, and had the same reaction (neutral to litmus). They were dialysed against distilled water for a week to reduce the concentration of sodium chloride. They were then evaporated to small volume, 2 cc.: B set to a firm jelly on cooling; A remained fluid. The jelly B was melted and the two solutions A and B were half saturated with ammonium sulphate. A dense white precipitate formed in both tubes, and was filtered off. The filtrates were free from protein. Precipitate A was soluble in cold water, precipitate B was insoluble in cold water but dissolved readily on warming.

Gelatin therefore cannot be recovered unchanged from alkaline solution.

Solutions of gelatin in caustic soda, made in the cold, were compared against control solutions in water for the presence of free ammonia and free amino-groups. No difference could be detected between the alkaline solution and the control, *i.e.* there was no evidence of break-down of the protein molecule at the moment of solution. It is important that the solutions should be examined immediately after solution, as the alkaline solution shows evidence of hydrolysis after two or three hours' standing at room temperature. The fresh gelatin-alkaline solutions were examined for their rotatory power. No change in optical activity of the gelatin was detected, and indeed from Dakin's experiments would hardly be expected.

V. THE BASIC AND ACIDIC PROPERTIES OF GELATIN.

The observation that gelatin solutions can dissolve considerably greater quantities of lime than can pure water is recorded by Heintz [1853], and the solubility of gelatin in acid or alkali in the cold by Kuhne [1888]. The first definite description of these compounds of gelatin with acids and bases respectively, as gelatin salts, appears to be in a paper by Nasse [1889] published in 1889. Pauli [1912, 1913] further postulated that gelatin salts were ionised in aqueous solution. A considerable amount of work has since been done on the subject and the present position may be summarised briefly as follows: gelatin can combine chemically with both acids and bases, the amount of acid or base combined with unit weight of gelatin being a function of the hydrogen-ion concentration. This is well shown in both Procter [1914] and Loeb's [1919, 1] recent experimental figures. This inter-relation of combined acid and reaction has been interpreted as an adsorption phenomenon, but it can equally well be deduced from the theory that gelatin is a multi-basic acid or a multi-acid base, which dissociates in stages according to the reaction in a way strictly analogous to the dissociation of phosphoric acid. Procter considers that gelatin acts as a di-acid base and has a molecular weight of 839. Consideration of the chemical evidence (see Appendix) shows that the molecular weight of gelatin must be of the order 10,000. A few rough experiments made by means of indicators during the course of this work have indicated that gelatin when acting as a base is not completely neutralised by hydrochloric acid until the reaction is at $P_H < 2.5$. At this reaction, and taking

10,000 as the molecular weight, the basicity is 8. Conversely in an alkaline system, the acid valencies of gelatin are not yet satisfied at $P_H = 13$, and the acidity at this reaction appears to be 28. This figure however is not reliable. Guttenberg [1896] and Berrar [1912] using different quantitative methods both reach the figure for gelatin, 10 atoms of nitrogen correspond to 1 molecule of acid. As Berrar states that the binding power of gelatin for acid is unaffected by excess of acid, this value may be taken to be a maximum. Assuming 133 nitrogen atoms in the gelatin molecule (see Appendix) the basicity of gelatin becomes 13.

The chemistry of the gelatin salts is at present under investigation, and it is hoped shortly to publish more satisfactory evidence of their constitution. The theory that gelatin forms ionisable salts which are more soluble in water than either the free acid or the free base is in accordance with all the evidence at present available and has been dealt with briefly here, as it is an essential part of the theory of gel structure which is discussed in Section VII of this paper. The further question of the nature of the ions to which these gelatin salts give rise in solution is also discussed.

VI. THE BEHAVIOUR OF SWOLLEN GELS IN AN ATMOSPHERE OF SATURATED WATER VAPOUR.

Masson [1905] has recorded for silk fibres that the maximum degree of swelling in liquid water or in saturated water vapour is identical, and that the rates of swelling in the two cases are very nearly so. Schroeder [1903] has stated that this is not the case with gelatin. He gives figures showing that dry gelatin in contact with water vapour will increase its weight up to about 140 % of the dry weight; removed to liquid water further increase up to 1200 % will occur. Conversely gelatin in equilibrium with, or even absorbing water from, liquid water will give up water if transferred to an atmosphere of saturated vapour. Schroeder's experiments were made under apparently controlled conditions, but his controls were not very rigid. Wolf and Buchner [1915] re-investigated the question of the equilibrium of gels in liquid and gaseous water and found that by conducting their experiments in a very reliable thermostat, and using vessels silvered on their internal surfaces, it was possible to transfer gelatin in equilibrium with liquid water into the saturated vapour phase without subsequent loss of weight occurring. With even the slightest temperature variation in the apparatus, loss of water from the swollen jelly promptly occurred. This loss however occurred equally from small basins of pure water suspended in the chamber, and Wolf and Buchner regard it as a distillation on to the walls of the containing vessel under a temperature gradient. It appears therefore to have been satisfactorily demonstrated that the equilibrium of gelatin gels in water and water vapour does not form an exception to the second law of thermodynamics as has been claimed. But in spite of the undoubted truth of Wolf and Buchner's results, and the disproof of Schroeder's phenomenon *per se*, it is hoped to demonstrate

that in systems consisting of acid or alkaline gelatin two separate equilibria do actually occur in the fluid and gaseous media, but that the existence of these two points follows naturally from Donnan's membrane potential theory [1911] and does not therefore form an exception to the second law of thermodynamics.

If gels which are swelling in dilute hydrochloric acid, or in caustic soda, are lifted out of the liquid into a vapour phase, they immediately contract and express fluid. The expulsion of water is frequently so great and so rapid that after three or four minutes in air, drops of liquid can be seen gathering at points all over the surface of the jelly even though the latter be kept in the horizontal plane. In the course of a few hours these beads of water may run together and flow away from the jelly, causing loss of weight, and if the gels are kept in an atmosphere of saturated vapour over a plane surface of water and at a steady temperature, further marked loss will continue to occur for three or four days, doubtless owing to the raised vapour pressure at the curved surface of the fluid drops. It is particularly wished to emphasise that the water does not merely drip away from the lowest edge of a suspended jelly, but is squeezed out at definite *loci* scattered evenly all over the free surface of the gel. This observation confirms the existence of definite pores in a jelly surface, which was postulated both by Bütschli [1896, 1898] and v. Bemmelen [1898]. It may be noticed that Hardy's figures of coagulated colloidal solutions all show an orientation of the solid particles at the surface, which would lead to the formation of pores. This phenomenon of recoil appears to be a good demonstration of the existence of elastic forces within the gel, and it will be shown below that these elastic forces must be localised in the solid framework. The amount of water squeezed out by a gel in this way is independent of the total amount of water in the gel. This is shown by the following experiment: discs of leaf gelatin were soaked in $N/100$ NaOH for 1, 2 and 5 days respectively, the swollen jellies were transferred to the same moist chamber, and weighed every 24 hours.

	I	II	III
Weight of moist gelatin, g.	= 0.0655	0.0590	0.0610
„ „ gelatin corrected for 20 % moisture, g.	= 0.0524	0.0472	0.0488
Volume of $N/100$ NaOH added	= 150 cc.	140 cc.	140 cc.
Days in $N/100$ NaOH	= 1	2	5
Weight on removing from NaOH, g.	= 1.8515	2.1410	2.754
Ditto calculated as percentage of dry weight of gelatin =	3530	4420	5630
Weight after 24 hours in moist chamber calculated			
as % of dry weight	= 2520	3590	4440
Do. after 48 hrs	= 2270	3160	3840
Do. „ 3 days	= 2180	3000	3550
Do. „ 4 days	= 1990	2900	—

These figures have been plotted and are shown on the curves in Fig. 5. It will be seen that the three curves are parallel. If the shrunken gels are again immersed in fluid the reaction is reversed, water being again absorbed.

Though the amount of water in a gel does not determine the amount lost in an atmosphere of saturated water vapour, the reaction of the fluid in which the initial swelling of the dry leaf gelatin has taken place is a very potent factor. By varying the reaction it is possible to determine at will whether a gel shall lose weight on being transferred to a vapour phase, gain weight, or remain at a steady equilibrium. Curves for acid gels of varying reaction are shown in Fig. 6, and for alkaline gels in Fig. 7. In these curves, the abscissa is time, and the ordinate the gain or loss in weight which occurs after transferring to the vapour phase. This is expressed in percentages of the dry weight of gelatin. Notice that in all cases the weight at transference is placed at the origin. In both figures a control curve has been plotted. This shows the change in weight of a disc of moist filter paper kept in the same apparatus. Under ideal conditions this should of course run parallel to the x -axis. It will be seen that in reality it falls, slowly and steadily.

Fig. 5.

Abscissa = time.

Ordinate = weight of gel expressed as percentage of dry weight of gelatin.

The initial weights of the gelatin discs and their weights after soaking for 48 hours in the experimental fluid are given below. The various weight changes in the saturated vapour phase can be read from the curves. 150 cc. of acid or alkali were used in all the experiments. The acid figures (see Fig. 6) are given first.

Concentration of hydrochloric acid, 150 cc. taken	$N/2$	$N/10$	$N/20$	$N/200$	$N/1000$	$N/10,000$	$N/100,000$
Weight of gelatin disc, g.	0.0676	0.0676	0.0870	0.0695	0.0604	0.0640	0.0650
Do. corrected for 20% water content, g.	0.0541	0.0541	0.0696	0.0558	0.0484	0.0512	0.0520
Weight after 48 hrs. in acid, g.	0.864	1.031	2.0268	2.169	1.430	0.922	0.788
Do. expressed as percentage of dry weight of gelatin	1590	1910	2920	3900	2960	1800	1510

The experimental quantities in the alkaline series (Fig. 7) were:

Concentration of NaOH, 150 cc. taken	N/10	N/20	N/100	N/200	N/1000	N/10,000	N/100,000	Dist. water
Weight of gelatin, g.	0.0622	0.0621	0.0620	0.0637	0.0581	0.0636	0.0622	0.0696
Do. corrected for 20 % water content, g.	0.0498	0.0497	0.0496	0.0510	0.0465	0.0509	0.0498	0.0557
Weight after 48 hours in NaOH, g.	1.617	1.135	1.206	1.297	0.835	0.501	0.495	0.551
Do. expressed as percentage of dry weight of gelatin	3250	2280	2440	2545	1790	990	996	990

It can be seen from Figs. 6 and 7 how very powerfully the reaction of the fluid influences the behaviour of the gel in the saturated vapour. Though the water loss is considerable in some of the acid systems, it is even exceeded in the alkali systems. In the experiment with N/200 HCl (Fig. 6) 100 units

Fig. 6.

Abscissa = time.

Ordinate = change of weight in saturated vapour calculated as a percentage of the dry weight of gelatin.

of gelatin were holding at transference 3900 units of water; 650 of these, equalling one-sixth of the water content of the jelly, were lost in three days. In the alkaline series the greatest loss again occurs at the concentration N/200. In the experiment shown in Fig. 7, 100 units of gelatin were holding 3500 units of water; 1700 units of water, equalling nearly a half of the total water content, were expressed in three days. After three or four days the weight curves all run parallel to the filter paper control. Further loss of weight may therefore be attributed to experimental error. It is interesting that the reaction causing maximum swelling in the liquid systems should coincide with the reaction allowing maximum recoil in the vapour phase. It should be noted that the fluid expressed by the gels is not pure water, but contains gelatin

in solution. From acid gels the fluid has an acid reaction; from alkaline gels an alkaline. The reaction of the expressed fluid is not the same as the reaction of the external medium, but lies nearer to the neutral point. This fluid is the "internal phase" of the gel.

Gels which have been swollen in hydrochloric acid or caustic soda of normality $N/20$ or greater all show further absorption of water on being transferred to the saturated vapour. (In the $N/20$ NaOH curve, shown in Fig. 7, there is loss of water followed by absorption.) In some cases the gels

Fig. 7.

Abscissa = time.

Ordinate = change of weight in saturated water vapour calculated as percentage of dry weight of gelatin.

ultimately liquefy. These curves are starred on Figs. 6 and 7. It can be shown here, as in solution in strong acid or caustic soda, that the gelatin has not been hydrolysed but has only gone into solution. It can be recovered from the acid solutions, but not from the alkaline ones.

The behaviour of the swollen gels in a vapour phase seems to be most easily interpreted on the theory that it is determined by a solid framework in the gel, which possesses elastic properties. The stretching force in the gel may be taken to be the osmotic pressure of the gelatin salts in solution. The

forces of recoil appear to conform to Hooke's law, stress $\propto$ strain, i.e. they become greater the more swollen the jelly. Osmotic forces and elastic counter-forces will therefore increase in relation to each other, and in the experiments shown in Figs. 6 and 7 it will be seen that with increasing concentration of acid or alkali—up to a reaction corresponding to a concentration $N/200$ for the strong acid hydrochloric, and the strong base caustic soda—that the greater the swelling in the fluid, the greater the recoil in the vapour. Notice that the acid and alkaline systems, though similar, are not symmetrical: the greatest swelling is reached in acid solutions, the greatest recoil in the alkaline ones. As acid and base become more and more concentrated, the gelatin passes to a greater and greater extent from the solid external phase to the fluid internal phase. This gradual corrosion of the solid framework is accompanied by a decrease of elastic properties, until finally they vanish, the framework ceases to exist and the whole system becomes a hydrosol.

The recoil of swollen jellies on transference to a system of saturated water vapour has been quoted as an exception to the second law of thermodynamics. It can however be anticipated from Donnan's membrane theory, which is based on an application of the second law. Donnan has shown that if a system containing in solution an ionisable crystalloid and an ionisable colloid, which dissociates giving one colloidal ion, be separated into two phases by a membrane impermeable to the colloidal ion, then the crystalloid will distribute itself unequally across the membrane, the phase of greater concentration of the crystalloid being the phase free from colloid. Consider the system gelatin jelly in equilibrium with a hydrochloric acid solution. This can be represented as:

where c_2 and c_3 represent the concentrations of hydrochloric acid in the jelly and liquid phases respectively, and where c_1 represents the concentration of the gelatin salt. Both salt and acid are assumed to be completely dissociated. Hydrolysis of the gelatin salt will be suppressed in the presence of free acid. If c_1 , c_2 , c_3 represent ionic concentrations, then c_3 must always be greater than c_2 owing to the electrostatic repulsion between non-diffusible G, the gelatin ion, and diffusible H. But now consider what must happen if the acid solution in phase II be replaced by saturated water vapour. This system is unstable since the electrostatic forces between G and H are no longer balanced. A certain fraction of the hydrogen ions from I, with their accompanying chlorine ions, will therefore be transferred across the membrane, and for this to be possible water in the liquid form must be transferred too. Notice that the greater the ratio c_1/c_2 , the greater will be the fraction of c_2 which has to move from I to II in order to adjust the equilibrium, and the greater therefore the loss of water from the swollen gel. Although the weakening of the osmotic forces brought about in this way within the jelly will partially account for

the diminished volume, it is important to notice that the escape of large quantities of liquid water only occurs in gels in which it may be presumed that the elastic framework is still intact. The water is therefore partly expressed under the action of the recoil of the gel.

VII. THEORY OF GEL STRUCTURE DEDUCED FROM A STUDY OF GELATIN SWELLING.

Though the gel state is easily recognised in the laboratory, by its appearance and properties, the molecular mechanism which underlies these has been very variously pictured. Three types of mechanical models have been postulated, all differing in their physical conceptions, and all offering evidence in their own support. These are:

- (1) The model of a gel as a two-phase solid-liquid system.
- (2) The model of a gel as a two-phase liquid-liquid system.
- (3) The model of a gel as a one-phase (presumably fluid) system.

In the last model a molecular network is generally thrown through and round the system, in order to cope with the anomaly, that though there is only one phase and that fluid, yet it has lost the property of flowing! The theory that gels in equilibrium with fluids are one-phase structures has been strongly advocated by Procter. It is also the theory finally accepted by Brailsford Robertson after a general survey of the literature of the subject. The one-phase theory of gel structure has been held by Pauli for gelatin, by Hayes [1914] for pecten, etc. The chief line of argument in its favour used by its supporters is that it is sufficient to explain the various properties of gels, and that the appearance of a second phase at setting is an unnecessary assumption.

The two-phase liquid-liquid theory of gels was postulated by Ostwald [1905, 1909]. It is very easy to show that oil and water, for example, can be made, in certain proportions, to form stiff emulsions that will not flow in a test-tube. These emulsions, however, have not really the solid properties of gels. They cannot, for instance, be handled like blocks of gelatin jelly, retaining their shape the while. Hatschek [1916] has shown by mathematical reasoning that the liquid-liquid theory is untenable.

The two-phase solid-liquid theory of gel structure appears in several forms. Hardy, basing his belief on microscopical evidence, states that in most cases gelation consists of the breaking up of the sol into an open solid framework, with an interstitial fluid phase. The surface of the jelly on this theory would appear as a solid membrane perforated by numerous pores. This structure has also been postulated by van Bemmelen and by Andersen [1914] for gels of silica, and is accepted by Freundlich [1909]. In this theory, which is the one accepted by the writer, both solid and liquid phases are continuous.

Another form of the solid-liquid theory of gels is that of Bradford [1918]. Bradford's conception of the gel state is that the solid phase is discontinuous, and he compares a gel in structure to a pile of shot. He states that gels cannot have a rigid framework since they flow slowly and take on the form

of the containing vessel. This observation has not been confirmed during the course of the present work, in fact it has constantly been noticed that thin flat discs of gelatin maintain a rigid attitude when immersed in fluid, and keep perfectly flat for months, even though not lying in a horizontal plane, and unsupported except at the upper and lower edges. Gelatin discs which have been placed in a suspended crucible in order to swell in an experimental fluid will, if they grow sufficiently large, lift themselves right out of the crucible, rather than accommodate themselves to its limitations by bending. Throughout the course of this work, both in liquid and gaseous media, if flowing was observed in a jelly, it was always a sign that liquefaction of the gel was immanent. The theory of the discontinuity of the solid phase of gels appears to be upheld by Arisz [1914].

The process of separation of the solid phase from the liquid has been compared to the process of crystallisation, from the early days of the study of colloids. Both Frankenheim [1835] and von Nägeli [1858] state that the solid part of a gel is crystalline, and the same theory is held by von Weimarn [1908], Levites [1908] and Bradford. Hardy has called the external phase a solid solution, and has shown that it contains water, the amount of the latter depending on the rate of cooling. If the water can be regarded as present in the solid phase as water of crystallisation, and if the existence of several hydrates of gelatin is postulated, then the variation of water content with rate of cooling remains in harmony with the crystalline theory of the solid phase. Moreover, if the external phase is solid then there must be a definite amount of adsorbed water at the solid-liquid interface [see Parks, 1902, 1903]. Any variation in the interfacial area, such as is brought about by variations in the cooling rate, will therefore again affect the water content of the solid phase. Osborne [1918] has recently examined the problem as to whether the solid phase of a gel is crystalline or amorphous. His results are not very conclusive.

Although it is obvious that both the "liquid-liquid" theory and the "pile of shot" theory of gel structure would lead to the formation of highly viscous fluids which would closely simulate gels, yet the theory that gels possess a continuous solid framework appears to explain most satisfactorily the elastic properties dealt with in Section VI of this paper. This continuous framework forms a *locus* for the contracting forces which have been demonstrated to exist in the gels.

There remains to be considered the liquid phase of the gel. Hardy showed this to be a solution of gelatin in water, and Procter brought forward very satisfactory evidence that in a gelatin-hydrochloric acid system the gelatin is present as a soluble salt. Procter's theory of gelatin swelling, though now well known, is summarised here for the sake of clearness. Gelatin is an amphoteric substance which possesses the power of combining with acids to form di-acid, ionisable salts. These dissociate to form one diffusible ion, the anion, and one non-diffusible ion, the gelatin cation. At equilibrium in

a system consisting of gelatin jelly plus acid external fluid, the ionic products on each side of the jelly-water interface are equal, but the sums of the diffusible ions in the two systems are unequal, owing to the unbalanced diffusible anions of the jelly salt which are retained in the jelly phase. This is of course an application to a special case of Donnan's general theory of membrane equilibria [1911]. This inequality of diffusible ions in the two systems causes an osmotic pressure in the jelly, which is greater than the osmotic pressure in the external fluid by a quantity which Procter calls e . This osmotic excess e is the force which causes the jelly to swell, but there must be some other force opposing e , since otherwise the jelly would swell to infinity. The origin of this opposing force is not very clear in Procter's paper, where it is assumed that the jelly only forms a single phase, but if a continuous solid framework is accepted as an integral part of the gel structure, then the counter-force, $-e$, is readily located in the elastic properties of this framework. If e becomes sufficiently great, the framework is stretched beyond its breaking point, $-e$ vanishes, and e does cause the jelly to swell to infinity, in other words to dissolve in the external fluid. The volume occupied by a gel in an acid solution therefore is that due to the resultant of two sets of forces, osmotic and elastic, located respectively in the two phases of the gel. Loeb [1919, 2] has considered it due to the resultant of two sets of forces, osmotic and electric. The obvious importance of the electric forces is included in Donnan's original theory. One common misinterpretation of Donnan's theory might be mentioned here. It has been held that Donnan's "membrane potential" implies that there must be a charge at the surface of the membrane, and various workers, including Ehrenberg [1913], have endeavoured in vain to demonstrate this. An examination of Donnan's theory, however, shows that the membrane potential is due to an electric repulsion between the non-diffusible ions and the diffusible ions of the same sign. This leads to a condition of electric strain across the membrane, but not to polarisation in the ordinary sense of the term. Procter claims to have detected a potential of a few millivolts at a gelatin-water interface. The theory of acid-gelatin equilibrium can also be applied to alkaline-gelatin equilibrium, though the qualification must be added that the former equilibrium is reversible, the latter is not. This distinction is probably one of considerable significance in biological problems, especially in view of the fact that the muscle colloids have been shown in freshly excised muscles to be a protein-acid system [Jordan Lloyd, 1916]. It should be noticed before leaving the subject of the gelatin salts that Procter considers that the jelly cation of gelatin hydrochloride is non-diffusible and contributes nothing to the osmotic pressure. But the fact that the internal phase of swollen jellies can be shown to contain gelatin hydrochloride as a non-gelling compound, shows that the colloidal ion must be diffusible and must therefore exert an osmotic pressure. Brailsford Robertson believes that protein salts ionise across the middle of the protein molecule, giving two colloidal ions. If this view were correct, the distribution of free hydrochloric acid across

a jelly water interface would result in equal concentration on both sides. But in Procter's experimental results, the concentration of the acid in the external medium is always greater, at equilibrium, than in the jelly itself, a result which follows naturally if gelatin hydrochloride gives rise to one colloidal and one crystalloidal ion.

A gelatin gel may therefore be taken to consist of two phases, solid and liquid, and two chemical states of gelatin, *viz.* gelatin *per se* and gelatin in the form of soluble salts. Such gels therefore are three component systems, the components being water, gelatin, and an acid (or base).

It is necessary to consider the evidence that there must be some third component—an electrolyte—in a gel system. The most convincing work on the subject is that of Jordis [1902] on colloidal silicic acid. Jordis shows that the nearer silicic acid comes to chemical purity the more insoluble it becomes, and that ultimately when chemically pure it is practically insoluble in hot or cold water, and cannot be made to pass into the colloidal state. This only occurs in the presence of traces of acid, alkali, or organic groups. Loeb [1918] has shown recently for gelatin that at the iso-electric point it is an inert substance, insoluble in cold water, which moreover releases neither hydrogen nor hydroxyl ions, *i.e.* it has all the characteristics of an insoluble solid precipitate. Consider the case of a hot solution of gelatin cooling at a reaction $P_H = 4.6$. The iso-electric gelatin will be precipitated at numerous crystallisation centres, the solid drops will run together to form a framework, but since there can be no osmotic forces in the system (the only available source being the + and - ions of the dilute acid, to which the pores of the framework may safely be presumed to be freely permeable), the framework will contract under the action of its own surface forces, and the internal phase will be squeezed out. In short the system will show minimal swelling. That minimal swelling does coincide with the iso-electric point has been shown by Chiari [1911]. The properties of iso-electric gelatin have not been studied to any extent, probably owing to the extreme difficulty of keeping the system at the iso-electric point, and free from electrolytes. The purest water obtainable is still an electrolyte, and is alkaline to gelatin, and therefore will react with it to form salts. Much work, especially by Dheré [1913], Dheré and Gorgolewski [1910], Nasse [1889], Mörner [1889], etc., has been devoted to preparing ash-free gelatin, and studying its properties. Ash-free gelatin, however, is not necessarily iso-electric, and the fact that Dheré states ash-free gelatin to be electro-negative is sufficient to show that he was dealing with an alkaline system, and not with pure iso-electric gelatin. It is therefore justifiable in view of the work of Loeb, Chiari and others, to deduce that the gel state as a two-phase system cannot exist as a stable system at the iso-electric point.

It is now necessary to consider the fate of the system in strongly acid and strongly alkaline media, respectively. It has been shown during the course of this work that in the presence of sufficient acid or base the hydrogel

becomes a hydrosol. This process is reversible in an acid system, irreversible in an alkaline one. Gelatin, therefore, cannot form gels even in concentrated solutions, if it is present entirely as a gelatin salt. The process of gelation is therefore pictured as follows: gelation will only occur on the cooling of a sol which contains in solution iso-electric gelatin, and gelatin salts in equilibrium with free electrolytes. As the sol is cooled the insoluble iso-electric gelatin is precipitated in a state of suspended crystallisation and forms a solid framework throughout the system. The more soluble gelatin salts remain in solution, and by their osmotic pressure keep the framework extended. Gels therefore are two-phase systems, the solid phase consisting of iso-electric gelatin, the liquid of gelatin in the salt form.

This paper forms a preliminary part of an investigation undertaken on behalf of the Medical Research Committee on the equilibrium of the tissues and body fluids. I have to thank Professor Hopkins for much encouragement during the course of the work.

APPENDIX.

ON THE MOLECULAR WEIGHT OF GELATIN.

The molecular weight of gelatin determined by physical methods has been estimated at values ranging from 800 to 31,000. The following estimations are quoted:

	Mol. wt. of gelatin
Schutzenberger and Bourgeois [1876].	1836
Paal [1892]	900
Procter [1914]	839
Berrar [1912]	823
Biltz, Bugge and Mehler [1916]	5500 to 31,000 for different varieties

Considerations based upon the most accurate estimations of individual amino-acids lead to a figure for the reacting weight of the order of 10,000. The value of the evidence which is given below should be duly weighed. The evidence of the distribution of nitrogen in the gelatin molecule is taken from van Slyke's [1912] estimations.

	% of Total nitrogen
{ Ammonia nitrogen	2.25
{ Melanine „	0.07
{ Cystine „	0.00
{ Arginine „	14.7
{ Histidine „	4.48
{ Lysine „	6.32
Mono-amino „	56.3
Non-amino „ = proline + oxyproline	14.9
	<hr/> 99.02

In order to reduce these percentage figures to others which will show the relations in terms of nitrogen atoms, it is necessary to multiply throughout

by some factor arbitrarily determined. Now the histidine molecule contains three atoms of nitrogen, the arginine four, and the lysine two. The atomic ratios therefore must be such that the histidine nitrogen atoms form some multiple of 3, the arginine of 4, and the lysine of 2. Histidine, being present in very small quantity, may be taken as a convenient basis of calculation. If 1 histidine grouping is present in the gelatin molecule then there must be 3 histidine nitrogens. The percentage histidine value 4.48 can be reduced to 3 by multiplying it by 0.665. If, however, 0.665 is taken as the common factor, both ammonia and arginine yield figures corresponding to fractional (half) molecules. If 2 histidine groupings are assumed, the factor becomes 1.33. Multiplying throughout by 1.33 we obtain:

Ammonia nitrogen	...	...	...	...	2.9 = 1 × 3 approximately	
Arginine	„	...	...	...	19.7 = 4 × 5	„
Histidine	„	...	...	...	6.0 = 3 × 2	„
Lysine	„	...	...	...	8.4 = 2 × 4	„
Mono-amino	„	...	...	...	75.2 = 1 × 76	„
Proline + oxyproline nitrogen	...	...	...	...	20.0 = 1 × 20	„
Total	...	...	...	...	132.2 = 133	„

These figures are sufficiently satisfactory to justify the assumption that 1 gelatin molecule contains 133*x* nitrogen atoms distributed amongst: 3*x* ammonia (amide) groupings, 5*x* arginine, 2*x* histidine, 4*x* lysine, 76*x* mono-amino, and 20*x* proline + oxyproline groupings. In the absence of further evidence, *x* may be taken as unity. Two of the figures call for special comment—the lysine value, 8.4 instead of 8.0 is too high, but since the lysine is estimated by difference in van Slyke's calculation, it will carry as a positive error the negative error from both histidine and arginine. The mono-amino figure, 75.2 has been approximated to 76 instead of 75, in order to bring the total from the various groupings to the necessary value of 133.

Nitrogen may be taken to form 18.0 % of the total weight of dry gelatin. The following analyses of purified gelatin are quoted:

Paal [1892]	...	...	...	...	N = 18.12 % (ash content = 0.07 %)
Sadikoff [1903]	...	...	...	...	N = 17.47 Kjeldahl's method
„ [1903]	...	...	...	...	N = 18.18 Dumas' method
Schutzenberger and Bourgeois [1876]	...	...	...	...	N = 18.3
Mulder [1843]	...	...	...	...	N = 18.3
v. Name [1897]	...	...	...	...	N = 17.81
Chittenden and Solley [1891]	...	...	...	...	N = 18.0

Mean = 18.0 % error = ± 2 %

If 133 atoms of nitrogen form 18.0 % of the weight of the gelatin molecule, then the lowest weight of gelatin which can act as a chemical individual must be $\frac{133 \times 14 \times 100}{18.0} = 10,344$, or approximately 10,300. The error in the mean of the analyses given above falls within ± 2 %; the error in van Slyke's analyses is of the order ± 1 %; the total error in the computed value is therefore of the order ± 3 %.

SUMMARY.

1. Gelatin is an amphoteric substance which can exist as the free base (or acid), the so-called neutral gelatin, or as a salt in combination with an acid or a base. The molecular weight of gelatin is about 10,000, or some multiple of 10,000.

2. Pure neutral gelatin is only stable at the iso-electric point ($P_H = 4.6$); it is slightly soluble in hot water, but insoluble in cold water; but it has the power of combining with water (? as hydrates, or as solid solutions).

3. At $P_H < 4.6$ gelatin acts as a base and combines with hydrochloric acid to form soluble, ionisable salts—hydrochlorides. Gelatin is a multi-acid base, and combines with increasing quantities of acid as the reaction of the fluid becomes more and more acid. At about $P_H \approx 2.5$ gelatin exists mainly in the salt form.

The reaction: gelatin + hydrochloric acid $\rightleftharpoons$ gelatin hydrochloride is reversible.

4. At $P_H > 4.6$ gelatin acts as an acid and combines with soda to form soluble, ionisable salts—so-called gelatinates. Gelatin is a multibasic acid and combines with increasing quantities of base as the reaction of the fluid becomes more alkaline. At $P_H \approx 13$ gelatin exists mainly in the salt form.

Gelatin acts more readily as an acid than as a base; its dissociation constant for H' is greater than for OH' , and its valency towards bases is greater than towards acids.

The reaction: gelatin + soda $\rightarrow$ sodium gelatinate is not reversible.

5. The formation of keto salts in acids and enol salts in alkalies is postulated, ordinary gelatin being taken as a keto compound.

6. The figures given above apply to a system at 20° .

7. Gels are two-phase structures, which can only be formed in the first place from a fluid system (*i.e.* by cooling). They consist (1) of a solid framework of precipitated neutral gelatin, with which is combined a certain amount of water; (2) of an interstitial fluid which is a solution of gelatin in the salt form (either acid or basic).

8. The volume of any system in the gel condition is determined by the equilibrium between two opposing sets of forces. These are:

(1) The elastic forces of the solid framework which tend to make the volume contract.

(2) The osmotic forces of the ionised gelatin salts dissolved in the interstitial fluids which tend to make the volume expand.

9. Neutral gelatin at the iso-electric point cannot make a stable gel, since the constituent of only one phase is present. The gel formed on cooling will be free to contract till it is solid throughout, *i.e.* it will no longer be a gel.

Gelatin in the salt form cannot make a gel, since the constituent of only one phase is present. Gelatin in this form will form only sols with water.

10. The proportion of neutral gelatin to gelatin salts in any system is determined by the reaction of the system.

11. The volume occupied by a gelatin gel swelling in a fluid medium depends on: (1) the ratio of the mass of the dissolved electrolyte to the mass of the gelatin; (2) the geometrical form of the gel; (3) the hydrogen ion concentration of the medium, etc. Gels which are swelling in a weakly acid (or alkaline) medium lose water on being transferred to an atmosphere of saturated vapour. The amount of water lost by the gel is a function of the reaction of the previous external fluid. This water loss is shown to follow from the elastic properties of the gel, and to be in accordance with Donnan's membrane theory.

12. Van Bemmelen's theory that the surface of a gel is to be regarded as a solid membrane, perforated by pores, is confirmed by observation.

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