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Evidence of a structure in gelatin gels.[1]

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The question as to whether or not a colloid gel has a definite structure is extremely important to the biologist because of its bearing on the structure of protoplasms.

Bancroft[2] recently reported some unpublished results of a Mr. Cartledge in which gelatin gels of different concentration were dried down to 96 per cent. gelatin and then these dried sheets were allowed to again imbibe water. It was found that "each swelled rapidly to the original concentration and then took up water slowly," or in other words, a film of dried gelatin made from 8 per cent. gelatin gel was still potentially an 8 per cent. gel and very different from a dried film from a 16 per cent. gel. Arisz[3] had previously reported data showing a marked difference in imbibition capacity of gelatin gels of different concentrations and also of discs of uniform concentration but of different thickness.

Inasmuch as the experiments cited by Bancroft have such a profound bearing on all colloid-chemical studies in which gelatin has been used, it appeared worth while to attempt to duplicate Cartledge's results. Accordingly a series of gels was prepared as follows:

Weighed quantities (10, 15, 20, 25, 35 and 40 grams) of "Bacto" gelatin[4] were added to 100 c.c. of distilled water in clean pyrex flasks. After soaking for 15 to 30 minutse the flasks were placed in a hot water bath and allowed to remain until all of the gelatin had dissolved to form a homogeneous solution. A measured quantity (25 c.c.) of this solution was then poured into petri dishes 89 mm. in diam., thus ensuring the same thickness of the

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[1] Published with the approval of the Director as Paper No. 311, Journal Series, Minnesota Agricultural Experiment Station.

[2] Bancroft, W. D., "Applied Colloid Chemistry," 1921, p. 251.

[3] Arisz, L., *Koll. Beih.*, 1915, vii, 51–6.

[4] Air-dry gelatin as received from manufacturer.

gelatin gel in each instance. After standing for 12 hours duplicate rectangles 5 x 2.5 cm. in surface area were cut and placed on watch glasses to dry in a current of warm air (30°–40° C.) to a moisture content which did not exceed 3.5 per cent. The rate of moisture loss was followed by frequent weighings of the gelatin plates during the drying process, but no marked differences in rate of moisture loss were observed. The dried sections were then placed

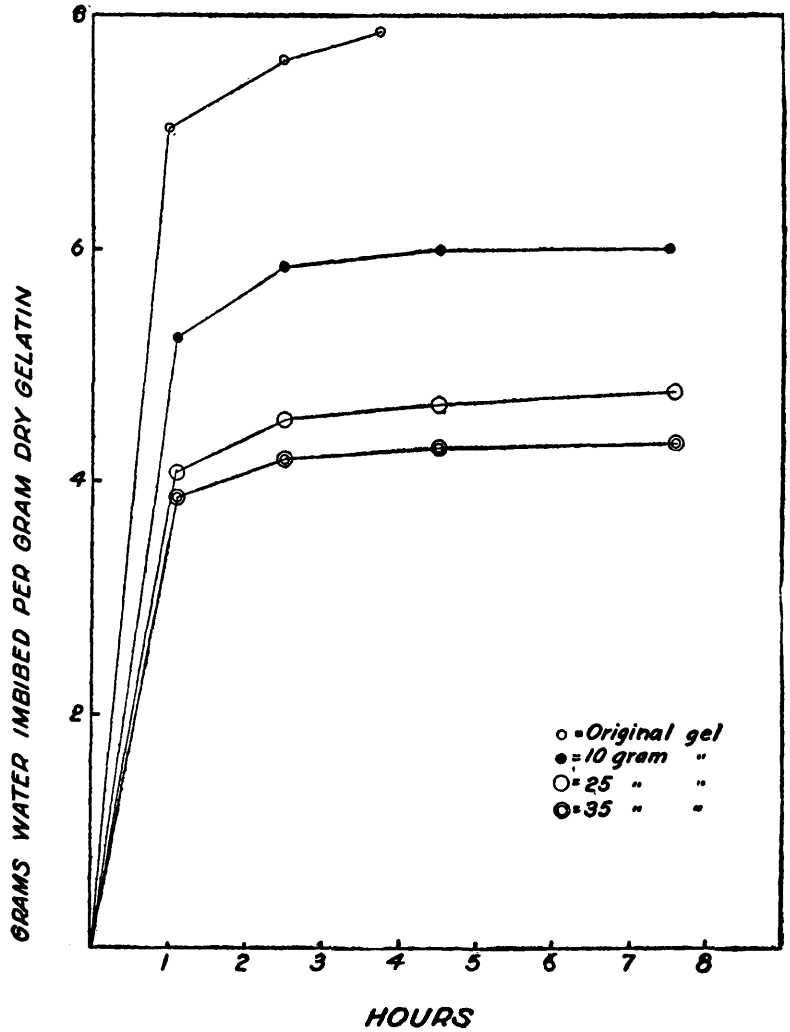

FIG. 1. Showing imbibition curves (in water) for sections of dried gelatin prepared from gels of different initial concentrations.

in distilled water and allowed to reimbibe moisture. The rate of swelling was followed by weighing the discs at frequent intervals after removing surface water by blotting with neutral filter paper. The data calculated in *grams water imbibed per gram dry gelatin* are shown in Fig. 1.

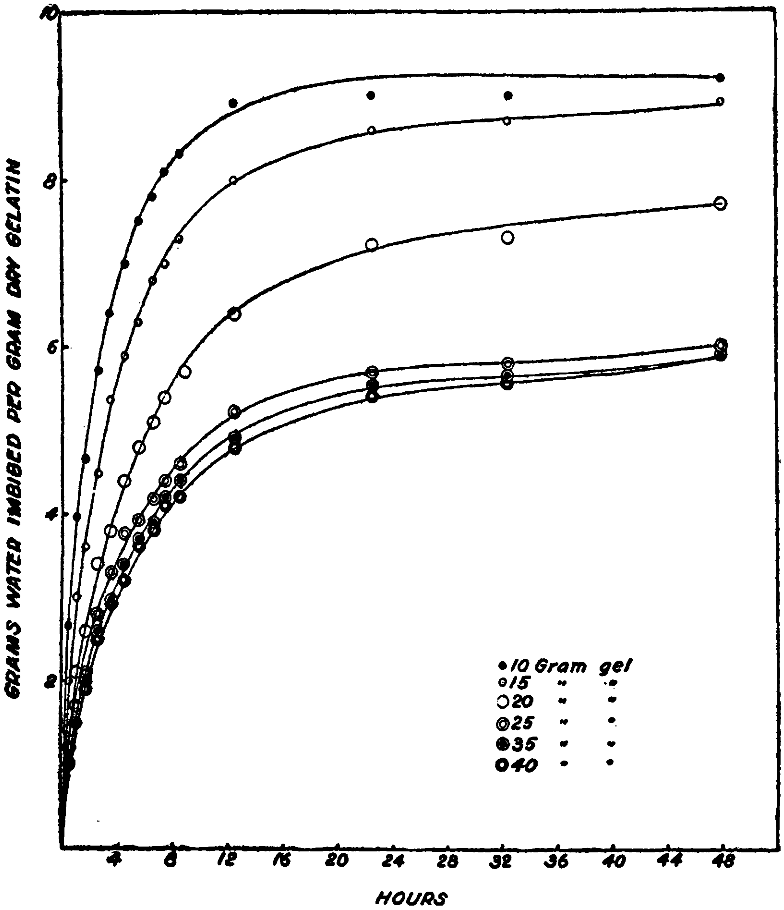

FIG. 2. Showing imbibition curves (in water) for dried sections of a 10 per cent. gel and a 40 per cent. gel. Dried sections of equal surface area and of equal thickness.

While these curves appear to indicate marked differences in the dried plates, depending on the original concentration of the gel, such a conclusion is open to the criticism that the dried plates, while of equal surface area, were necessarily of unequal thickness.

We therefore prepared another series of 10 per cent. and 40 per cent. gelatin gels (10 grams air-dry gelatin in 90 grams water and 40 grams gelatin in 60 grams water) and poured the 10 per cent. gel 4 times as deep in the petri dish as was the 40 per cent. gel. These on drying should give gelatin plates of equal area and thickness. After drying these plates they were allowed to imbibe water as in the above experiment. Figure 2 shows the imbibition curves obtained. Here again there is a marked effect due to the initial concentration of the gel. Similar discs were placed in *N*/25 lactic acid (P_H 2.49). It is well known that acid solutions greatly influence rate of imbibition and amount of swelling of

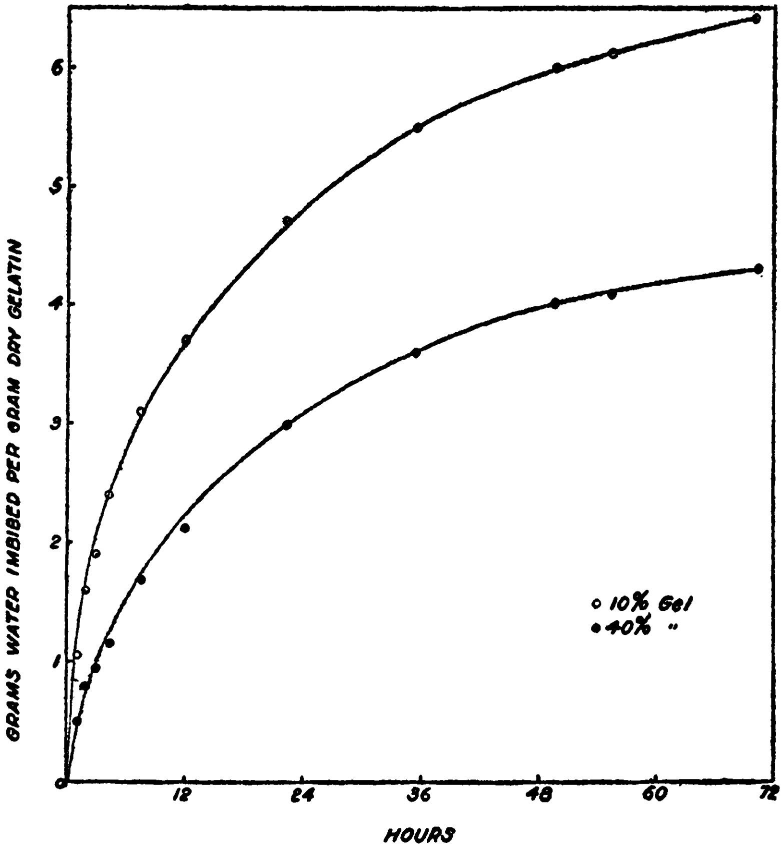

FIG. 3. Showing imbibition curves (in *N*/25 lactic acid) for dried sections of a 10 per cent. gel and a 40 per cent. gel. Dried sections of equal surface area and equal thickness.

proteins on the acid side of the isoelectric point. Fig. 3 shows the very marked differences which were obtained.

In order to be perfectly sure that the shape or thickness of the gelatin plate was not responsible for the differences in swelling, portions of dried plates from 10-gram, 25-gram and 35-gram gels, as well as portions of the "original" Bacto gelatin, were ground and sieved, those particles passing through a 2-mm. sieve and remaining on a 1-mm. sieve being retained for experimental work. A weighed quantity of these "granules" were placed in a Gooch crucible and allowed to imbibe water. At frequent intervals the crucibles were removed from the water, centrifuged at low speed for 2 minutes in order to remove excess moisture and weighed. Fig. 4 shows the form of the imbibition curves.

In order to ascertain whether the lower imbibing capacity of the gels containing the most gelatin might be due to the dehydrating effect of electrolytes in the gelatin "ash," which of course would be present in increasing amount as the concentration of the gelatin increased, a series of experiments was conducted in which the swelling plates were allowed to imbibe in (1) the same portion (100 c.c. of distilled water), and (2) in frequent changes of distilled water. If electrolytes were present they should have been dialyzed out in series (2). No differences in excess of experimental error were observed; in fact, a slightly greater imbibition was noted in series (1).

The effect of hydrogen-ion concentration upon the physical state of gelatin has been pointed out by many investigators, but it is not clear that we are dealing with any appreciable changes in hydrogen-ion concentration. It is impossible to secure by direct measurement the hydrogen-ion concentration of the dried gelatin plates which are immersed in the distilled water, and we have no means of being sure that it is identical with that of the gel from which the plates were prepared. Electrometric measurements on a 5 per cent. gel gave a P_H of 5.19 and the same value was obtained for the 10 per cent. gel. The higher concentrations of gelatin were so viscous that electrometric determinations were not attempted. Inasmuch as there was no change between the 5 per cent. and 10 per cent. gel we believe that it is safe to assume an initial P_H of approximately 5.2 for all gels before drying. This is slightly on the alkaline side of the isoelectric point of gelatin.[1]

The hydrogen-ion concentration of the water in which the plates were immersed was probably between P_H 5.0 and 6.0. The water as it came from the still was a fair grade of conductivity water and was free from carbon dioxide and ammonia. Naturally it afterward absorbed some carbon dioxide from the air. Kendall[2] has recently presented data showing the rapidity with which this takes place. According to his work distilled water is about P_H 5.7 when in equilibrium within the carbon dioxide of laboratory air. We have tried a number of times to determine the P_H of distilled water, as used in these experiments, but have failed to secure sharp readings on the potentiometer because of the slight conductance of the water, our values ranging between P_H 5.0 and 6.0.[3] Colorimetric measurements of hydrogen-ion concentration could not give accurate readings due to the fact that the indicators are all acids or bases which have greater ionizing power than has the water which is being measured.

If we assume, therefore, that the hydrogen-ion concentration of the dried gelatin plate is identical with that of the original gel from which it was prepared, *i.e.*, P_H 5.2, and that the distilled water was in equilibrium with the carbon dioxide of laboratory air, *i.e.*, P_H 5.7, we should still have no appreciable effect of hydrogen-ion concentration on our experimental results for *the same water and gelatin were used in all experiments.* We doubt whether the above assumptions as to P_H values are justified, but we do feel that the differences in the swelling of dried gelatin plates which we have described are not due primarily to differences in hydrogen-ion concentration.

Conclusions.—The above data appear to indicate that gelatin gels have a structure and that this structure is more or less fixed at the time that gelation takes place. It would appear that the gelatin aggregates or micelles are more and more interlaced at increasing concentrations of gelatin. This structure is apparently not appreciably altered by drying at a temperature below the

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[1] Loeb, J., *J. Gen. Physiol.*, 1918, i, 39.

[2] Kendall, J., *J. Amer. Chem. Soc.*, 1916, xxxviii, 2460.

[3] As a matter of fact the P_H of the distilled water is probably not an important factor since the water is so feebly buffered that a mere trace of acid or alkali will change the hydrogen-ion concentration through a wide range.

"melting" point of the gel. The fact that drying does not markedly influence the gel structure appears to argue against the formation of micelles by the adventitious coming in contact of dispersed particles of gelatin, for certainly many gelatin particles must touch each other in the dried sheet, but apparently they do not cohere to each other with any appreciable force, certainly not with a force at all comparable with the force of coherence between particles or micelles originating at the time of gelation. This

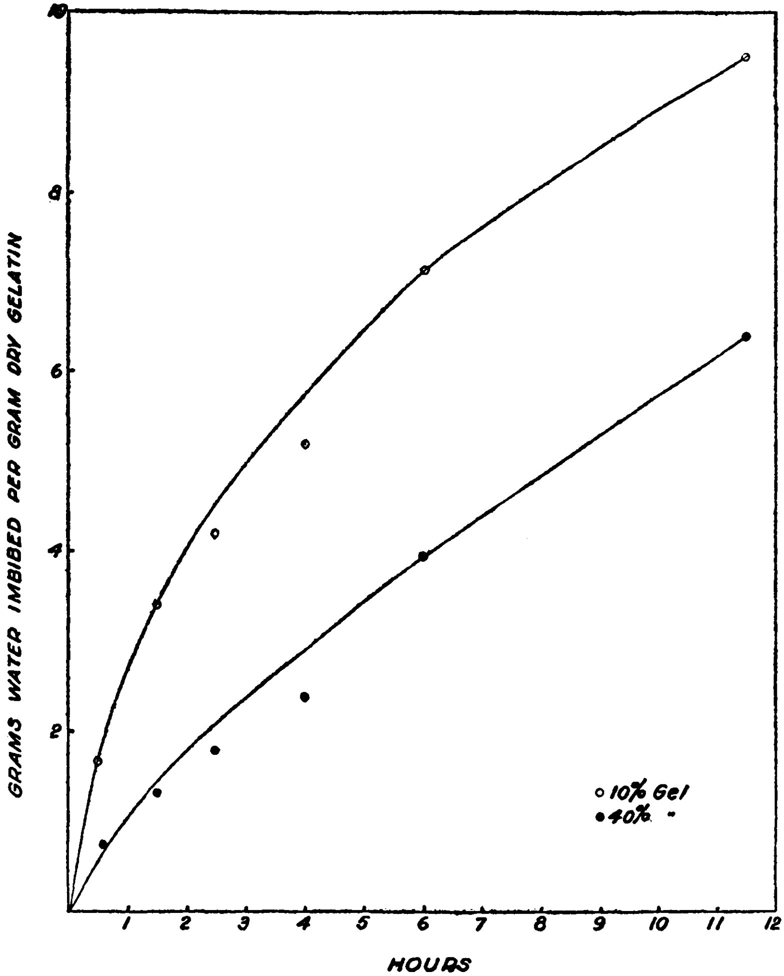

FIG. 4. Showing imbibition curves (in water) for granules of equal average size of dried gelatin prepared from gels of different initial concentration.

might be explained by a crystal structure where the crystals melt or soften at the gelation temperature. The micelles would then be formed by the solidification of crystals and later when micelle touched micelle the hardened surface of the crystal would prevent cohesion. We have recently shown[1] that a crystal gel may be very dilute and yet possess considerable rigidity so that a crystal structure is not incompatible with the properties of gelatin gels.

These experiments likewise show the marked influence that hysteresis may have on experiments where gelatin is involved. This is particularly noticeable in Fig. 4 where uniform-sized particles prepared from gels of different concentration were used. Inasmuch as a dried sheet of gelatin apparently reflects the structure of the original gel from which it was prepared, the production of different samples of dry gelatin possessing uniform physico-chemical properties would appear to be extremely difficult. It is possible that these experiments may account for the differences between the experimental data of various workers.

It would be interesting to know whether alcohol precipitation from sols of differing concentration produces gelatin particles of uniform physico-chemical properties. It is our intention to investigate this problem in the near future.

ADDENDUM, MARCH 1, 1922.

Since the above MS. was prepared, Sheppard and Elliott[2] have published on the same question. They do not find it necessary to assume a gel structure and believe that the different swelling rates are due to "casehardening" or surface drying effects. We believe that our experiment where uniform sized gelatin particles were employed excludes such an explanation and consequently prefer to adhere to the structure theory outlined above.

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[1] Gortner, R. A., and Hoffman, W. F., *J. Amer. Chem. Soc.*, 1921, xliii, 2199.

[2] Sheppard, S. E., and Elliott, F. A., *J. Amer. Chem. Soc.*, 1921, xliv, 373.