HYSTERESIS IN SORPTION

XV. Hysteresis in the Sorption of Water on Casein, Egg Albumin and Gelatin

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INTRODUCTION

SOME years ago, the problem of hysteresis in sorption appeared to be inexplicable. Recent investigations on this phenomenon in this laboratory, have conclusively shown the phenomenon to be real and reproducible.[2, 3, 4] Moreover, the interesting phenomena of drift of the hysteresis loop[6] and disappearance of the hysteresis loop[7] have been discovered. It has also been shown that the cavity concept is the general explanation of the hysteresis effect and the various phenomena connected with it. In the light of the cavity concept the phenomenon of the disappearance of the hysteresis effect initially exhibited by the elastic gels has been attributed to the property of the swelling of such gels in solvating liquids. The importance of the elasticity of gels which swell on the imbibition of solvating liquids has been shown by studies in sorption on organo gels such as rice (a monocot),[3, 7] dhal (a dicot)[7] and gum arabic (a plant exudate).[9] The generality of the phenomenon has been established by further work on the sorption of water on certain proteins such as casein, egg albumin and gelatin presented in this paper.

EXPERIMENTAL

The McBain-Bakr quartz fibre spring technique[1] described in an earlier paper[2] was used in the present investigations.

Proteins

Casein.—Kahlbaum's "casein nach Hammersten" was employed.

Denatured casein.—Hammersten casein was treated with boiling absolute alcohol for one hour and dried in an air oven at 75° C. for one and half hours. The product obtained was found to be insoluble in aqueous sodium acetate (the original casein was soluble) but soluble in alkali.

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Egg albumin.—Merck's "soluble egg albumin" was employed.

Denatured egg albumin.—A solution of egg albumin (about 10%) in water was heated for one hour on a boiling water-bath, to denature the protein. The precipitated albumin was filtered, washed with water and dried at 40° C. for 2 hours in vacuum.

Gelatin lumps.—A 20% solution of gelatin (Merck's gold label) in boiling water was allowed to set in a test-tube. The gel was removed by plunging the tube in boiling water for a few seconds and suddenly inverting it. The cylindrical piece of gelatin was cut into lumps (approximately a cm. cube). These were dried slowly at room temperature in partial vacuum (about 10 cm. pressure) for 4 days.

Dehydration of proteins

In the case of casein and egg albumin, particles passing through 20-mesh and retained by 30-mesh sieves were used. When particles of this size were not available (*e.g.*, Kahlbaum's sample of Hammersten casein), the fine powder was made into a thick paste, dried in vacuum for two hours at the temperature of dehydration. The mass obtained was then ground and particles of the above-mentioned size selected.

Dehydration of each protein was effected by heating to an appropriate temperature in vacuum for six hours. The temperature of dehydration was 60° C. in the case of normal and denatured casein and 40° C. in the case of normal and denatured egg albumin.

In the case of the gelatin lump, the dehydration was effected at the temperature of the thermostat (30° C.).

Air-free water

Distilled water was kept in the bulb attached to the sorption tube and the air was flushed out in vacuum for half an hour.

Air-free ethyl alcohol

Ethyl alcohol was obtained by distilling absolute alcohol twice over metallic calcium in an all-glass distillation apparatus. The bulb was almost filled with alcohol and the air in the bulb was flushed out by allowing a portion of alcohol to evaporate in vacuum. During sorption and desorption measurements, the possibility of the grease on the stopcock being removed by the solvent action of alcohol was avoided in the same way as described for carbon tetrachloride.[5]

Sorption and desorption of water vapour

A suitable amount of the dehydrated protein was placed in the bucket attached to the spring and was degassed for 5 hours at 10^{-2} mm. attained by means of Cenco Hyvac pump. After degassing, a series of sorptions and desorptions of the vapour of liquid on the protein at 30° C. were conducted. In all the experiments sufficient time was allowed for the attainment of equilibrium. With water, about 10 hours were found to be necessary for securing equilibrium and with ethyl alcohol only one hour.

RESULTS

Sorption of water

Casein-water.—Casein takes at the end of the 1st, 2nd and 3rd sorptions, 33·0 gm. 33·6 gm. and 37·4 gm. of water respectively per 100 gm. at saturation pressure. The initial hysteresis loop is reduced in size in the 2nd cycle of sorption and desorption and entirely disappears in the 3rd (Fig. 1).

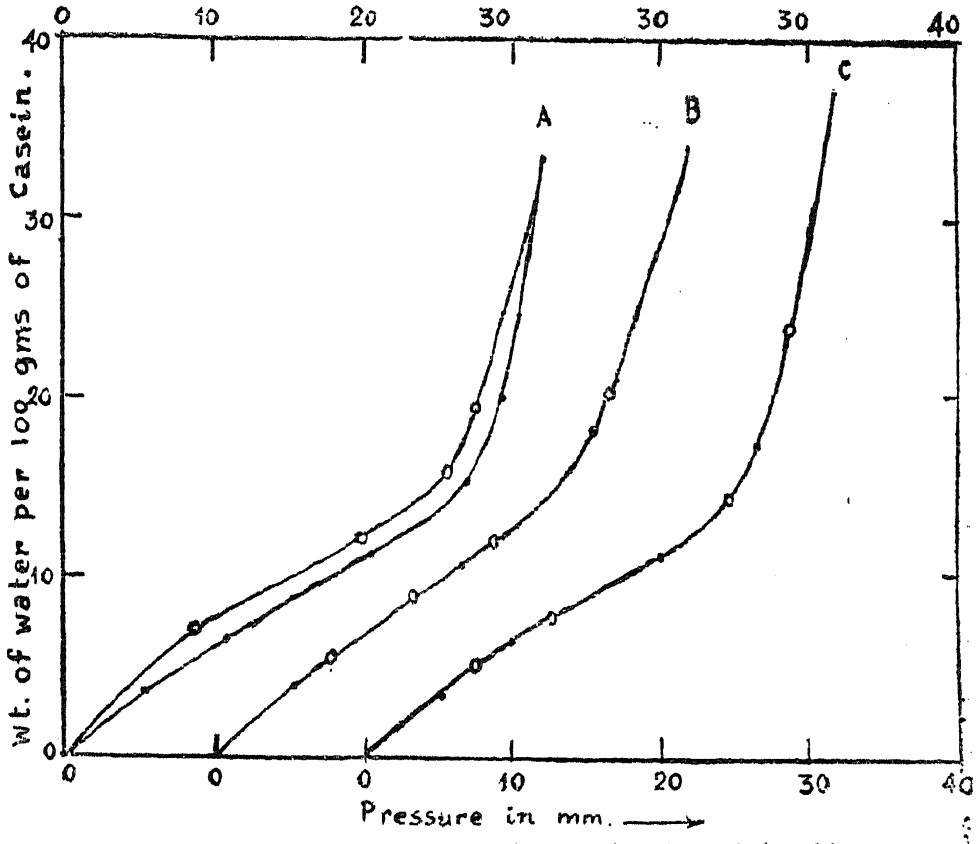

FIG. 1. Casein-water (A 1st cycle, B 2nd cycle, C 3rd cycle)

Denatured casein-water.—The sorptive capacity of denatured casein for water, at the end of the three successive sorptions are 32·7 gm., 34·0 gm. and 34·3 gm. of water respectively per 100 gm. The hysteresis loop obtained in the first cycle disappears in the subsequent cycles, Fig. 2.

Egg albumin-water.—Egg albumin takes at saturation pressure, 62·8 gm., 68·8 gm. and 81·2 gm. of water per 100 gm. at the end of the 1st, 2nd

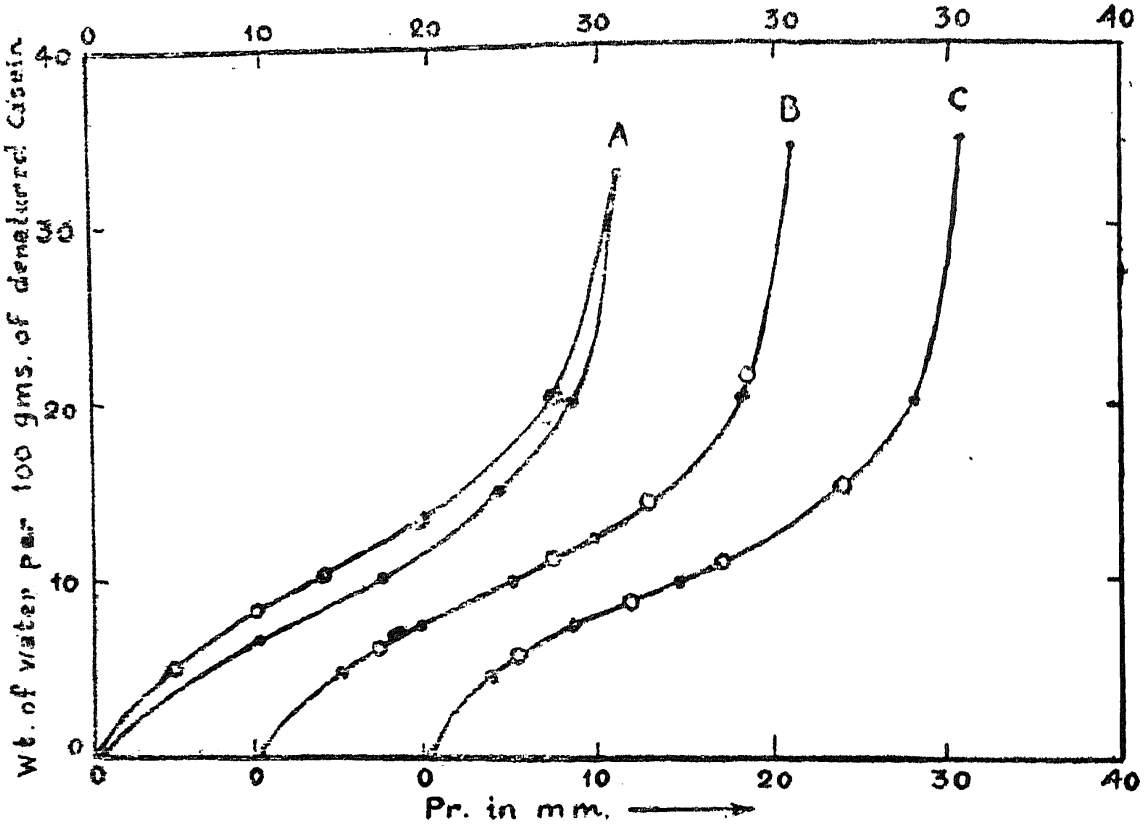

FIG. 2. Denatured casein-water (A 1st cycle, B 2nd cycle, C 3rd cycle)

and 3rd sorptions respectively. The initial hysteresis loop disappears in the 2nd and 3rd cycles (Fig. 3).

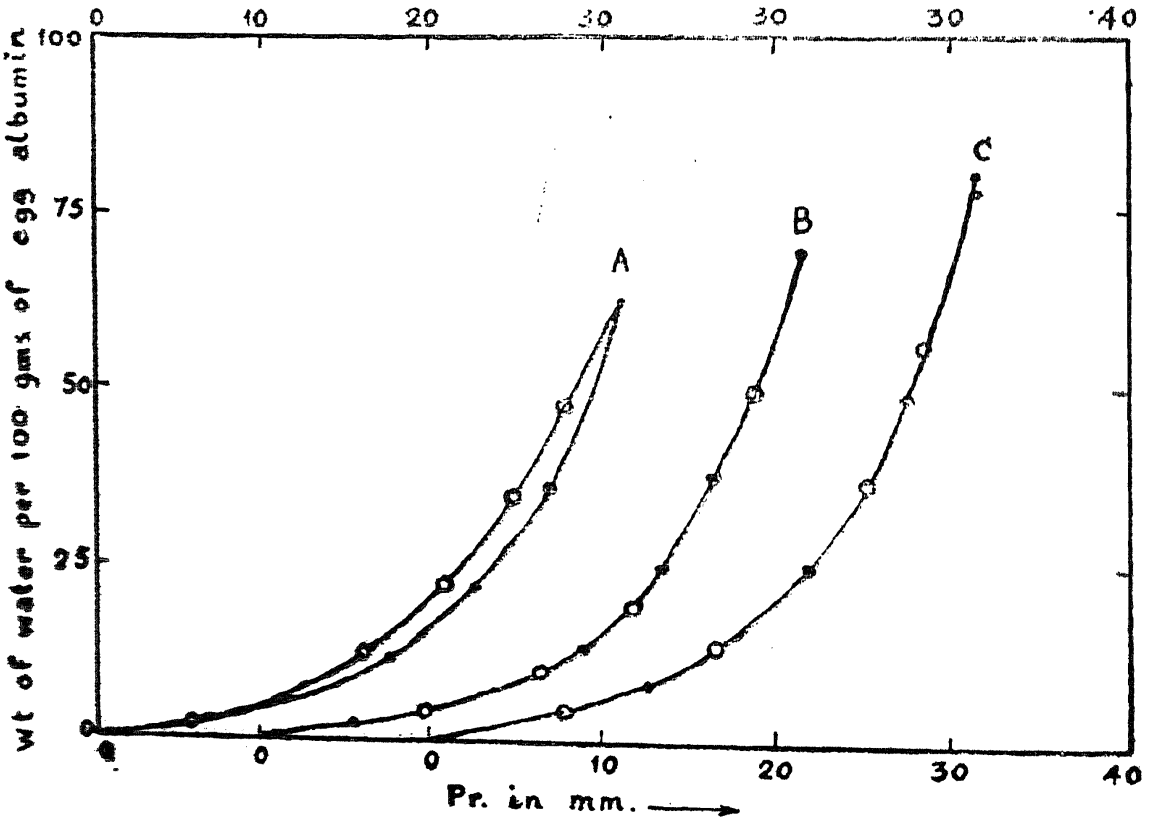

FIG. 3. Egg albumin-water (A 1st cycle, B 2nd cycle, C 3rd cycle)

Denatured egg albumin-water.—The sorptive capacities in the 1st, 2nd and 3rd cycles are 38·4 gm. 45·6 gm. and 47·4 gm. per 100 gm. of the denatured protein. There is no hysteresis loop at all even in the 1st cycle (Fig. 4).

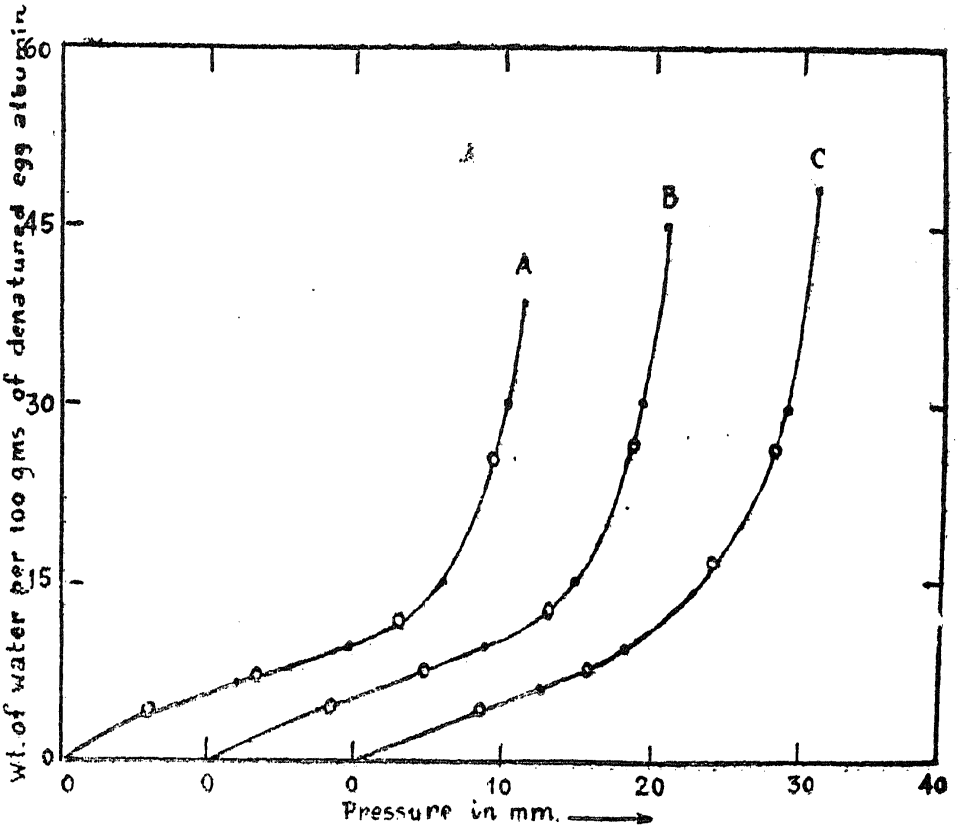

FIG. 4. Denatured egg albumin-water (A 1st cycle, B 2nd cycle, C 3rd cycle)

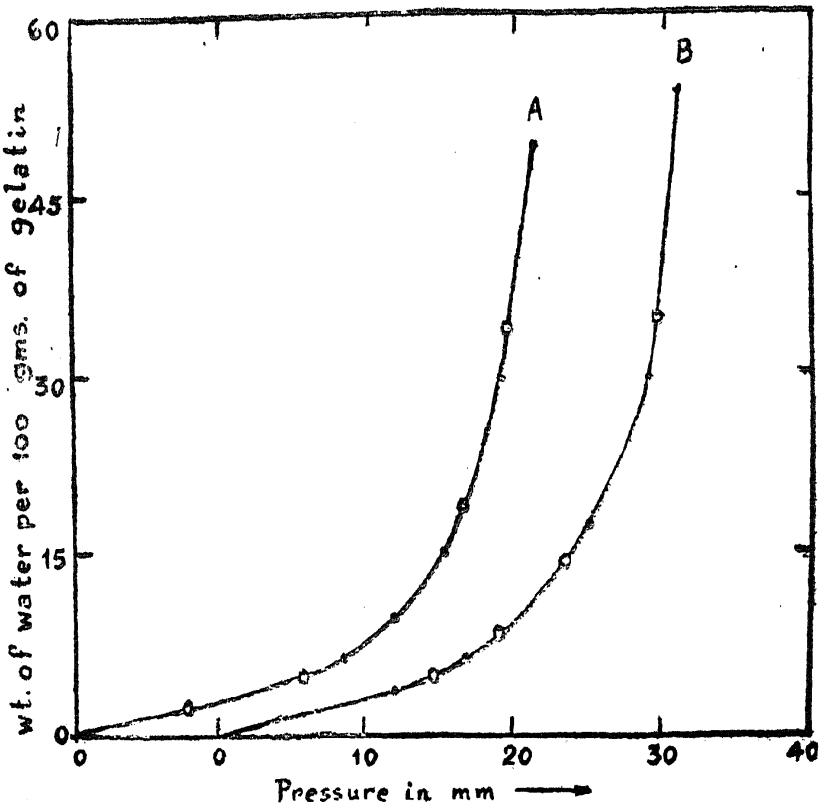

FIG. 5. Gelatin lump-water (A 1st cycle, B 2nd cycle)

Gelatin lump-water.—Sorptive capacities at saturation pressure at the end of the 1st and 2nd sorptions are 48·7 gm. and 55·4 gm. per 100 gm. There is no hysteresis loop even in the 1st cycle (Fig. 5).

Sorption of ethyl alcohol

The sorption at saturation pressure of ethyl alcohol was measured on casein, denatured casein, albumin, denatured albumin and gelatin. Casein and denatured casein sorbed 1·8 c.c. and 9·9 c.c. of alcohol per 100 gm. of protein. Albumin and denatured albumin show no sorption at all. The gelatin lump takes no alcohol.

Discussion

Swelling property and disappearance of the hysteresis effect

The relation between the swelling property of adsorbents and the disappearance of the hysteresis effect in sorption was indicated in the studies in the sorption of water on rice,[8] dhal[7] and gum arabic.[9] The present studies on casein, egg albumin and gelatin give additional support to this relation. That cavities with constricted necks entrap water during desorption and cause hysteresis effect has been established. In the light of the cavity concept, rigid porous adsorbents should show permanent and reproducible hysteresis effect on successive sorptions and desorptions and instances of remarkable permanence and reproducibility have already been recorded.[2, 4] With elastic gels which swell on the imbibition of solvating liquids, such permanence and reproducibility are not to be expected. Proteins in general swell in water and become elastic. Casein and albumin (normal as well as denatured) when dehydrated have a comparatively rigid structure. Their stable cavities entrap water and cause hysteresis. Near the saturation pressure, the gels will be in swollen condition and the cavity walls elastic. At this stage conditions are favourable for the disappearance of the cavities. The disappearance of the cavities however, is a process taking time. With some elastic gels the hysteresis loop disappears after the 1st cycle of sorption and desorption and with other gels at the end of the 2nd or 3rd cycle. It is to be noted that during desorption, fresh cavities are not formed but cavities still existing will remain intact. If the swelling gel is kept at saturation pressure for a sufficiently long time (days and even months), it is probable that the number of cycles of sorptions and desorptions at the end of which the hysteresis effect disappears is diminished. As a rule in all elastic adsorbents which swell on the imbibition of solvating liquids, there should be either no hysteresis loop or the loop initially exhibited must disappear on successive sorptions and desorptions. With nonsolvating liquids however there should

be permanent and reproducible hysteresis effect. This is illustrated in rice-carbon tetrachloride system.[7]

The observations on the gelatin lump are of great interest. In this adsorption experiment with a single solid lump of gelatin, free from pores, no hysteresis effect was noticed in the very first cycle of sorption and desorption. This is to be expected on the basis of the cavity concept. Denatured albumin behaves similarly, showing no hysteresis loop in the first cycle though it was activated at the same temperature (40° C.) as the normal albumin. Either cavities have all collapsed during denaturation or fresh cavities are not produced during dehydration, of the denatured protein.

Experiments on the sorption of ethyl alcohol on casein, albumin and gelatin are interesting. The sorptive capacity is negligibly small. Alcohol is a nonsolvating liquid for casein, albumin and gelatin and there is no swelling in this liquid. Consequently sorption is very low with casein and there is no sorption at all with egg albumin.

Another feature is the progressive increase in the sorptive capacity for water of the proteins on successive sorptions and desorptions. Gelatin, normal and denatured casein and albumin exhibit this property. Such a behaviour has been noticed in rice, dhal and gum arabic and is attributed to the loosening of the gel structure.

Denaturation and Sorptive Capacity

The sorptive capacities of denatured casein and denatured albumin are lower than those of normal casein and albumin.

The subject of denaturation of proteins has attracted considerable attention during recent years. It is a well-known fact that proteins undergo change in their properties on denaturation. They become less soluble and their swelling gets less. It is probable that on denaturation, casein and egg albumin become less hydrophilic and swell in water to a smaller extent and consequently have lower sorptive capacities than normal casein and egg albumin.

The high sorptive capacity of denatured casein for ethyl alcohol (9·9 c.c. per 100 gm.) in comparison with the low value for normal casein (1·8 c.c. per 100 gm.) also indicates that on denaturation the proteins become less hydrophilic and show a tendency to adsorb more of the nonsolvating liquids.

Summary

A series of sorptions and desorptions of water vapour at 30° C., on gelatin, casein, egg albumin, denatured casein, denatured egg albumin have

been conducted and in these systems either there is no hysteresis loop at all or the loop initially exhibited disappears on successive sorptions and desorptions.

These results indicate that the swelling of the adsorbent on imbibing the solvating liquid is responsible for the disappearance of the hysteresis effect.

Experiments on the sorption of ethyl alcohol at saturation pressure on the above proteins at 30°C. show that the nonsolvating liquid is either not adsorbed at all or taken up to a very small extent.

With casein and egg albumin, the sorptive capacities for water are lower in the denatured forms of the proteins. These results indicate a decrease in hydrophilic character of the proteins on denaturation.

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