Conclusion.

When silica is present the peroxide method has a tendency to give high results. With removal of the flocculent silica which partly precipitated out, results checked the Benedict method in percentage to the third decimal place. Previously the peroxide method checked to the second decimal place.

The technique of the modified Benedict method has been adapted to facilitate the estimation of sulfur in feeds and feces without requiring undue attention and care. The method of solution of the sample by the Benedict method gives results which check the peroxide method when silica is removed, within an average of 3.17 and 4.17%, respectively, for feces and feeds.

The source of a high value for the blank determination due to sulfate present in the reagents and the need of procuring reagents of sufficient purity for the Benedict method have been pointed out. Not undue quantities of reagents are required for the small amounts of barium sulfate recovered. The modified method is short; the processes are few and require little attention.

The writer acknowledges his obligation to Dr. V. K. Krieble and A. W. Mangum for advice given in the use of their modified sodium peroxide method.

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THE MOLECULAR MECHANISM OF COLLOIDAL BEHAVIOR. II. THE SWELLING OF FIBRIN IN ALKALIES.

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In a previous article,[1] Tolman and Stearn have studied the swelling of fibrin in acid solutions, and correlated the swelling with the amount of acid adsorbed from the solution. On the basis of their results, they advanced the theory that a piece of fibrin covered with water is a fibrous, spongelike structure with many minute pores or pockets which are themselves full of water. The addition of acid is followed by the adsorption of hydrogen ions to form a double layer on the surface of these pockets with a consequent increase in their size owing to electrostatic repulsion. The addition of a neutral salt to the solution, or the addition of further acid after the limit of adsorption has been reached, is followed by a decrease in swelling, since further ions added arrange themselves, in accordance with well known electrical principles, in such a way as to neutralize the original electrostatic repulsion. The addition of a neutral salt to the solution also leads to further adsorption of acid owing to the fact that the neutralization of the electrostatic forces makes it easier for further hydrogen ions to attach themselves to the walls of the pockets.

[1] THIS JOURNAL, 40, 264 (1918).

In the present article similar experiments are described which were made with fibrin in alkaline solutions; these have led to similar conclusions as to the molecular mechanism of the swelling produced by alkalies. Owing to the amphoteric nature of its amino acids, fibrin has a tendency to adsorb hydroxide ion from an alkaline solution, just as it adsorbs hydrogen ion from an acid solution, and hence all the phenomena produced by acids can be duplicated with alkalies. The article presents data on the swelling and adsorption in solutions of sodium, potassium and ammonium hydroxides, in mixtures of sodium hydroxide with potassium chloride and potassium sulfate, in sugar and raffinose solutions, and in acetic acid. The work with the sugar and raffinose solutions was performed in order to get an idea as to the adsorption of water itself. The results with acetic acid are presented since they differ from those reported by Tolman and Stearn.

Experimental Methods.

In order that the results of this work should be comparable with those in acid solutions, the procedure previously used[1] was duplicated as nearly as possible, with a few improvements.

Materials.—The blood fibrin[2] was purified by treatment with 0.1 *N* hydrochloric acid and subsequent removal of the acid by continuous washing with distilled water; it was then dried in a current of warm air. Samples of blood fibrin vary as to toughness and resistance to grinding, and a sample having maximum toughness was chosen. Fibrin which does not show this property markedly swells so readily that no supernatant solutions remain for sampling.

The saccharose was the ordinary cane sugar of commerce. The raffinose was an imported sample. The alkalies and salts were ordinary "C. P." reagents.

Measurement of Swelling and Adsorption.

The swelling of the fibrin was measured in special flat bottomed test-tubes using a 0.3 g. sample of the powdered fibrin and 15 cc. of the desired solution. This ratio of materials is the same as that used in the adsorption measurements.

The adsorption experiments were carried out in 250 cc. ground glass-stoppered bottles, using 2 g. of powdered fibrin and 100 cc. of solution. After standing 24 hours, with occasional shaking, the supernatant liquid was drawn off and the alkali titrated with standard acid. If on standing the protein went into solution, as was the tendency in the solutions of alkali of higher concentration, the supernatant liquid was drawn off without shaking. The number of mols of alkali, A, adsorbed per g. of fibrin was calculated by the formula

[1] *Loc. cit.*

[2] Merck's.

$$A = \frac{(C_o - C)V}{W}$$

where C_o is the original concentration in mols per liter, C the final concentration after the adsorption has taken place, V the volume in liters of the solution employed, and W the weight of the fibrin in grams.

In the determinations of negative adsorption (adsorption of water) using sugar solutions, a small amount of phenol was added to the solution to prevent bacterial decomposition. The sugar concentrations were measured with a Lippich Large Universal Polariscope. The percentage of sugar may be obtained from the polariscope readings by the well known formula

$$P = \frac{100\ \alpha}{d\ \alpha_o\ l}$$

where α is the rotation, d the density, α_o the specific rotation and l the length of tube. The number of mols of water, A' adsorbed per g. of fibrin can then evidently be calculated by the formula

$$A' = \frac{(P_2 - P_1)M}{18P_2W} = \frac{(\alpha_2 - \alpha_1)M}{18\alpha_2W}$$

where M is the weight in g. of the solution, P_1 the original concentration, P_2 the final concentration of the sugar, W the weight of fibrin in g., and α_1 and α_2 the original and final rotations.

Experimental Results and Discussion.

Acids.—There is a well known lack of exact reproducibility in experimental results obtained with colloids, owing largely to the great effect which the past history of a colloidal material has upon its immediate behavior. For this reason it seemed desirable as a preliminary to this work to check some of the results of Tolman and Stearn,[1] using the new sample of fibrin. Swelling and adsorption data for the new sample of fibrin were obtained in solutions of hydrochloric acid and nitric acid. The curves obtained were of the same form as those reported by Tolman and Stearn, the adsorption being somewhat higher and the swelling a little less.

With acetic acid, a maximum adsorption of about 0.00095 mol was found at a concentration of about 1.0 N. This differs from the result previously reported in which no maximum was found; this divergence may be due to the great difficulty of obtaining reproducible results in this kind of work. It is felt, however, that the new result is more reliable, particularly because of the use of glass-stoppered bottles in carrying out adsorption experiments instead of finger bowls covered with watch glasses. The new curves for swelling and adsorption are given

[1] *Loc. cit.*

in Fig. 1, concentrations being plotted as abscissas and swellings or adsorptions per g. of fibrin as ordinates.

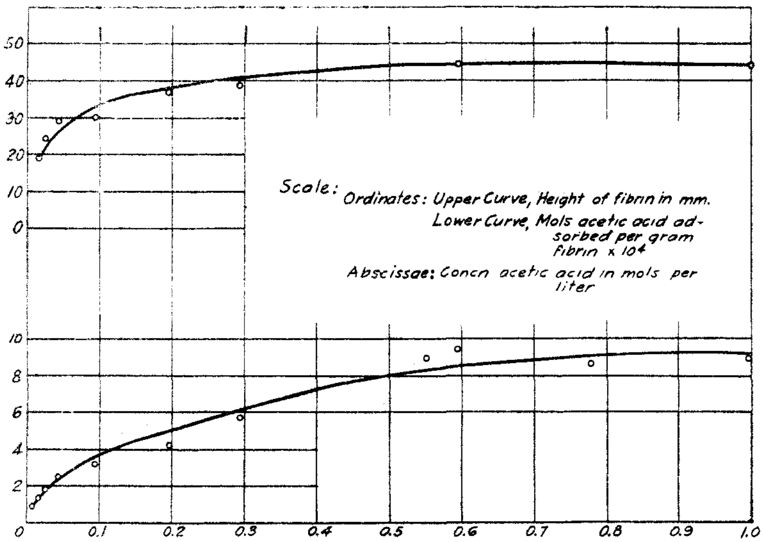

Fig. 1.

Strong Alkalies.—The upper two curves in Fig. 2 show the swelling of fibrin over a range of concentration in solutions of sodium and potassium hydroxides. It is to be noted that the swelling reaches a maximum at a very low concentration and with further increase of concentration

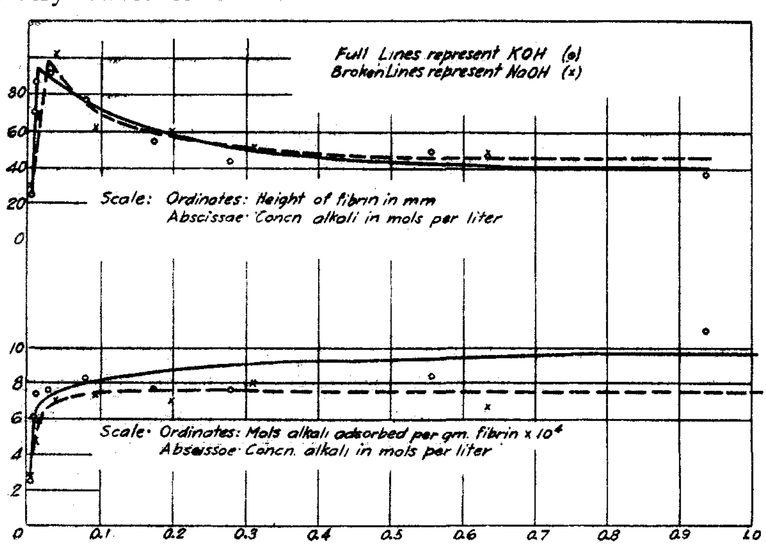

Fig. 2.

there is a reduction in swelling. The two lower curves represent the adsorption of alkali over the same range of concentrations. These are similar to the adsorption and swelling curves obtained in strong acids and may be similarly explained. Increasing the concentration of the alkali, beyond that at which those points on the protein molecule having the greatest affinity for the hydroxyl ions are satisfied, does not result in further adsorption but only increases the ionic concentration of the solution. As long as the number of hydroxyl ions adsorbed increases, the electrostatic forces produced by the adsorbed layer increase. Hence swelling increases as long as adsorption continues. When the maximum adsorption is reached, the further ions added arrange themselves in such a way as to neutralize the electrostatic forces, and subsequent reduction of swelling takes place.

Weak Alkalies.—The upper curve in Fig. 3 shows the swelling of fibrin in ammonium hydroxide up to 0.8 *N*. We note that the swelling gradually increases without reaching a maximum. This curve coincides with

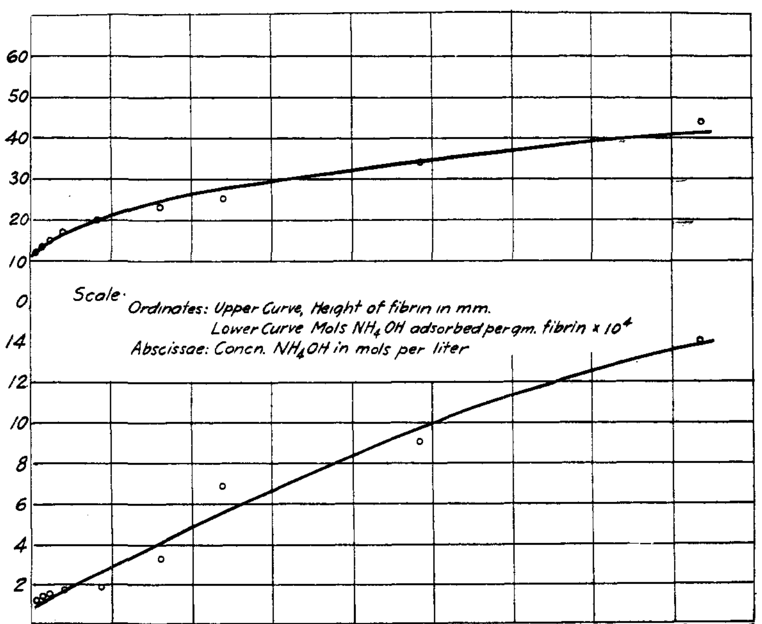

Fig. 3.

the swelling curves for weak acids. On the basis of the theory this is again explained by the fact that with the weak electrolyte there are few ions available for neutralizing the electrostatic field.

The lower curve represents the adsorption of ammonium hydroxide by the fibrin. It is not known whether the wide deviation of some of the points from the curve drawn has any significance or is merely an illustration of the difficulty of obtaining reproducible results in this kind of work. A further set of experiments with ammonia would have been made, except for lack of time and for the exhaustion of the particular sample of fibrin in question. It may be noted that this irregularity occurs with a weak alkali and that a lack of reproducibility in the results obtained in different experiments with a weak acid (acetic) has already been noted. It is felt that the *continued* swelling which is obtained with *weak* acids and *weak* alkalies may cause a breaking down of the fibrin structure which accounts for the variable results.

Mixed Alkalies and Salts.—The effect of additions of potassium chloride and potassium sulfate in reducing the swelling of fibrin in approximately 0.0145 *N* sodium hydroxide solution is shown in the upper two curves of Fig. 4. We see that the addition of a neutral salt produces

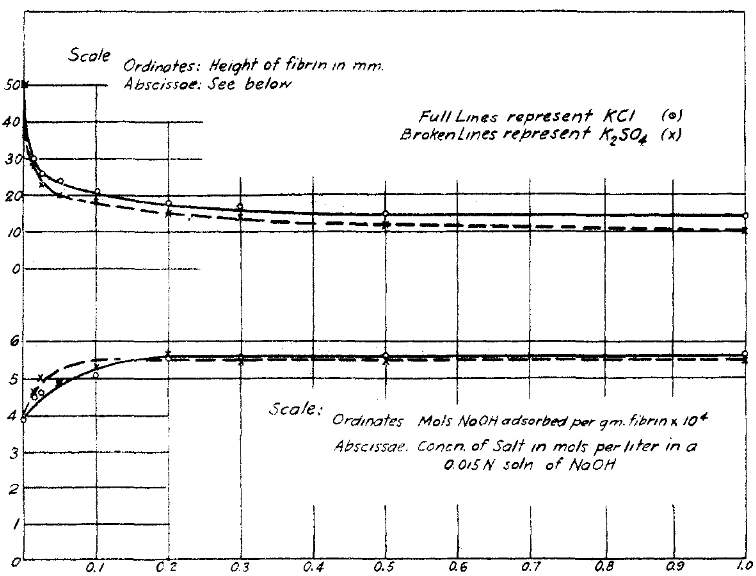

Fig. 4.

the same effect in lowering the amount of swelling, as increasing the concentration of the alkali itself beyond the saturation point. This agrees with the results obtained in acid solutions. In any case the controlling factor is the concentration of the free ions which so arrange themselves as to neutralize the original electrostatic repulsion. It is of particular interest to note that mol for mol, potassium sulfate is more effective in reducing the swelling than potassium chloride. This is to be expected because of the greater electrical neutralizing power of a mol of the sulfate over a mol of the chloride.

The effect of the neutral salts on the adsorption of hydroxyl ions is also to be noted in the lower two curves of Fig. 4. With solutions 0.0145 *N* with respect to sodium hydroxide the effect of the salt up to a certain concentration is to force the adsorption. This phenomenon, which was also observed in acid solutions, lends additional support to our theory that an electrostatic field is set up by the adsorption of the hydrogen or hydroxyl ions. Such a field would tend to prevent the further adsorption of ions, and if the ions of the neutral salt tend to neutralize this electric field, further adsorption will result. Here again the potassium chloride curve lags behind the potassium sulfate curve. This result is also obviously in accordance with our theory. This effect of the neutral salt is also shown in the curves of Fig. 5 which are similar

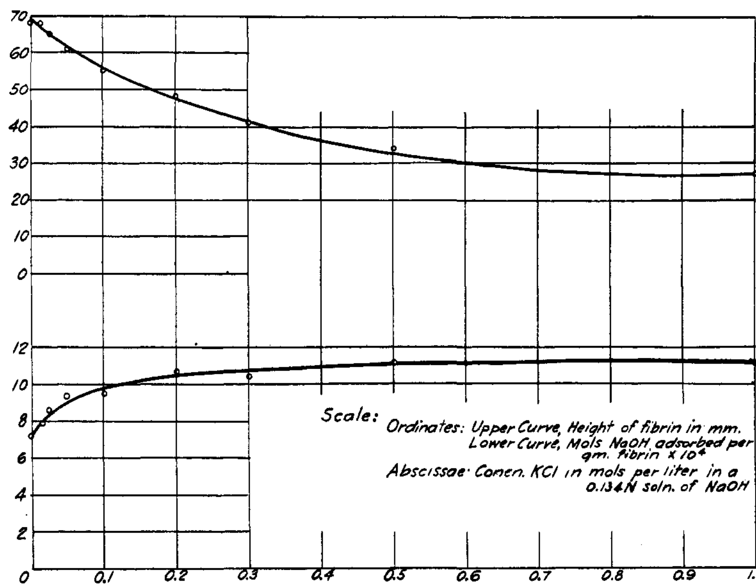

Fig. 5.

to the curves of Fig. 4 except that the concentration of sodium hydroxide is 0.134 *N*. We see that the adsorption is forced even beyond the maximum for the higher concentrations of pure strong alkali.

Sugar Solutions.—Tolman and Stearn[1] found that with fibrin in sodium chloride solutions there was an increase in concentration after 24 hours, indicating that there is at least more of a tendency to adsorb water than sodium chloride. In order to try to determine the amount of water actually adsorbed, cane sugar was used as a reference substance; from the measured change of concentration the amount of water adsorbed was calculated. It is to be noted first in the upper curve of Fig. 6 that the

[1] *Loc. cit.*

sugar solution had no effect on the swelling of the fibrin. In the lower curve where the abscissas give the concentration in mols per liter of saccharose and the ordinates the mols of water adsorbed per gram of fibrin, the adsorption value is noted to have nearly a constant value of about

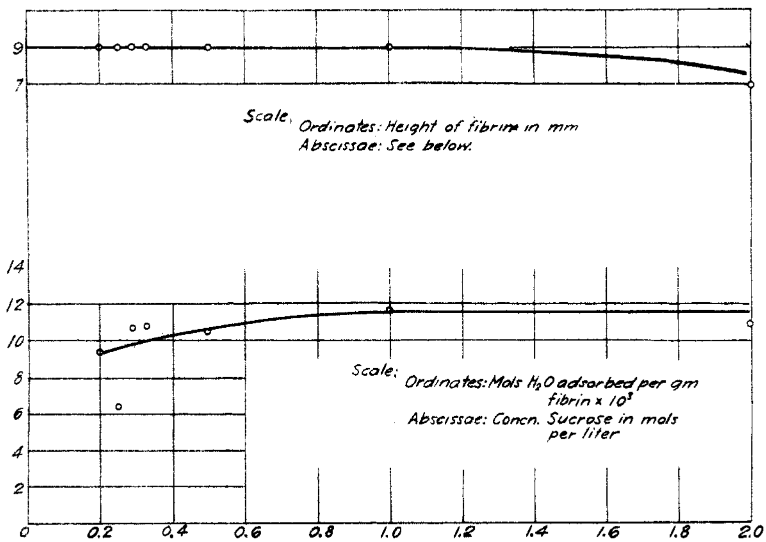

Fig. 6.

0.01, a value about 10 times the molal adsorption of acid or alkali per g. fibrin. A small sample of raffinose was available and used for a single measurement at a molal concentration of 0.225; with this reference substance a value of 0.02 mol of water adsorbed per g. of fibrin was obtained.

The evidence indicates that the phenomena is, as would be expected, merely a matter of the relative adsorption of water and the sugars and the results can have no absolute significance. In view of the adsorption of water in sugar solutions we should expect water also to be adsorbed in acid and alkali solutions. Assuming the amount of water adsorbed to be the same as in the cane sugar solution, a simple calculation shows that the amount of water would not affect the concentration of our acids or alkalies appreciably.

Conclusions.

(1) The behavior of fibrin in solutions of strong alkalies and of ammonia, and the behavior on the addition of neutral salts, is similar to the behavior in solutions of strong acids, and can be explained by the theory originally advanced by Tolman and Stearn.

(2) Fibrin adsorbs water from solutions of non-electrolytes.

The work described in this article was performed in the chemical laboratory of the University of Illinois.

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