Anal. Calcd. for $C_7H_4O_6NSHg_2Na$: Hg, 62.8. Found: 63.1.

Trimercury Derivative.—A solution of 6.37 g. (1 molecular equivalent) of the dimercury compound in 15 cc. of warm water is treated with a hot solution containing 0.8 g. (2 equivalents) of sodium hydroxide, and then with a boiling solution of 3.18 g. (1 equivalent) of mercuric acetate in 15 cc. of water. The resulting greenish-colored solution when poured into alcohol gives a greenish-yellow powder. It dissolves in water giving a solution with a slightly alkaline reaction.

Anal. Calcd. for $C_7H_4O_6NSHg_3Na$: Hg, 70.3. Found: 70.5.

The stability of these substances varies with the number of mercury atoms present. The mono derivative is the most stable. All are very soluble in water and are decomposed at once by hydrogen sulfide. Dil. acids precipitate the acid substance. Concd. acids and boiling alkalies react drastically. Attempts to introduce a fourth mercury atom invariably failed. In several cases, green products, not very soluble and having a mercury content of about 73%, were obtained.

Summary

New mercury compounds of succinimide, phthalimide and saccharin have been prepared and described. The linkage of the mercury is of a new type, intermediate in stability between that in ordinary mercury salts of oxygen acids and organic mercury compounds.

DETROIT, MICHIGAN

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES, COLUMBIA UNIVERSITY, NO. 464]

THE THERMOLABILITY OF COLLAGEN[1]

BY ARTHUR W. THOMAS AND MARGARET W. KELLY

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The discovery of the second point of minimum swelling of gelatin at *P*H 7.7 by Wilson and Kern,[2] and a corresponding second point of minimum swelling of calfskin at *P*H 7.6 by Wilson and Gallun[3] has aroused new interest in the chemical behavior of these proteins in solutions on the alkaline side of *P*H 4.7 or 5.0, the accepted iso-electric points.

The absorption spectrum of gelatin measured at different hydrogen-ion concentrations by Higley and Mathews[4] likewise shows two points of minimum: the first at *P*H 4.69, corresponding with the "first iso-electric point," and the second at *P*H 7.65, corresponding with the "second iso-electric point."

In extended studies of the rate of fixation of vegetable tannins by hide substance at room temperature as a function of the hydrogen-ion concentra-

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[1] Presented before the Division of Leather Chemistry at the 66th meeting of the American Chemical Society, Milwaukee, Wis., September 10–14, 1923.

[2] Wilson and Kern, THIS JOURNAL, **44**, 2633 (1922).

[3] Wilson and Gallun, *Ind. Eng. Chem.*, **15**, 71 (1923).

[4] Higley and Mathews, THIS JOURNAL, **46**, 852 (1924).

tion of the solutions, Thomas and Kelly[5] showed that the degree of fixation at a maximum at *P*H 3, dropped to a minimum at *P*H 5 (the "first isoelectric point"), rose again when a Sörensen value of about 8 was reached and then abruptly fell off with further increase in alkalinity.

Since the tannin particles are negative, the decrease in rate of fixation from *P*H 3 to 5 was readily explained by the Procter-Wilson[6] theory as due to the decreasing positive charge of the collagen. The rise in rate of fixation between *P*H 5 and 8 was unexplainable until the discovery of the second point of minimum swelling of gelatin and of collagen (the mother substance of gelatin).

Since Smith[7] postulated two forms of gelatin in solution, a "sol" form stable at temperatures above 35° and a "gel" form stable under 15°, Wilson and Kern[2] suggest a reflection of such a condition in collagen, arguing that at the temperature of the experiments by Thomas and Kelly, the fixation of tannin was due to the presence of a preponderance of the form of collagen analogous to the "sol" form of gelatin, which being cationic at *P*H 5 to 7.6 should combine with negatively charged tannin in that range.

The work of Smith[7] and of Miss Lloyd[8] shows that the change of gelatin from "gel" form to "sol" form also takes place with increase in the Sörensen value of the solution. Consequently, the *rise* in tannin fixation from that of *P*H 5 to that of *P*H 8 found by Thomas and Kelly was probably due to the increasing proportion of the positively charged modification of collagen analogous to the "sol" form of gelatin.

If a collagen undergoes an internal change with increasing temperature, like gelatin, and since negatively charged tannins combine only with cationic collagen, then if one had the "sol"[9] form only, the tannin fixation curve should not show a minimum at *P*H 5 but should progressively decrease to approximately *P*H 8.

Since Smith[7] gives 35° as the "gel"-to-"sol" transition temperature for gelatin and while Davis and Oakes[10] state it to be 38°, it was decided to adopt 40° as the working temperature.

Materials and Technique

Tannic acid (gallotannin) and extract of wattle bark were selected as tanning agents, the former because it is the closest obtainable approximation to a pure tannin and the latter because of its low content of non-tannins.

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[5] Thomas and Kelly, *Ind. Eng. Chem.*, **15**, 1148 (1923).

[6] Procter and Wilson, *J. Chem. Soc.*, **109**, 1327 (1916).

[7] Smith, THIS JOURNAL, **41**, 135 (1919).

[8] Lloyd, *Biochem. J.*, **14**, 147 (1920).

[9] The terms "sol form" and "gel form" are admittedly subject to criticism as applied to collagen, but are used for convenience and for lack of better ones.

[10] Davis and Oakes, THIS JOURNAL, **44**, 464 (1922).

Portions of defatted hide powder equal to 2.000 g. of absolutely dry substance were placed in a series of 400cc. bottles and covered with 200 cc. of phosphate buffer solutions (phosphoric acid and a mixture of phosphate and sodium hydroxide were employed at the extreme acid and alkaline ranges) of the same Sörensen values as used for the tanning solution later. These mixtures of hide substance and buffer solutions were kept in the ice box overnight, then filtered through cloth and the solutions squeezed from the hide powder. The hide powder was quantitatively replaced in the bottles, which were then immersed in a water thermostat at 40° ± 0.01°, and 100cc. portions of tanning solutions (containing 4 g. of total solids) previously adjusted to the desired Sörensen values, and at 40°, were poured in. The bottles were rotated in the thermostat for two hours after which they were filtered quantitatively on Wilson and Kern extractors.[11] The tanned specimens were first washed with distilled water at 40° to 50°, then at room temperature until all soluble matter had been removed. They were then air-dried, dried in a vacuum at 100° for 16 hours and weighed. The increase in weight was the amount of tannin fixed.

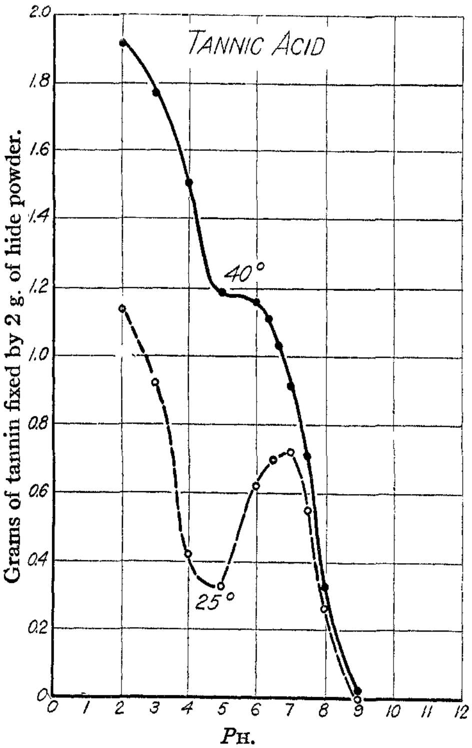

Fig. 1.

The results are given in Figs. 1 and 2. For comparison the curves showing the degree of fixation at 25° are included. It is seen that the curve of tannin fixation for gallotannin falls rapidly to *P*H 8, there being no fixation on the alkaline side thereof. On the acid side of this Sörensen value, collagen combines with negatively charged tannin particles at 40° and must therefore be cationic. It does not combine with tannin particles on the alkaline side thereof and hence must be anionic; or a value of approximately *P*H 8 is the iso-electric point of the "sol" form of collagen. A very slight degree of fixation is noted at *P*H 9. This is due to the well-known fact that the basic properties of an amphoteric substance are not entirely suppressed on the alkaline side of its iso-electric point. Furthermore, small amounts of phenolic substances which are oxidized to compounds of the nature of quinone act as tanning bodies in alkaline solution.

The 25° curve given in Fig. 1 is not strictly comparable with the 40°

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[11] Wilson and Kern, *J. Ind. Eng. Chem.*, **13**, 772 (1921).

curve, since the time of tannage for the former was six hours instead of two hours. This difference in time alters the amount of tannin fixed, but not the shape of the curve.

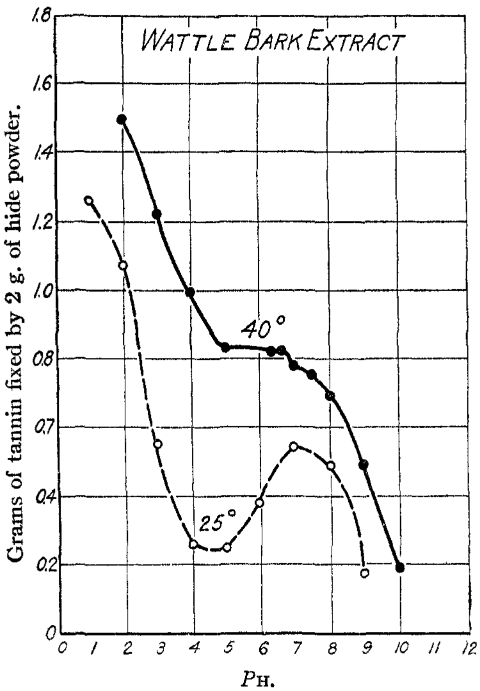

Fig. 2.

The curves for tannage with wattle bark show the same pronounced difference as those for gallotannin tannage. The tanning action in solutions at *P*H 9 and 10 is due to the non-tannins oxidizing to quinone-like bodies.[12] The 25° wattle-tannage curve was obtained with a solution of wattle bark containing 6.1 g. of total solids per 100 cc. The time of tanning was 24 hours.

A result exactly like that shown in Fig. 1 was obtained when hide powder was treated with buffers, all at *P*H = 7.5, prior to the tannage with gallotannin.

The authors wish to express their appreciation to A. F. Gallun and Sons Company for grants in aid of this investigation.

Summary

Collagen exists in two modifications analogous to the "gel" and "sol" forms of gelatin. The latter form is developed in solutions at 40°, the iso-electric point of which is indicated by the process of vegetable tanning to be at approximately *P*H 8.

NEW YORK, N. Y.

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[12] Thomas and Kelly, *Ind. Eng. Chem.*, **16**, 925 (1924).