Summary

1. The acetyl-benzoyl derivatives of certain bases having one reacting group on a side chain have been tested for the possibility of migration of acyl.

A. Benzoylation of 2-hydroxy-3,5-dibromobenzylacetanilide gave a stable O-benzoyl-N-acetyl derivative.

B. When the acetyl and benzoyl radicals were introduced in both orders into *o*-aminobenzyl alcohol, isomeric mixed diacyl derivatives were obtained and no rearrangement was observed.

2. The acetyl-benzoyl derivatives of 4′-amino-4-hydroxy-diphenyl were obtained in isomeric forms that showed no tendency to rearrange. This behavior opposes the theory that Positions 4 and 4′ of diphenyl derivatives are fixed relatively as suggested by the Kaufler-Cain formula.

3. Only one acetyl-benzoyl derivative of 8-amino-1-naphthol was obtained although the radicals were introduced in both possible orders, which shows that a molecular rearrangement occurred in one case and indicates that Positions 1 and 8 have a relationship approaching that of an *ortho* compound.

IOWA CITY, IOWA

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES, COLUMBIA UNIVERSITY, No. 499]

THE BEHAVIOR OF DEAMINIZED COLLAGEN. FURTHER EVIDENCE IN FAVOR OF THE CHEMICAL NATURE OF TANNING[1]

BY ARTHUR W. THOMAS AND STUART B. FOSTER

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Studies on vegetable tanning have focused attention on the important part played by the amino groups of the collagen molecule in vegetable tanning, and considerable evidence in support of a chemical theory of vegetable tanning has been accumulated.[2] Briefly stated, this theory holds in part, that in the vegetable tanning process the conversion of raw hide to leather is due to chemical combination between the amino (and possibly imino) groups of the complex collagen cations and the anions of the complex weak organic acids, the tannins, the rate of combination being a function of the Sörensen (P_H) value of the tannin solution.

It was thought that additional evidence in behalf of the chemical theory of tanning might be acquired through an investigation of the behavior of collagen in whose molecular structure the free amino groups had been replaced by less reactive groups such as hydroxyl. Granting the validity

[1] Read before the Division of Leather and Gelatin Chemistry, 69th Meeting of the American Chemical Society, April 6-10, 1925, Baltimore, Md.

[2] (a) Procter and Wilson, *J. Chem. Soc.*, **109**, 1327 (1916). (b) Wilson, *J. Am. Leather Chem. Assoc.*, **12**, 108 (1917); (c) **15**, 374 (1920). (d) Thomas and Kelly, *Ind. Eng. Chem.*, **15**, 1148 (1923); (e) **16**, 800, (f) 925 (1924).

of the mechanism of leather formation as outlined above, such an alteration of the collagen molecule should change its chemical properties in a definite and measurable manner. Such a study has been undertaken and the results are reported here as a further contribution to the chemical theory of vegetable tanning.

Materials Used.—In all except the swelling experiments, American Standard Hide Powder was used as the source of hide protein, hereinafter referred to as collagen. The hide powder for deaminization was used as received; that required for comparison tanning experiments had been sifted to remove 100-mesh particles, then thoroughly extracted with chloroform to remove fat, and air-dried. The moisture in all stock samples was determined by heating at 100° in a vacuum oven for 16 hours, and all weights of hide powder are reported on this moisture-free basis.

Pieces of calf-skin were furnished by John Arthur Wilson from the stock used by him in a determination of the points of minimum plumping.[3]

As sources of tannin five different commercial extracts were employed, namely, wattle, oak and hemlock barks and quebracho and gambier extracts. A concentrated stock solution of each was prepared by dissolving in water at 85°. After the liquid had cooled, insoluble and coarse suspended matter was removed by centrifuging.

The Preparation of Deaminized Collagen

Introductory.—Nitrous acid was first used as a deaminizing agent for proteins in 1885 by Loew,[4] but Skraup[5] and his co-workers have been largely responsible for perfecting the methods used in preparing deaminized protein products. Casein, gelatin, albumin, serum globulin and edestin were subjected to a deaminizing treatment and the products of hydrolysis studied.

Dunn and Lewis[6] deaminized casein using a slight modification of Skraup's method and found that the nitrogen content of deaminized casein from various samples ranged from 0.22 to 0.68% lower than the values obtained for the original material.

The work thus far mentioned was primarily concerned with the effect of deaminization on the products of subsequent hydrolysis. However, in 1914, Blasel and Matula[7] deaminized gelatin according to Skraup's method and showed that the deaminized protein was still capable of combining with hydrochloric acid. Recently, Hitchcock[8] has published a

[3] Wilson and Gallun, *Ind. Eng. Chem.*, **15**, 71 (1923).
[4] Loew, *J. prakt. Chem.*, **31**, 129 (1885).
[5] Skraup, *Monatsh.*, **27**, 853 (1906); **28**, 447 (1907).
[6] Dunn and Lewis, *J. Biol. Chem.*, **49**, 327 (1921).
[7] Blasel and Matula, *Biochem. Z.*, **18**, 417 (1913–1914).
[8] Hitchcock, *J. Gen. Physiol.*, **6**, 95 (1923).

quantitative study of the combining capacity of ordinary and deaminized gelatin from hydrochloric acid. He prepared deaminized gelatin by Skraup's method, at room temperature instead of at 40°, a preliminary study having indicated that the higher temperature induced a slight hydrolysis. Analysis showed an almost exact agreement between the difference of the total nitrogen values for ordinary and deaminized gelatin, and the percentage of amino nitrogen removed in the Van Slyke analysis.

Procedure.—While the proteins deaminized by previous workers had been in solution, it seemed reasonable to expect that even an insoluble protein, such as collagen, in contact with nitrous acid would lose nitrogen. Accordingly, the following procedure adapted from Hitchcock's experience with gelatin was adopted.

One hundred g. of hide powder was suspended and allowed to soak in about 1 liter of water; 500 cc. of a solution containing 100 g. of sodium nitrite was added, and finally 70 g. of glacial acetic acid. The mixture was stirred frequently to expedite the removal of gas. At the end of 24 hours the liquid was poured off and the hide powder, now a canary-yellow, was washed nearly free from acid with tap water. It was next covered with sodium chloride crystals to reduce swelling and force out more of the acid solution. The salt and acid were removed by repeated washing with cold water. Dehydration was accomplished with 95% alcohol and then the powder was dried in a current of air and allowed to reach equilibrium with atmospheric moisture. The treatment with sodium chloride was omitted in the preparation of a second lot.

Analysis of the final product for total nitrogen by the Kjeldahl method gave 17.32% while the original hide powder contained 17.81%, a loss of 0.49% of nitrogen. This difference in nitrogen content characterizes the deaminized collagen and may be taken as an index of the extent of deamination although no quantitative claims in this connection can be made. It is freely admitted that the extent of deamination is not known. However, recourse may be had to Hitchcock's results which are offered as presenting an important analogy. His deaminized gelatin was examined for amino nitrogen by both the Van Slyke analysis and the formol titration of Sörensen, but none was found by him. The difference between total nitrogen for gelatin and deaminized gelatin was 0.56%, which was almost exactly equal to the percentage of amino nitrogen removed in the Van Slyke analysis. Furthermore, the deaminized collagen[9] as prepared for this investigation conformed to the descriptions of deaminized proteins given in the literature by being yellow in color, as freshly made, but rapidly turning brown on exposure to light. In consideration of the above facts, it is believed justifiable to assume that the nitrous acid treatment of collagen has removed amino nitrogen in a manner similar to the well-known primary aliphatic amine reaction.

[9] The terms "deaminized collagen" and "deaminized calf-skin" as hereinafter used refer only to hide powder and skins which have undergone the nitrous acid treatment described above.

The Iso-Electric Point of Deaminized Collagen

Using American Standard Hide Powder and the dye technique, Thomas and Kelly[10] found the iso-electric point of the proteins of hide substance to be at hydrogen-ion concentration equivalent to P_H 5. This value coincided with the observed location of the point of minimum rate of tannin fixation on the same material. Inasmuch as the tanning experiments with deaminized hide powder revealed a displacement in this point of minimum toward the acid side of P_H 5.0, and swelling experiments upon deaminized calf-skin indicated a similar shift, it was deemed important to locate the iso-electric point of the deaminized hide powder by a third and independent method. The dye technique of the above-mentioned authors was used throughout with a few minor changes in details. The acid dye was Acid Black[11] (blue dye) and methylene blue served as the basic dye, such dark colored dyes being required on account of the brown color of the deaminized product. It will be recalled that Loeb[12] has shown that proteins combine with cations only on the alkaline side of the iso-electric point, and with anions only on the acid side.

Procedure.—One-half g. portions of deaminized hide powder were covered with 50 cc. of buffer solutions of various hydrogen-ion concentrations. The mixtures were allowed to stand overnight, when 5 cc. of dye solution containing 1 g. of dye per liter was added to each. At the end of 12 hours the dyed powders were filtered off, uncombined dye being partially removed by washing with the respective buffer solutions, and were then transferred to conical centrifuge tubes where the washing was completed by centrifuging and decanting. Since hide substance is a mixture of proteins, a sharp iso-electric point was not obtained, but rather a range of hydrogen-ion concentration between the extremes of which the iso-electric point of deaminized hide substance is located. The results showed the iso-electric point to be in the range of P_H 3.7 to 4.2.

Swelling Experiments with Deaminized Calf-Skin

Introductory.—When animal skins are immersed in dilute solutions of acid or alkali, an increase in thickness accompanied by a marked increase in the resistance of the skin to compression is observed. This type of swelling is called "plumping," and in common with some other properties of proteins, is a function of the hydrogen-ion concentration of the solutions with which the skins are in contact. Wilson and Gallun[3] in a detailed study of the plumping of calf-skins found two points of minimum and suggested that those two points indicated the iso-electric points of two forms of the protein of calf-skin. Inasmuch as theoretical considerations predicted a different behavior for deaminized skin, experiments were performed which closely followed the technique of Wilson and Gallun but in which deaminized calf-skin replaced the raw skin used

[10] Thomas and Kelly, THIS JOURNAL, **44,** 195 (1922).

[11] Calco Chemical Company.

[12] Loeb, "Proteins and the Theory of Colloidal Behavior," McGraw-Hill Book Co., New York, **1925.**

by them. While Kaye and Jordan Lloyd[13] have deaminized pieces of dried goat skin and compared their swelling with that of untreated skins, the observations were made at such wide intervals of Sörensen values that no valid conclusions could be drawn.

Procedure.—Pieces of wet-salted calf-skin were freed from sodium chloride by repeated washing with cold water and subjected to the nitrous acid treatment employed in the preparation of deaminized collagen. The same proportions of the reagents were used, but to insure thorough penetration, the skins were allowed to soak overnight in the sodium nitrite solution before the addition of the acetic acid. The mixture, at room temperature, was stirred frequently and at the end of 24 hours the skins were washed nearly free from acid and kept under distilled water in a refrigerator at 7° until used. The skins were a canary-yellow, but on exposure to sunlight the color changed to brown. The deaminized material was found to contain 17.2% of nitrogen, while the raw skins contained 17.8%, both values being on the absolutely dry basis. The amounts of ash on this basis were 0.90% in the deaminized and 0.30% in the original raw skins.

The technique for the swelling experiments was essentially that of Wilson and Gallun. Phosphate buffer solutions were prepared by mixing 0.1 *N* orthophosphoric acid with 0.1 *N* sodium hydroxide solution in previously determined proportions necessary for the desired Sörensen values which ranged from 2.0 to 11.0. A Perkins paper gage was used to measure the thickness, or strictly speaking, the compressibility of the skin. It was sensitive to 0.001 inch (0.0254 mm.) and had a plunger with a circular base 5 mm. in diameter and a fixed base of the same dimension opposite the plunger. Readings were taken three minutes after the plunger had been dropped on the skin.

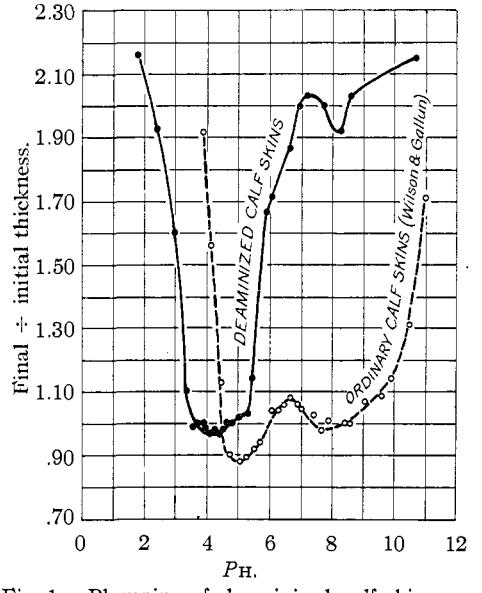

Fig. 1.—Plumping of deaminized calf skin as a function of P_H value.

Two or three pieces of deaminized skin, the thickness of which had been measured as described, were placed in each of a series of bottles and covered with 100 cc. of the buffer solutions. The bottles were kept in the refrigerator at 7°, the solutions being replaced daily until there was practically no change in their Sörensen values. Four days were usually sufficient. When equilibrium had been established, the skins were removed, blotted quickly with filter paper and their thicknesses again measured. The results appear in Table I and graphically in Fig. 1. The ratio of the final to the initial thickness may be taken as a measure of the degree of plumping and this number is plotted as a function of the equilibrium P_H value as determined electrometrically.[14] A sufficient number of measurements were made at

[13] Kaye and Jordan Lloyd, *Biochem. J.*, **18,** 1043 (1924).

[14] The Sörensen values were measured with the aid of a saturated potassium chloride calomel half-cell, junction with the solution being effected through a saturated

each point on the curve to equalize experimental variations due to differences in the initial skin thickness.

TABLE I

PLUMPING OF DEAMINIZED CALF-SKIN

Final P_H value of soln.	Ratio Final / Initial thickness	Final P_H value of soln.	Ratio Final / Initial thickness
1.80	2.16 (6)[a]	4.81	1.00 (5)
2.46	1.93 (6)	5.14	1.02 (7)
3.05	1.60 (11)	5.32	1:03 (4)
3.40	1.10 (11)	5.48	1.14 (7)
3.60	0.99 (5)	5.92	1.67 (6)
..	.....	6.15	1.72 (6)
3.79	1.00 (16)	6.64	1.87 (2)
3.94	0.99 (6)	6.79	2.00 (8)
4.06	.97 (10)	7.23	2.03 (12)
4.25	.98 (8)	7.79	2.00 (12)
4.42	.97 (5)	8.33	1.92 (11)
4.58	.98 (6)	8.60	2.03 (8)
4.67	1.00 (3)	10.70	2.15 (9)

[a] Numbers in parentheses indicate the number of observations of which the ratio given is the average.

The data are best examined by comparing the curve with that published by Wilson and Gallun[3] for ordinary calf-skin, and which is inserted in the figure. They found two points of minimum plumping, one at P_H 5.1, and the second at P_H 7.7 and considered these values to be in harmony with other "minimum" properties of collagen reported in the literature. The values obtained with the deaminized skins likewise reveal two points of minimum plumping, but here the first, is in the neighborhood of P_H 4.0 and the second near P_H 8.3. Thus is offered another demonstration of the dependence of a property of protein such as collagen on the relation between its acidic and basic dissociations. Considering the first point of minimum, the rendering of the protein less basic through the loss of nitrogen would be expected to shift this point, which is the region of minimum chemical activity, toward the acid side. The rapid rise of the curve on the alkaline side of P_H 5 to a high maximum at P_H 7.2 was unexpected. This may mean that the hydroxy groups substituted for the amino groups by the action of the nitrous acid are acidic in character. If so, the swelling of deaminized calf-skin in alkaline solutions should be greater than that of raw calf-skin by virtue of the fact that its greater number of acid groups results in the formation of a larger amount of the sodium salt of the protein and consequently greater swelling. The tanning curves show, as will be seen, the same steep rise through the same P_H range.

potassium chloride salt bridge. P_H was calculated from the equation, $P_H = (E - 0.2466)/0.000198T$.

Tannage of Deaminized Collagen

Thomas and Kelly[2d] have shown that the rate of fixation of vegetable tanning materials by collagen is a function of the Sörensen value of the tanning solution. In each of their experiments a minimum was always obtained in the neighborhood of *P*H 5, the iso-electric point of collagen. On either side of this minimum the curves rise to a maximum at Sörensen values of 1.0 to 3.0 on the acid side and to a lower maximum at *P*H 7.0 to 8.0 on the alkaline side, beyond which there is a drop in each case. The important conclusion from this work was that the vegetable tanning process is essentially the combination of collagen cations with tannin anions to form an insoluble substance, the union taking place chiefly at the amino groups. If this conclusion is valid, then a reduction in the number of amino groups in the collagen molecule should cause a corresponding decrease in the rate of tannin fixation, and in the total amount fixed, as well. Furthermore, since the removal of amino nitrogen would make the collagen less basic, then the point of minimum fixation should occur at a hydrogen-ion concentration more acid than that corrresponding to *P*H 5. The following experiments were performed in order that this hypothesis might be tested. The Wilson and Kern modified method[15] was used for the determination of the tannin fixed.

Experimental.—Portions of deaminized hide powder equivalent to 2.000 g. of absolutely dry material were placed in 400cc. bottles and covered with 200cc. portions

TABLE II

FIXATION OF TANNINS BY 2 G. OF DEAMINIZED HIDE SUBSTANCE

Time of action, 24 hours

*P*H of tanning soln.	Wattle Bark Ex. T.s.[a] = 41.1 g./l. D.h.p.[b]	[d]	O.h.p.[c]	Quebracho Ex., T.s. = 26.4 g./l. D.h.p.	O.h.p.	Hemlock Bark Ex. T.s. = 58.2 g./l. D.h.p.	Gambier Ex. T.s. = 32.5 g./l. D.h.p.	Oak Bark Ex. T.s. = 34.4 g./l. D.h.p.
1.0	0.86	1.48	1.55	0.89	1.16	0.40	0.26	0.35
2.0	.67	1.10	1.46	.52	1.29	.50	.17	.47
3.0	.44	0.90	1.12	.50	1.12	.32	.16	.32
3.5	.41	.87	0.99	.53	0.87	.33	.19	.29
4.0	.47	.94	.65	.55	.69	.38	.19	.33
4.5	.54	1.03	.62	.57	.67	.47	.21	.38
5.0	.58	1.14	.52	.65	.50	.54	.24	.41
6.0	.69	1.22	.56	.61	.57	.59	.26	.42
7.0	.82	1.40	.59	.75	.58	.62	.30	.44
8.0	.88	1.59	.76	.74	.75	.62	.29	.49
9.0	.56	0.64	.70	.38	.49	.41	.26	.27
10.0	.19	.28	.37	.10	.20	.17	.04	.10

[a] T.s. = total solids.

[b] D.h.p. = deaminized hide powder.

[c] O.h.p. = ordinary hide powder.

[d] Values in this column are for two weeks' action.

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

of tanning extracts, the Sörensen values of which varied from 1.0 to 10.0. The adjustment to the desired hydrogen-ion concentration was made by addition to the given amount of stock extract, before dilution to volume, of the required amount of hydrochloric acid or sodium hydroxide, these amounts having been previously determined by electrometric titrations of a sample of the extract. The final concentrations of the extracts with respect to total solids were such that an excess of tannin was always present, the amount necessary being taken from the concentration curves of Thomas and Kelly.[16]

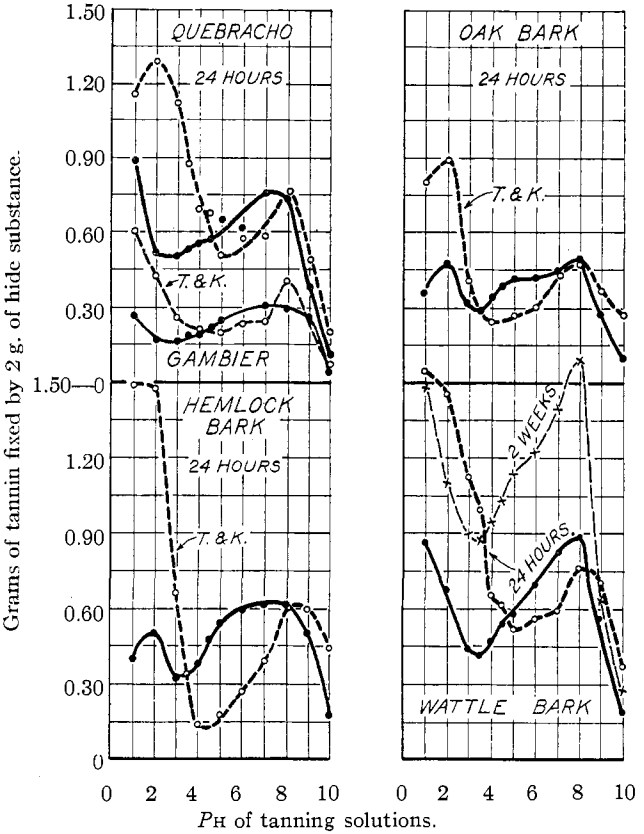

Fig. 2.—Fixation of tannins by deaminized hide substance as a function of P_H value (continuous lines). The broken lines depict the same action by raw hide substance, inserted for comparison.

The bottles containing the mixtures of hide powder and tanning extracts were rotated for 24 hours at room temperature; then the tanned samples were filtered in Wilson and Kern extractors, washed free from tannins and "non-tans" with distilled water and dried in a current of air. The dried samples were transferred to weighing bottles and desiccated for 16 hours at 100° in a vacuum, the increase in weight being taken as the amount of tannin fixed. In some cases a comparison experiment was performed with ordinary hide powder. In the case of wattle extract, a two-weeks experiment was included. The results are collected in Table II and graphically presented in Fig. 2.

[16] Thomas and Kelly, *Ind. Eng. Chem.*, **15**, 928 (1923).

The curves obtained with ordinary hide powder and tanning extracts have been explained by Thomas and Kelly with the aid of the Procter-Wilson theory[17] of vegetable tanning. The theory, it will be recalled, provides a satisfactory working hypothesis for tannin fixation in the range from P_H 2.0 to P_H 7.7, which is, in brief, a belief that on the acid side of its iso-electric point at P_H 5, collagen exists in cationic form and combines with the tannin anions functioning as weak organic acids to form a definite compound. The rise in the rate of fixation between P_H 5 and P_H 7.7 is ascribed to the presence of increasing amounts of the "B" form[18] of collagen which is positively charged and hence can unite with the tannin ions that are negatively charged in this range.

Now, considering the curves obtained with the deaminized hide powder, it is seen that they are of the same general shape as those obtained with the ordinary hide powder, but with the following differences.

1. In each case the point of minimum fixation has shifted from the region of P_H 4 to 5, to P_H 3.0 to 3.5.

2. The amounts of tannin fixed by the deaminized hide powder are consistently less than the corresponding quantities fixed by the regular hide powder at P_H values below 4.0.

3. The rate of fixation increases rapidly on the alkaline side of P_H 3.5, rising to a maximum at P_H 7 or 8 and dropping sharply at P_H 9.0 and 10.0. While deaminization has lowered the combining capacity of collagen on the acid side of P_H 3.5, there is an apparent increase in this property on the alkaline side of this point.

The new point of minimum as well as the decrease in the amount of tannin fixed between the range of P_H 1.0 to 4.0 are in accord with the prediction made at the outset of the experiments. Removal of amino nitrogen thus rendering the collagen molecule less basic has moved the iso-electric point toward the acid side and inasmuch as this point is taken to be that hydrogen-ion concentration where the sum of the cation and anion concentrations derived from an amphoteric substance is at a minimum, then a reduction through deaminization of the number of cations will act to raise the hydrogen-ion concentration corresponding to the new minimum sum. Since the chemical activity of a protein is at a minimum at its iso-electric point, then a change in the point of minimum fixation of tannin should accompany a change in the location of the iso-electric point. In view of the complexity of the reacting substances, no stoichiometric relationship can be evaluated, but a most striking analogy may be found in Hitchcock's work on gelatin, to which reference has been made. Loeb[12] has shown that the iso-electric point of a protein coincides

[17] Wilson, "The Chemistry of Leather Manufacture," Chemical Catalog Co., New York, **1923.**

[18] Wilson and Kern, THIS JOURNAL, **44,** 2633 (1923).

with a minimum of osmotic pressure. Hitchcock[8] measured the osmotic pressure of a 1% solution of deaminized gelatin and found the point of minimum at P_H 4.0. Repeated measurements by Loeb with ordinary gelatin have always placed this point at P_H 4.7, the generally accepted iso-electric point of gelatin at temperatures under 40°.

The maximum combining capacity of 1 g. of this deaminized gelatin for hydrochloric acid was found to be 0.00044 mole.[19] The corresponding value for gelatin is 0.00089 mole. The difference between these two values is 0.00045. The number of equivalents of amino nitrogen removed in the deaminizing of 1 g. of gelatin was found to be 0.00040. Considering the limitations of the method employed, Hitchcock believes that the loss in combining capacity for hydrochloric acid of 1 g. of gelatin on being deaminized is chemically equivalent to the number of amino groups removed, thus constituting a new indication that the union of protein with acid is chemical combination.

In consideration of the close relationship between gelatin and collagen and the fact that tannins are weak, organic acids, the data recorded in the preceding paragraph are believed to justify the conclusion drawn from the tanning experiments with deaminized collagen, namely, that the vegetable tanning process is a case of chemical combination between collagen cations and tannin anions, the former behaving like ammonia and the latter as weak, organic acids, to form insoluble salts which may be designated as tannates of collagen. Obviously, no claim can be made that this union takes place only at the amino groups. There are undoubtedly many imino groups in the collagen molecule which are concerned with tannin fixation. It is claimed, however, that these studies have shown that the amino groups play an important part in the reaction.

The increase, in the rate of fixation of tannin, on the alkaline side of the point of minimum to a high maximum at P_H 7.0–8.0 is in harmony with the results of the swelling experiment. It is difficult to explain this greater fixation of tannin unless the hypothesis of the existence of the "B" form of collagen[19] is accepted. Then it would appear that more of this modification arises in deaminized collagen.

Tannic Acid and Deaminized Hide Powder

Quantitative studies of the tanning action of tannic acid have shown that it acts like commercial vegetable tanning extracts. The rate of tannage as a function of the Sörensen value shows that the action is identical with that of commercial tannins on the acid side of P_H 7 to 8. To compare the action of tannic acid on deaminized hide powder with these results, the following experiment was performed.

Deaminized hide powder was tanned with solutions containing 25 g. of

[19] Hitchcock, *J. Gen. Physiol.*, **4**, 733 (1921–1922).

TABLE III

FIXATION OF GALLOTANNIN BY 2 G. OF DEAMINIZED HIDE SUBSTANCE

PH of tanning soln.....	1.0	2.0	3.0	3.5	4.0	5.0	6.0	7.0	8.0	9.0	10.0
Tannin fixed, 24 hrs.....	1.13	0.64	0.50	0.48	0.57	0.92	1.14	0.98	0.58	0.14	0.07

tannic acid per 200 cc., in the same manner as described in the case of the commercial tannins. The results are shown in Table III and graphically in Fig. 3.

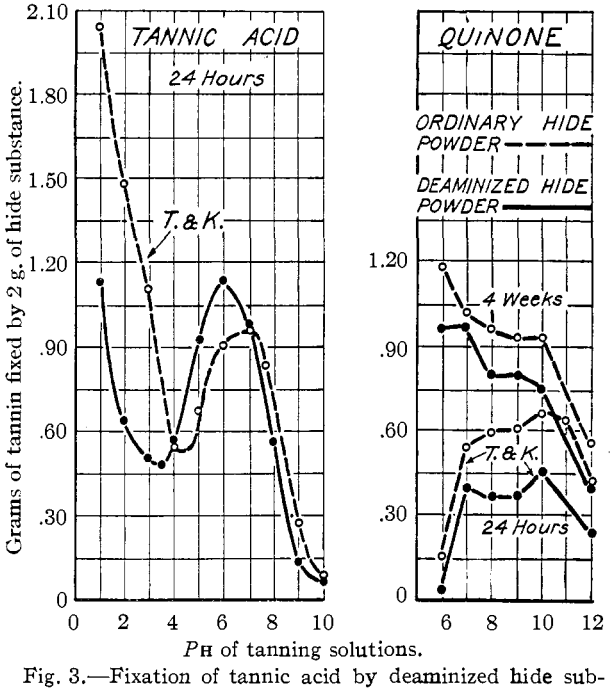

Fig. 3.—Fixation of tannic acid by deaminized hide substance and quinone tannage of deaminized hide substance (continuous lines). Tannage of raw hide substance inserted for comparison (broken lines).

The curve from these data closely resembles that obtained with commercial extracts and confirms the conclusion drawn therefrom. There is the same point of minimum at PH 3.5 with the rise to a maximum on both sides. The PH curve for the combination of tannic acid with ordinary hide powder previously published by Thomas and Kelly[2e] is inserted for comparison. Again, there are noted the shift in the point of minimum toward the acid side of the iso-electric point of ordinary collagen, the decrease in the total amount of fixation on the acid side of this minimum, and the apparently anomalous increase on the alkaline side thereof.

Effect of Deamination on Quinone Tanning

Quinone tans most rapidly in alkaline solutions, and the rate of fixation on hide powder has been shown in this Laboratory to be greatest at PH

8 to 10. When deaminized hide powder is subjected to the action of quinone under the same experimental conditions maintained in the previously reported study of quinone tannage by Thomas and Kelly,[2f] the results are found to be as expected.

Portions of 2.74 g. of quinone were placed in wide-mouthed pint bottles; 200cc. portions of phosphate buffer solutions were poured in and when the quinone had dissolved, portions of deaminized hide powder equal to 2.000 g. of absolutely dry substance were added. The bottles of one set were rotated for 24 hours at room temperature. A second set was rotated for the same length of time and then allowed to stand in a dark room with daily shaking for four weeks. In the latter case, a comparison experiment was run in parallel, using ordinary defatted hide powder. At the end of the designated period, the contents of the bottles were filtered and washed free from quinone in Wilson and Kern extractors. The tanned samples were air-dried, then dried in a vacuum for 16 hours at 100° and the increase in weight was taken as the amount of "quinone fixed."[20] The 24 hours' curve of Thomas and Kelly[2f] for quinone tanning of ordinary hide powder is inserted in the figure for comparison.

TABLE IV

QUINONE TANNAGE OF 2 G. OF DEAMINIZED HIDE SUBSTANCE

	Grams of "quinone fixed"		
	Deaminized hide powder		Ordinary hide powder
*P*H of buffer	24 hours	4 weeks	4 weeks
6.0	0.04	0.96	1.21
7.0	.39	.97	1.02
8.0	.36	.80	0.96
9.0	.36	.80	.93
10.0	.45	.76	.93
12.0	.24	.39	.56

For both periods, the rate of fixation on the deaminized collagen is controlled by the same conditions which govern the rate found for quinone and collagen, as is shown by the very close similarity between the two sets of curves. The amounts of quinone fixed in a given time interval are consistently less with the deaminized material. This fact lends additional support to Meunier's belief[21] that in tanning by quinone, quinone first oxidizes the collagen as it does aromatic amines, the oxidized collagen then combining with part of the remaining quinone. In accordance with this view, deaminized collagen could not be oxidized to the same extent as the untreated material and hence less quinone would be fixed.

The increase in the rate as well as in the total amount of "quinone fixed" obtained in four weeks may be due to changes which took place in the quinone solutions on standing. Even those solutions adjusted to *P*H 6 and 7, while a clear yellow when freshly prepared, were a muddy brown in a week's time. The more alkaline solutions quickly turned a dark brown,

[20] The dark color of the tanning solutions ranging from a muddy brown to a brownish-black make it evident that they contained substances other than benzoquinone.

[21] Meunier, *Compt. rend.*, **146**, 987 (1908); *Collegium*, **1908**, 195; **1909**, 58, 319; **1914**, 523; *Cuir*, **3**, 772 (1914).

indicative of a chemical change. The formation of condensation products which may have combined with the collagen, or which due to lowered solubility were not removed in the washing operation, is a possible explanation of the higher fixation at P_H 6 and 7. However, if this is true, the error introduced is constant for both kinds of collagen and would not affect the observed difference in the height of the two curves.

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Conclusions and Summary

Treatment of animal skin with nitrous acid yields a product of diminished nitrogen content. The iso-electric point of such "deaminized collagen" is in the region P_H 3.5 to 4 as revealed by the dye technique, swelling and point of minimum rate of tannage. "Deaminized" calf-skin shows two points of minimum plumping, P_H 4.0 and P_H 8.3. It swells in solutions of P_H 6 to 10 to a remarkable extent in comparison with raw skin.

From a purely chemical point of view, the more acid character of "deaminized collagen" in comparison with collagen is to be expected upon removal of nitrogenous groups. If the combination between skin and organic tannins is fundamentally a chemical reaction between the nitrogenous groups of the protein molecule and the acidic tannins, then collagen which has been impoverished in its nitrogenous groups should show a diminished rate of tanning in acid solutions and a shift in the minimum rate to a more acid region. This has been found to be true.

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[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

THE KJELDAHL-PREGL METHOD APPLIED TO NITRO COMPOUNDS

BY ADALBERT ELEK AND HARRY SOBOTKA

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Kjeldahl[1] found that it was possible to convert 60 to 80% of the nitrogen of potassium nitrate into ammonium sulfate by the digestion with sulfuric acid if three parts of sugar were added to one part of the nitrate. Von Asboth[2] obtained quantitative results by this method on nitro and cyano compounds. For nitrates, however, the sugar had to be replaced by benzoic acid. For the analysis of nitro, azo and similar compounds by digestion with sulfuric acid, others suggested the addition of phenol

[1] Kjeldahl, *Compt. rend. Lab. Carlsberg*, **2**, 1, 12 (1883); *Z. anal. Chem.*, **22**, 366, 381 (1883).

[2] Von Asboth, *Chem. Centr.*, [3] **17**, 161 (1886).