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.

NEW YORK, N. Y.

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

THE KJELDAHL-PREGL METHOD APPLIED TO NITRO COMPOUNDS

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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).

and zinc dust,[3] phenol, resorcinol or phloroglucinol alone,[4] zinc alone,[5] potassium persulfate and zinc dust,[6] stannous chloride[7] and sulfur.[8]

The use of sugar suggested by von Asboth is not to be found in most analytical handbooks and this method has apparently fallen into disuse. In the analysis of nitro-uracil xyloside[9] we noticed that the sugar component of this compound is almost sufficient to reduce the nitrogen of the nitro group. We never had obtained satisfactory results by the various methods mentioned above, which involve a tedious additional operation and require up to six hours. We, therefore, tried the addition of glucose in the analysis of various substances which otherwise would have had to be analyzed by the Dumas method. The accuracy and range of the method is readily seen from the results given in Table I, where aliphatic and heterocyclic as well as isocyclic nitro compounds were tested. One g. of glucose when added to 0.1 g. of sample was found to be sufficient; digestion and distillation may be carried out in the usual manner.

TABLE I

RESULTS WITH TYPICAL NITRO COMPOUNDS, USING GLUCOSE

	Substance	Formula	Wt., g.	0.1 *N* acid, cc.	Nitrogen, % Calcd.	Found	Glucose, g.
(1)	Nitrobenzoyl chloride	$C_7H_4O_3ClN$	0.1000	5.40	7.70	7.56	1
(2)	β-Nitrosonaphthol	$C_{10}H_7O_2N$	.0960	5.50	8.09	8.00	1
(3)	Nitropropanol (liq.)	$C_3H_7O_3N$	.1116	10.50	13.33	13.26	1
(4)	Nitroquinoline	$C_9H_6O_2N_2$	.0920	10.40	16.02	15.82	1
(5)	Picric acid	$C_6H_3O_7N_3$	.0924	12.10	18.37	18.32	2
			.0924	12.05		18.25	1
(6)	*m*-Nitro-aniline	$C_6H_6O_2N_2$	.0993	14.35	20.21	20.23	1
(7)	Nitro-uracil	$C_4H_3O_4N_3$	.1000	19.15	26.75	26.81	1
			.1000	19.10		26.74	2

On account of the great advantage offered by Pregl's[10] micro methods as to economy of both time and material, we developed von Asboth's modification of the Kjeldahl method for micro-analysis; 3–10 mg. of the sample was weighed in a small weighing tube and dropped into the flask. After addition of 50–100 mg. of glucose, 1 g. of potassium sulfate and a small crystal of copper sulfate, 3 cc. of sulfuric acid was added and digestion and distillation were carried out according to Pregl. In order to make certain that a sufficient amount of alkali had been added, a few drops of

[3] Jodlbauer, *Chem. Centr.*, [3] **17**, 433 (1886).

[4] Margosches and Scheinost, *Ber.*, **58**, 1850, 1857 (1925).

[5] Dafert, *Z. anal. Chem.*, **27**, 222 (1888).

[6] Milbauer, *ibid.*, **42**, 725 (1903).

[7] Krüger, *Ber.*, **27**, 1633 (1894). Flamand and Prager, *Ber.*, **38**, 559 (1905).

[8] Eckert, *Monatsh.*, **34**, 1964 (1913).

[9] Levene and Sobotka, *J. Biol. Chem.*, **65**, 469 (1925).

[10] Pregl, "Quantitative Organic Microanalysis," Trans. by E. Fyleman, Blakeston and Co., Philadelphia, **1925**.

alizarin sulfonate were used as an indicator. The digestion did not involve a loss of time as compared with the usual Pregl method. The addition of a few drops of alcohol after decolorization seemed to be unnecessary with our modification. The digestion was complete after 40 minutes. Therefore, a little more than one hour was required for one estimation.

TABLE II

RESULTS IN MICRO-ANALYSES

	Substance	Wt., mg.	*N*/70 acid, cc.	Per cent. of nitrogen Found	Calcd.
1.	Nitrobenzoyl chloride.....	11.274	4.31	7.64	7.70
2.	β-Nitrosonaphthol.....	8.238	3.37	8.18	8.09
3.	Nitropropanol.....	9.152	6.20	13.49	13.33
4.	Picric acid.....	3.280	3.02	18.41	18.37
5.	*m*-Nitro-aniline.....	5.460	5.58	20.44	20.21
6.	Nitro-uracil.....	4.474	5.96	26.65	26.75

Excellent checks were obtained on dried samples, although in numbers 1,2 and 4 of Table II, samples were taken from stock bottles containing substances not labelled C. P. This fact might help to overcome the objection of industrial chemists to micro-analysis on account of the alleged danger in taking minute samples.

Summary

A micro Kjeldahl method is described which gives satisfactory results for the determination of nitrogen in nitro, azo and other similar compounds.

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[CONTRIBUTION FROM THE POLARIMETRY SECTION, BUREAU OF STANDARDS, UNITED STATES DEPARTMENT OF COMMERCE][1]

RELATIONS BETWEEN ROTATORY POWER AND STRUCTURE IN THE SUGAR GROUP. XII.[2] THE PREPARATION AND PROPERTIES OF PURE ALPHA-METHYL *d*-LYXOSIDE

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It was shown in Part VII[3] that the rotation of methyl-*d*-lyxoside which Van Ekenstein and Blanksma[4] have recorded ($[\alpha]_D = +40.2$ in water) is much smaller than the fairly concordant values that were calculated for the pure alpha form of this substance by three independent ways, namely, 66 (derived from the rotation of α-*d*-lyxose), 66 (from that of α-benzyl *d*-lyxoside), and 61 (from that of α-methyl *d*-xyloside). As

[1] Published by permission of the Director of the Bureau of Standards, U. S. Department of Commerce.

[2] Part XI was published in THIS JOURNAL, **48**, 288 (1926).

[3] Hudson, *ibid.*, **47**, 272 (1925).

[4] Van Ekenstein and Blanksma, *Z. Ver. Deut. Zuckerind.*, **58**, 114 (1908).