moment desired to word his articles in such a way that the reader would be left under the impression that a "pure poison" could be obtained in this way. The whole object of this work was to isolate the poison in some fraction or fractions, and study these fractions with the view (1) of measuring the toxicity and finding a method ($KMnO_4$) for curing the wounds, and with the aims (2) of isolating and synthesizing the real poison or poisons. The work was discontinued because the very small amount of "poison" in the $100 worth of "total poison or tar" available made the further prosecution of the problem appear too expensive. We should indeed be glad to see anyone with the necessary funds continue this research and synthesize the pure poison or poisons. Success in this direction would mean much toward the solution of a problem which causes a great deal of human suffering.

Summary.

1. McNair's reasoning that the limbs of *Rhus diversiloba* should contain the same toxic and nontoxic constituents found in the leaves and flowers of another species, *Rhus toxicodendron*, in another locality and climate is against all the well-known evidence because:

(*a*) The botanical differences in species may often be detected only with difficulty while the chemical constituents may vary widely.

(*b*) The same species gives different substances in different localities and climates.

(*c*) The constituents found in the leaves of a given species are generally *not* identical with those found in the *limbs of the same plant*, much less of a different species under different conditions.

2. It is highly probable that Syme's "purified poisonous tar, gum or wax" was a mixture of toxic and nontoxic materials. Syme's "purified" the material as far as possible and when it gave out suspended the work on account of the expense. Although his description of the "purified poisonous tar, gum, or wax" and its reactions was in some places perhaps confusing, Syme did not believe that his "purified poison" was not a mixture. It is highly desirable to have the studies on all these toxic plants continued.

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THE NONPROTEIN NITROGENOUS CONSTITUENTS OF FEEDINGSTUFFS.

BY H. S. GRINDLEY AND H. C. ECKSTEIN.

Received May 25, 1916.

When the Van Slyke method for the determination of the chemical groups characteristic of the different amino acids of proteins was first

proposed as a method for the estimation of the free and the combined amino acids and the free and the combined amides of feedingstuffs,[1] it was realized that it would be necessary, in order to establish the accuracy of the method, to demonstrate that the nonprotein nitrogenous constituents of feedingstuffs do not lead to inaccurate experimental results or to results that falsify the interpretation of the experimental data so obtained. The investigation here reported was undertaken as a necessary step in this direction. The preliminary results of this work were reported at the New Orleans meeting of this Society.[2] Since that time Hart and Bentley[3] have published a paper relating to this same subject.

Briefly stated, the method finally adopted for the work here reported was as follows:

Ten to twenty-five grams of the feedingstuff, placed upon a four-inch Büchner funnel, were washed repeatedly with cold ammonia-free water until the filtrate measured about 2400 cc. The extract was diluted to 2500 cc.

(*a*) **The Total Soluble Nitrogen** was determined in 200 cc. portions of the extract in duplicate.

(*b*) **The Free Ammonia** was determined in a 500 cc. portion of the extract by the Van Slyke vacuum method.

The native proteins were separated in a 1250 cc. portion of this extract by heating it to boiling and by adding, drop by drop, while constantly stirring 7.5 cc. of colloidal ferric hydroxide[4] (containing 5% of Fe_2O_3). The solution was then boiled for one minute and 1.0 cc. of a solution of crystallized magnesium sulfate (made by dissolving $MgSO_4.7H_2O$ in an equal weight of water), was added to coagulate the excess of colloidal ferric hydroxide. The solution was again boiled for one minute. After standing overnight, the solution was filtered through folded filters and the precipitate was thoroughly washed with hot water. The filtrate and washings after being diluted to 1500 cc. were used for the following determinations:

(*c*) **The Total Nitrogen** not precipitated by the colloidal ferric hydroxide was determined in duplicate in 200 cc. portions.

(*d*) **The Free Ammonia** was determined in one portion of 500 cc. by the Van Slyke vacuum method.

(*e*) **The Free Amino-Acid Nitrogen** was determined by slightly acidifying the residual mixture left in the distilling flask from the above determination in (*d*) with hydrochloric acid, filtering and washing. The filtrate and washings were evaporated in a porcelain dish on the steam

[1] THIS JOURNAL, 37, 1778 (1915); 37, 2762 (1915).

[2] See *Science*, 42, 70 (1915) for abstract of paper.

[3] *J. Biol. Chem.*, 22, 477 (1915).

[4] Merck's.

bath to about 15 cc. The concentrated solution was washed into a 25 cc. measuring flask and diluted to the mark. The amino-acid nitrogen was determined in duplicate in 10 cc. portions of this solution by the Van Slyke nitrous acid method.

(*f*) **The Free and Combined Acid-Amide Nitrogen** were determined in a 500 cc. portion of the solution by adding enough of concentrated hydrochloric acid to give a concentration of 20% and then boiling under a reflux condenser for 12 hours. The excess of hydrochloric acid was removed by evaporation *in vacuo* and the ammonia determined by the Van Slyke vacuum method. One-half of the weight of the free ammonia nitrogen determined above in (*b*) subtracted from the weight of the ammonia nitrogen obtained in this determination gave the weight of the free and combined acid-amide nitrogen in one-half the quantity of the feedingstuff originally taken for the analysis. The free ammonia nitrogen as determined in the original water extract was considered more accurate than the corresponding determination in the filtrate from the colloidal iron precipitation, as the heating of the solution during the latter precipitation, clearly hydrolyzed, in part, the free acid amides present with the liberation of ammonia.

(*g*) **The Humin Nitrogen,** which was formed in the above determination by boiling the aqueous extract of the feedingstuffs with 20% hydrochloric acid for 12 hours, was filtered off and washed as per the Van Slyke method. The nitrogen in the humin precipitate was determined by the Kjeldahl method.

(*h*) **The Combined Amino-Acid Nitrogen** was determined by slightly acidifying the filtrate and washings from the above determination in (*g*) and then evaporating the resulting solution in a porcelain dish on the steam bath to about 30 cc. The concentrated solution was washed into a 50 cc. measuring flask and diluted to the mark. The amino-acid nitrogen was determined in duplicate in 10 cc. portions of this solution by the Van Slyke nitrous acid method. The free amino-acid nitrogen determined above in (*e*) subtracted from the free and combined amino-acid nitrogen obtained in this determination gave the combined amino-acid nitrogen.

(*i*) **The Residual Soluble Nitrogen,** which did not respond to any of the above tests, was obtained by subtracting the sum of one-half of the free ammonia nitrogen obtained in (*b*), the free amino-acid nitrogen obtained in (*e*), the free and combined acid-amide nitrogen obtained in (*f*), the humin nitrogen obtained in (*g*), and the combined amino-acid nitrogen obtained in (*h*) from the total nitrogen not precipitated by colloidal iron hydroxide obtained in (*c*).

It is possible that some of the humin nitrogen has its origin in the free amino-acid nitrogen. If this is true, then the residual soluble nitrogen

would necessarily be increased to an equivalent extent. At present, it is impossible to determine how much of the humin nitrogen comes from the free amino-acid nitrogen, and how much of it comes from the combined amino-acid nitrogen.

Much difficulty was experienced in obtaining a complete extraction of the food material with cold water. By the method described above the total nitrogen not precipitated by colloidal ferric hydroxide in a second extract following the first, and measuring 1000 cc., was reduced to less than 0.6% of the total nitrogen present in the alfalfa hay; to less than 0.5% of the total nitrogen of timothy hay; to less than 0.3% of the total nitrogen of the corn; and to less than 0.07% of the total nitrogen of the blood meal.

The use of colloidal ferric hydroxide for the separation of the proteins from the nonproteins was suggested by the recent use of this reagent for the separation of proteins from blood, milk, and partially digested proteins by Wolff,[1] Hill,[2] and Van Slyke,[3] *et al.*, Van Slyke, Vinograd-Villchur and Losee[3] say: "In experiments on Witte peptone and partially digested protein to be published later we have found, furthermore, that colloidal ferric hydrate not only lets all the amino acids go through

TABLE I.—THE NONPROTEIN NITROGENOUS CONSTITUENTS OF FEEDINGSTUFFS.

Results Expressed in Per Cent. of the Total Nitrogen of the Feedingstuffs.

	In the original extract.			In the filtrate from the colloidal iron precipitate.					
Feed.	Total nitrogen.	Free ammonia nitrogen.	Nitrogen precipitated by colloidal iron.	Total nitrogen.	Humin nitrogen.	Free amino-acid nitrogen.	Free and combined acid-amide nitrogen.	Combined amino-acid nitrogen.	Residual soluble nitrogen.
Alfalfa hay.....	28.58	1.07	11.07	17.51	1.40	5.08	3.40	3.10	3.46
Alfalfa hay.....	28.21	1.15	10.63	17.58	1.48	5.00	3.29	3.15	3.51
Average.....	28.40	1.11	10.85	17.55	1.44	5.04	3.35	3.13	3.49
Timothy hay.....	23.58	1.44	8.38	15.20	2.67	4.82	2.67	1.19	2.41
Timothy hay.....	23.92	1.49	8.27	15.65	2.70	4.84	2.60	1.14	2.88
Average.....	23.75	1.47	8.33	15.43	2.69	4.83	2.64	1.17	2.65
Blood meal.....	2.33	0.19	0.49	1.84	0.04	0.58	0.47	0.48	0.08
Blood meal.....	2.33	0.14	0.43	1.90	0.05	0.57	0.49	0.52	0.13
Average.....	2.33	0.17	0.46	1.87	0.05	0.58	0.48	0.50	0.11
Corn.....	7.17	0.66	1.55	5.62	0.24	2.16	1.04	0.57	0.95
Corn.....	7.74	0.70	2.06	5.68	0.36	2.17	1.08	0.51	0.86
Average.....	7.46	0.68	1.81	5.65	0.30	2.17	1.06	0.54	0.91
Clover hay.....	16.50	1.99	2.29	14.21	1.77	4.32	1.40	0.19	4.54
Clover hay.....	16.60	1.98	2.37	14.23	1.78	4.42	1.18	0.29	4.58
Average.....	16.55	1.99	2.33	14.22	1.78	4.37	1.29	0.24	4.56

[1] *J. Physiol.*, 49, 89 (1915).

[2] *J. Biol. Chem.*, 20, 175 (1915).

[3] *Ibid.*, 23, 381 (1915).

TABLE II.—THE NONPROTEIN NITROGENOUS CONSTITUENTS OF FEEDINGSTUFFS.

Results Expressed in Per Cent. of the Feedingstuffs.

	In the original extract.			In the filtrate from the colloidal iron precipitate.					
Feed.	Total nitrogen.	Free ammonia nitrogen.	Nitrogen precipitated by colloidal iron.	Total nitrogen.	Humin nitrogen.	Free amino-acid nitrogen.	Free and combined acid-amide nitrogen.	Combined amino-acid nitrogen.	Residual soluble nitrogen.
Alfalfa hay.....	0.751	0.028	0.290	0.461	0.037	0.134	0.089	0.081	0.092
Alfalfa hay.....	0.741	0.030	0.279	0.462	0.039	0.131	0.086	0.083	0.093
Average.....	0.746	0.029	0.285	0.462	0.038	0.133	0.088	0.082	0.093
Timothy hay....	0.202	0.012	0.072	0.130	0.023	0.042	0.023	0.010	0.020
Timothy hay....	0.205	0.013	0.071	0.134	0.023	0.042	0.022	0.008	0.026
Average.....	0.204	0.013	0.072	0.132	0.023	0.042	0.023	0.009	0.023
Blood meal.....	0.327	0.026	0.069	0.258	0.005	0.082	0.065	0.068	0.012
Blood meal.....	0.326	0.019	0.061	0.265	0.007	0.079	0.069	0.073	0.018
Average.....	0.327	0.023	0.065	0.262	0.006	0.081	0.067	0.071	0.015
Corn.....	0.103	0.009	0.022	0.081	0.004	0.031	0.015	0.008	0.014
Corn.....	0.111	0.010	0.029	0.082	0.005	0.031	0.015	0.006	0.015
Average.....	0.107	0.010	0.026	0.082	0.005	0.031	0.015	0.007	0.015
Clover hay.....	0.330	0.040	0.045	0.285	0.035	0.090	0.028	0.004	0.088
Clover hay.....	0.332	0.040	0.047	0.285	0.036	0.091	0.024	0.006	0.088
Average.....	0.331	0.040	0.046	0.285	0.036	0.091	0.026	0.005	0.088

TABLE III.—THE NONPROTEIN NITROGENOUS CONSTITUENTS OF FEEDINGSTUFFS.

Results Expressed in Per Cent. of the Total Soluble Nitrogen not Precipitated by Colloidal Ferric Hydroxide.

Feed.	Total nitrogen.	Free ammonia nitrogen.	Humin nitrogen.	Free amino-acid nitrogen.	Free and combined acid-amide nitrogen.	Combined amino-acid nitrogen.	Residual soluble. nitrogen.
Alfalfa hay.....	100.00	6.32	8.21	28.72	19.09	17.83	19.89
Timothy hay.....	100.00	9.53	17.43	31.30	17.11	7.58	17.07
Blood meal.....	100.00	9.09	2.67	31.02	25.67	26.74	5.88
Corn.....	100.00	12.04	5.31	38.41	18.76	9.56	16.11
Clover hay.....	100.00	13.99	12.52	30.73	9.07	1.69	32.07

into the filtrate, but that it also precipitates none of the intermediary products up to the albumoses, and none of these except some of complexity but little below that of the original proteins (proportion of amino nitrogen was but 6 to 7% of the total in the precipitated albumoses). As the precipitation of the native proteins themselves is complete, colloidal ferric hydrate appears especially well adapted to our purpose."

The character of the results obtained by the application of this method to the examination of a few feedingstuffs is apparent from the data given in Tables I, II, and III. In Table I, the nonprotein nitrogenous constituents of feedingstuffs are given expressed in per cent. of the total nitrogen of the feedingstuff. In Table II, the nonprotein nitrogenous constituents are expressed in per cent. of the feedingstuff. In Table III, the non-

protein nitrogenous constituents are expressed in per cent. of the total soluble nitrogen not precipitated by colloidal ferric hydroxide.

It is apparent that this method of the determination of the nonprotein nitrogenous constituents of feedingstuffs gives a fairly complete picture of the different forms of nitrogen represented in the so-called nonprotein nitrogenous constituents.

It is also apparent that the nonprotein nitrogenous constituents consist largely of the forms of nitrogen that result from the decomposition of proteins by hydrolysis. In other words, the sum of the amide nitrogen, the humin nitrogen, the free amino-acid nitrogen, the combined amino-acid nitrogen, and the free and combined acid-amide nitrogen represented in the nonprotein nitrogenous constituents form from 80% in the case of alfalfa hay to 94% in case of blood meal, of the nonprotein nitrogen. Further, it is probable that at least 50% of the residual soluble nitrogen not precipitated by colloidal iron represents the nonamino nitrogen present in the free and combined amino acids determined.

It is impossible at present to tell definitely what the remaining 3 to 10% of residual soluble nitrogen, which does not respond to any of the tests here applied, represents. However, it seems quite evident that only a small part, if any, of the nonprotein nitrogenous constituents of foods and feedingstuffs can in any way interfere with the application of the Van Slyke method for the determination of the chemical groups characteristic of the different amino acids of protein to the estimation of the free and combined amino acids and amides of feedingstuffs.

It is also evident that the so-called amide nitrogen of feedingstuffs is largely composed of free amino acids and peptide linkings. The nitrogen in these latter forms including the humin nitrogen constitute from 53 to 63% of the water-soluble nitrogen not precipitated by colloidal ferric hydroxide. Hart and Bentley[1] by the use of their method, which differs entirely from the method we here propose, reported 50 to 70% of the water-soluble nitrogen of immature and mature plants, that was not precipitated by boiling the slightly acidified extracts, as free amino acids and peptide linkings.

The free and combined acid-amide nitrogen varied from 17.11 to 25.67% of the soluble nitrogen not precipitated by colloidal ferric hydroxide. Hart and Bentley found the "free acid-amide nitrogen" by their method to be relatively small, seldom exceeding 20% of the water-soluble nitrogen, and more often being below 10%. Our results which represent the free and the combined acid-amide nitrogen are not directly comparable with those of Hart and Bentley which they considered to represent only the free acid-amide nitrogen. However, it does not seem probable, that the results they obtained for this determination represent merely free acid-

[1] *J. Biol. Chem.*, **22**, 477 (1915).

amide nitrogen, for it is a well-known fact that proteins and peptide linkings very readily yield ammonia when boiled with 20% hydrochloric acid.

In the work here reported the free ammonia nitrogen varied from 6.33 to 12.04% of the water-soluble nitrogen not precipitated by colloidal iron. Hart and Bentley found by their method that the free ammonia nitrogen of immature and mature plants rarely exceeded 5% of the water-soluble nitrogen, and in some instances was wholly absent. Their low results for ammonia were probably due to the direct extraction of the food materials with *hot* water. We have confirmed the results of Hart and Bentley that an extract of alfalfa hay prepared with boiling water contains no free ammonia, or at least only a slight trace. On the other hand, as shown in the Table III, a cold water extract of alfalfa hay contained 6.33% of its total soluble nitrogen not precipitated by colloidal iron, in the form of free ammonia. Further, an extract of alfalfa hay prepared by hot water slightly acidified (0.185% hydrochloric acid), contained 4.44% of its total soluble nitrogen, not precipitated by colloidal iron, as free ammonia. A water extract of alfalfa hay is distinctly alkaline to litmus paper.

Further studies to determine the amount and the nature of the nonprotein nitrogenous constituents of feedingstuffs are now under way in this laboratory.

URBANA, ILL.

THE ISOMERIC PENTACETATES OF GLUCOSAMINE AND OF CHONDROSAMINE.[1]

BY C. S. HUDSON AND J. K. DALE.

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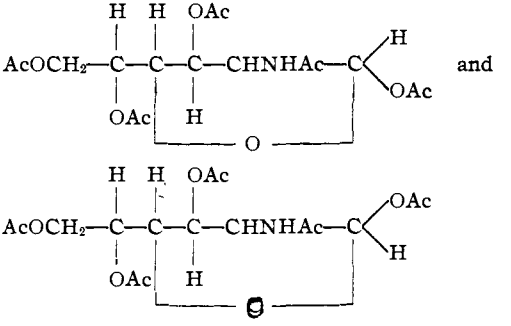
Two isomeric pentacetyl derivatives of glucosamine have been described by Lobry de Bruyn and Van Ekenstein.[2] If these compounds have the isomeric structures,



[1] Contribution from the Carbohydrate Laboratory, Bureau of Chemistry, United States Department of Agriculture.

[2] *Rev. trav. chim.*, 18, 83 (1899).