

behavior, without the addition of any form of activator. The minced gland itself furnishes all that is necessary to activate the trypsinogen.

Conclusions.

We have shown that it is possible to separate the true press juice from the minced pancreas, and best by aid of a powerful centrifuge operating at a speed of 3000 revolutions, or over, per minute. In the centrifugal separation the minced mass divides into three layers of distinct properties. The lower layer is largely protein, the middle layer a clear liquid, and the upper layer solid with much fat present.

The liquid layer has a marked acid reaction, as distinct from that of the so-called pancreatic juice, which reaction is nearly a constant for beef and hog glands. The juice is rich in phosphates and the reaction seems to be due to acid phosphate. This reaction may be as constant and characteristic as is that of the blood and may be expressed, approximately, by the relation,

$$C_H = 29 \times 10^{-7}.$$

CHICAGO, ILL.

[CONTRIBUTIONS FROM THE LABORATORY OF SOIL CHEMISTRY OF THE IOWA STATE COLLEGE EXPERIMENT STATION.]

AMINO-ACID NITROGEN OF SOIL AND THE CHEMICAL GROUPS OF AMINO ACIDS IN THE HYDROLYZED SOIL AND THEIR HUMIC ACIDS.

By R. S. POTTER AND R. S. SNYDER.

Received July 6, 1915.

This investigation was undertaken to correlate, if possible, the amounts of the various chemical groups¹ (1) in the soil with its humic acid, (2) in the soil and its humic acid with the kind of organic fertilizer previously applied to the soil, (3) in the soil and its humic acid with similar groups found in pure proteins, and (4) to compare the amounts of amino acid nitrogen, as such, in the soil with that found by hydrolysis. No study of just this nature has ever been made. Several investigators, in recent years, subjected the solution obtained by boiling the soil with strong acids to the Osborne and Harris-Hausmann method. Kelley and Thompson² analyzed the alkali extract and the humic acids of several soils by this same method.

Suzuki³ analyzed three humic acids rather extensively. Humic acid A was a Merck product, origin unknown to Suzuki. Humic acids B and C were prepared, respectively, from an unmanured soil and a compost

¹ Van Slyke, *J. Biol. Chem.*, 10, 15 (1910).

² *THIS JOURNAL*, 36, 438 (1914).

³ *Bull. Col. Tokyo*, 7, 513 (1907).

heap. Each acid was boiled for ten hours with strong hydrochloric acid. Besides other determinations, the amount dissolved, the amide, basic and nonbasic nitrogen were determined. 65-75% of the total nitrogen was dissolved by the acid, and in the extract 41-62% of the nitrogen was not precipitable with phosphotungstic acid. A large sample of the humic acid A was then hydrolyzed and the quantitative separation of the amino acids by the Fisher method¹ carried out. Small amounts of most of the protein amino acids were obtained. This work showed that at least part of the humic acid was of a protein nature. It is unfortunate that the origin of the acid was unknown to Suzuki. He states that, according to its general behavior, it probably was prepared from peat.

Since about half of the experimental work in connection with this investigation was completed, a paper has appeared by Lathrop² in which he gives analysis of "base goods" fertilizer by the Van Slyke method. The comparison of our results with those obtained by him will be of some interest and will be taken up in the experimental part of this paper.

The soils used in this work were obtained from experimental plots maintained by this Station and are located on the Wisconsin drift area. They consist of 1/20 acre plots situated side by side and separated by four foot alleys and are numbered 101 to 114. In this work soil from 101, 102, 103, 104, 106, 107, and 108 was used. There is a slight slope from plot 114 to 101. Previous to 1907 inclusively, the soils had received the ordinary farm cultivation. Since that time the soils used by us have received the following treatments:

Plot.	Cultivation.	Treatment per acre.
101.....	Timothy annually	Nothing added
102.....	Fallowed	2 T. peat annually
103.....	Fallowed	8 T. manure 1909-1913
104.....	Fallowed	8 T. clover 1909-1913
106.....	Fallowed	2 T. timothy annually
107.....	Fallowed	Nothing
108.....	Fallowed	2 T. oat straw annually

In addition to these soils a peat soil taken from one of the Station pastures was used. The soils were all collected during the winter of 1914-1915, immediately air dried and ground. In order to render the humus more soluble the soils were all extracted with 1% hydrochloric acid in the usual manner.

In Table I are given the amounts of nitrogen extracted by the acid, together with the total nitrogen of the soil.

¹ *Z. physiol. Chem.*, **33**, 151, 412 (1901).

² *J. Ind. Eng. Chem.*, **7**, 228 (1915).

TABLE I.

Soil.	Total nitrogen in per cent.	Nitrogen extracted by 1% HCl.	
		In per cent. of soil.	In per cent. of total N.
101.....	0.306	0.00490	1.60
102.....	0.336	0.00406	1.23
103.....	0.300	0.00546	1.82
104.....	0.270	0.00392	1.42
106.....	0.245	0.00406	1.66
107.....	1.234	0.00532	2.27
108.....	0.224	0.00504	2.27
Peat.....	1.770	0.01191	0.673

In considering the total nitrogen values, it must be remembered that there is a gentle slope down to plot 101 and hence there is a tendency for these plots to contain more nitrogen than those higher up. In the case of the results for the amount of nitrogen extracted by the 1% acid, the differences are not marked. Both the peat and the peat treated soil being low, shows that the peat has not lost its character after entrance to the soil.

The soils were in all cases hydrolyzed in the same way, namely, by boiling one part of soil with two parts of 22% hydrochloric acid for 48 hours. At the end of this period the mixture was allowed to cool somewhat, filtered and washed until free from chlorides. The filtrate, combined with the wash water, was evaporated to about 200 cc., made up to exactly 250 cc., and two 25 cc. portions were taken for analysis for total nitrogen. The 200 cc. remaining were divided into two portions of 100 cc. and the Van Slyke method, exactly as outlined by the originator, was applied to each portion. Cystin nitrogen was analyzed for in the case of two soils; but only a questionable trace was found in each case, so that for the remaining soils this determination was omitted. No doubt the cystin was decomposed by the long treatment with the acid.

It is not thought that the nitrogen as found by the Van Slyke method, working with such a complex as the soil, is in reality, all lysine, histidine, etc., nitrogen. It might be said that each group, as found, represents a class of compounds having the particular reaction by which the lysine, histidine, etc., nitrogen, respectively are determined. For extraction we have arbitrarily shaken 25 g. samples of the soils with 100 cc. of 1% sodium hydroxide for five hours. The mixture was then centrifuged in a high speed machine.¹

Most investigators, in preparing humic acids have precipitated the humus solutions by slight acidification with one of the mineral acids, but, as has been shown by Shorey,² upon neutralizing the filtrate from the humic acids another precipitate is given which contains nitrogen. This

¹ For description see *Iowa Sta. Bull.*, No. 124 (1911).

² U. S. Dept. Agr., *Hawaii Sta. Rept.*, 1906, p. 51.

is to be expected if the humus solution contains any protein material. In view of this and because of the rather general use of dilute acetic acid as a protein precipitant, it was decided to try the use of this reagent. Table II gives the results of some preliminary tests. One per cent. sodium hydroxide was the extractive reagent throughout. Twenty cc. portions were carefully neutralized with sulfuric acid and then sufficient 10% acetic acid added to give the desired strength. The mixture was heated at the boiling point for 15 minutes and then filtered by suction through paper pulp, and the precipitate washed with the same strength acid as the solution from which it had originated.

TABLE II.

Soil.	A.			103.			107.		
	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
Strength acetic acid (%).....	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
Nitr. ppt. in % total N.....	63.7	55.6	55.0	73.7	70.0	68.1	87.0	83.4	81.3

While it is possible that a weaker acid might have precipitated a greater amount of nitrogen, it was found to be extremely difficult to filter such a mixture.

The humic acids used in the subsequent analysis were prepared as follows: 150 g. of the acid extracted soil were placed in an 800 cc. bottle and shaken 5 hours with 1% sodium hydroxide and then immediately centrifuged. Precautions were taken to keep the loss of ammonia as low as possible. The extract so obtained was very carefully neutralized to litmus with sulfuric acid and then sufficient strong acetic acid to give a 0.5% solution was added. The mixture was heated at the boiling point for 15 minutes, and filtered by suction on a Buechner funnel containing two thicknesses of a good filter paper. After filtration the precipitate was transferred to a flask, 22% hydrochloric added and then the mixture was hydrolyzed for 48 hours. In some cases it was found to be impossible to transfer the humic acid to the flasks quantitatively. Where this was the case the procedure was as follows: A small portion of the alkali extract, usually 250 cc. was carried through the regular procedure, it always being possible to work quantitatively with such an amount. The hydrolysis was carried out and the humin and amide nitrogen determined. From the data so obtained the amount of extract in the larger sample was calculated.

After the completion of the hydrolysis the mixture was filtered and the undissolved residue washed free from chlorides. The residue was analyzed for total nitrogen. The filtrate was concentrated to about 200 cc., made up to exactly 250 cc., and two 25 cc. portions were taken for analysis of total nitrogen. The remaining 200 cc. were used for the determination of the chemical groups according to Van Slyke, in the case of soils 102, 103, 106, and 107 dividing it into two portions and carrying the analysis

out in duplicate. With the other soils the whole of the 200 cc. portion was used for one analysis. The precipitate found with the lime was analyzed for total nitrogen and to this was added the nitrogen from the insoluble residue, and the sum called humin nitrogen.

TABLE III.

Soil.	Extraction reagent.	Amide.	Melanin.	Arginine.	Histidine.	Lysine.
101.....	22% HCl	24.09	6.68	4.03	4.23	2.25
	1% NaOH	7.49	9.94	1.85	1.76	0.43
102.....	22% HCl	25.61	8.65	5.08	3.41	2.74
	1% NaOH	5.14	9.25	0.80	1.06	0.70
103.....	22% HCl	23.0	6.68	3.55	1.99	1.69
	1% NaOH	5.66	5.03	0.64	0.65	0.54
104.....	22% HCl	23.38	7.45	3.92	2.78	1.29
	1% NaOH	9.48	21.5	1.83	1.54	0.75
106.....	22% HCl	27.21	8.60	4.83	3.47	3.22
	1% NaOH	4.83	6.62	0.93	1.06	0.43
107.....	22% HCl	27.20	8.35	4.34	3.91	2.46
	1% NaOH	8.88	12.46	0.87	0.65	0.66
108.....	22% HCl	26.64	8.72	4.66	6.30	1.82
	1% NaOH	10.0	14.07	2.68	2.91	0.82
Peat.....	22% HCl	18.36	5.95	3.72	2.03	2.95
	1% NaOH	2.54	1.33	0.38	0.21	0.19

Soil.	Total basic.	Monoamino.	Nonamino.	Total nitrogen found.	Extracted.	Humic acid.
101.....	10.51	26.17	5.41	72.86	74.02	...
	4.05	7.34	2.95	31.77	42.0	32.1
102.....	11.23	24.62	3.85	73.96	75.12	...
	2.56	7.45	0.92	25.32	41.9	25.7
103.....	7.23	28.11	2.40	67.42	70.93	...
	1.83	6.72	1.15	20.39	28.6	21.0
104.....	7.99	27.21	5.00	71.03	74.31	...
	4.12	9.36	0.94	45.4	53.1	46.8
106.....	11.52	21.28	4.46	73.07	76.47	...
	2.42	5.54	1.68	21.1	31.8	21.0
107.....	10.71	21.01	3.91	71.18	71.31	...
	2.18	7.22	0.96	31.7	36.8	31.7
108.....	12.78	25.22	2.11	65.47	68.68	...
	6.41	9.80	1.0	41.28	52.2	43.0
Peat.....	8.70	31.72	6.15	70.88	71.37	...
	0.78	1.85	0.37	6.74	8.42	6.61

The results, together with those found by the analysis of the hydrochloric acid extract, are given in Table III, and are all expressed in per cent. of total soil nitrogen.

The results in Table III will be discussed only quite briefly. The amounts of nitrogen extracted by the strong acid are fairly constant; that extracted by the weak alkali varies widely and the variations are of no apparent significance. The amount of humic acid in the various soils is quite different. Whether this is due to some other differences, perhaps

physical, it is not possible to state. It is also possible that the use of such a small amount of weak alkali, relative to the amount of soil, used might have caused the differences. It was noticed that it required quite different amounts of sulfuric acid to neutralize the alkali extract for the different soils.

The most significant thing brought out by the table is the high amounts of humin nitrogen in the humic acids. Insofar as humin compounds are a sharply defined class of compounds it is hardly probable that all our humin belongs to it. Yet our material possessed many of the properties of this class of compounds. It was black, insoluble in all the organic solvents, dilute acids, salt solutions, and soluble in dilute alkali. In this connection a recent paper by Gartner and Blish¹ is of interest. Working with pure zein and gliadin they showed that the humin compounds had their origin in tryptophane, and that, contrary to the rather general belief, nitrogenous humins are not formed by the "adsorption of ammonia by nonnitrogenous humins formed from carbohydrates." In the soil there is, of course, carbohydrate material and it would seem that the amount of humin found by use would be too large to all come from the tryptophane. However, it may be that it is possible for other substances containing the NH group to form humin. The above investigators found that histidine did not form humin. In the continual degradation of the soil organic matter there would be a natural tendency for the humin to accumulate for, of course, this material once formed would be very resistant to further change.

The amounts of humin nitrogen found in the hydrochloric acid extracts is probably of no significance, for the amount found varied with the number of times the soil residue was washed. As long as there was any acid left the wash-water was clear, but towards the end of the washing brownish material was taken out, due no doubt to a passing over to the colloidal state. This brown material carried a great deal of nitrogen with it.

Because of the varying and abnormally high amounts of humin nitrogen in the acid extract and in the humic acids, for the purposes of better comparison the results have been calculated on the basis that amide + basic + monoamino + nonamino = 100. This will permit of the more active groups being compared. The calculation is found in Table IV. In the same table the results, calculated to the same basis, for four proteins are given. These values are taken from Van Slyke.²

The most noteworthy thing brought out by the comparison of the values for the soil and the proteins is the highness of the values for the amide nitrogen, and the lowness of the basic and nonbasic nitrogen. It

¹ THIS JOURNAL, 37, 1630 (1915).

² *Loc. cit.*

is a somewhat general property of the vegetable proteins to yield high amounts of amide nitrogen but none so far examined have given more than gliadin. It is rather surprising that soil should have such a relatively high content of amide nitrogen, as it is the nitrogen of this group which is the most quickly hydrolyzed upon boiling proteins with acids. One would think that the organic matter in soil which has been subjected to an extended period of decay, would be rather deficient in this group. Perhaps the synthetical operations more than overbalance the decomposition. In this connection it would be interesting to have some data on the nitrogen distribution of the bacterial proteins, but so far as we know no such data is to be had at present.

TABLE IV.

Soil.	Material.	Amide.	Arginine.	Histi- dine.	Lysine.	Total basic.	Mono- amino.	Non- amino.
101.....	HCl ext.	36.4	6.08	6.40	3.40	15.9	39.0	8.7
	Humic acid	34.2	8.50	8.08	1.99	18.6	33.8	13.4
102.....	HCl ext.	39.2	7.78	5.22	4.20	17.2	37.7	5.32
	Humic acid	32.0	4.92	6.59	4.34	15.9	46.4	5.8
103.....	HCl ext.	37.8	5.83	3.28	2.77	11.89	46.2	4.11
	Humic acid	36.9	4.18	4.21	3.50	11.9	43.7	7.5
104.....	HCl ext.	36.8	6.18	4.37	2.02	12.9	42.8	7.9
	Humic acid	39.6	7.65	6.45	3.16	17.3	39.2	3.9
106.....	HCl ext.	42.2	7.50	5.38	4.99	17.85	33.0	6.9
	Humic acid	33.4	6.39	7.30	2.97	16.7	38.3	11.6
107.....	HCl ext.	43.3	6.90	6.22	3.92	17.0	33.4	6.2
	Humic acid	46.2	4.54	3.41	3.43	11.4	37.6	4.95
108.....	HCl ext.	39.9	6.97	9.44	2.74	19.15	37.8	3.15
	Humic acid	36.8	9.88	10.70	3.02	23.5	36.0	3.7
Peat.....	HCl ext.	28.3	5.72	3.13	4.56	13.4	48.8	9.45
	Humic acid	46.6	7.04	3.83	3.48	14.3	34.2	5.0
	Gliadin	25.5	5.77	5.26	0.76	13.05	52.5	8.6
	Edestin	10.18	27.8	5.90	3.97	39.2	48.8	1.75
	Fibrin	8.63	14.4	5.01	11.95	32.3	56.3	2.8
	Hemoglobin	5.44	7.98	13.2	11.3	32.5	59.1	3.0

On comparing the relative proportions of the various groups in the acid extract and the humic acids there are no noteworthy differences. In fact, with few exceptions there is a rather surprising similarity. To us it means that the organic matter extracted by alkali is of no very different character than the organic matter of the soils as a whole. This, together with the fact proved by Fraps and Hammer¹ that, upon adding organic matter to soil, at the end of a year's time there is no more material extracted with dilute ammonia than at the beginning of the period, proves quite conclusively that the determination of the amount of humus as found by the various methods is of no particular value in the study of a soil.

Nothing very significant can be deduced from the variations in the

¹ *Tex. Bull.*, 129 (1910).

different soils. The treatments have not been continued for long enough time to change materially the character of the original organic matter. Since these plots are a permanent feature of this Station it is hoped that similar analyses in after years will throw more light on the subject.

As to the question—just what is the constitution of humic acid? The idea that humic acid is a definite compound or even the mixture of a few compounds has been exploded long ago. According to the investigations of Suzuki, Kelley and the data here presented it contains material of a protein nature. Weak alkali solutions extract more protein material from seeds than any other extracting reagents and, according to Osborne,¹ usually in an unaltered condition. The form they are in when precipitated with acids depends upon the amount of acid added. In the soil, where there is undoubtedly a comparatively high content of dead bacterial cells, which would contain relatively high amounts of nucleoproteins, it would seem that part of the precipitate obtained on adding acid to the alkali extract would be made up of combinations of protein material with nucleic acid² as well as other organic acids. Of course the protein might also be precipitated unchanged or as a salt of the acid used for precipitating it.

The free amino acid and the peptide nitrogen in the unhydrolyzed soil⁴ was determined by the Kober method³ as we have applied it to soils. These results, which are given in Table V, are interesting in that they show how small the amount of amino acid and peptide nitrogen is in comparison to that present in the organic matter after hydrolysis. Lathrop,⁵ in the discussion of his results on the "base goods" fertilizer, seems to hold that such compounds as histidine would be of direct significance in plant nutrition especially when added to the soil in the "base goods." In view of the fact that the amino acid nitrogen in all soils examined by us is so small makes such a possibility seem quite remote to us. These compounds would probably be adsorbed to an extent at least as great as ammonia, so that they would be present in the soil solution in but the merest traces. The recent work of Upson and Powell,⁶ shows that many compounds, quite toxic to plants growing in water culture, are not at all or at most only slightly toxic when used in soil culture. It evidently is the investigator's intention to extend their experiments, and the results for the beneficial compounds will be awaited with interest.

¹ "The Vegetable Proteins," 1912, p. 18.

² Nucleic acid was found in many soils by Shorey, U. S. Dept. Agr., Bureau of Soils, *Bull.*, 88 (1913).

³ THIS JOURNAL, 35, 1546 (1913).

⁴ To appear shortly in the *J. Ind. Eng. Chem.*

⁵ *Loc. cit.*

⁶ *J. Ind. Eng. Chem.*, 7, 420 (1915).

TABLE V.

Soil.	Free amido acid nitrogen.	Polypep- tide ni- trogen.	Soil.	Free amino acid nitrogen.	Polypep- tide ni- trogen.	Soil.	Free amino acid nitrogen.	Polypep- tide ni- trogen.
101	0.30	0.56	104	0.40	0.92	108	0.65	1.50
102	0.29	0.88	106	0.24	0.74	Peat	0.071	0.26
103	0.16	0.84	107	0.29	0.71			

Conclusions.

1. The amount of nitrogen precipitated from a neutralized alkali extract of soil varies, in a qualitative way, inversely with the strength of the acid.
2. The amount of humin nitrogen, as found by the Van Slyke method, extracted by dilute alkali from soil is very high when compared to the amounts in proteins.
3. Dilute alkali does not extract any typical class of organic compound from the soil.
4. Before definite conclusions are drawn from the remainder of the Van Slyke results it is believed that analyses on these same plots in future years should be awaited.
5. The amount of amino acid and peptide nitrogen in soil is found to be very small when compared to the amounts of amino acids formed by hydrolysis.

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[CONTRIBUTION FROM THE HARE CHEMICAL LABORATORY, UNIVERSITY OF PENNSYLVANIA AND LABORATORY OF PHYSIOLOGICAL CHEMISTRY, COLLEGE OF MEDICINE, UNIVERSITY OF ILLINOIS.]

A DISTURBING FACTOR IN BARFOED'S TEST.¹

BY WILLIAM H. WELKER.

Received July, 1915.

Barfoed's test has been used extensively in biological chemistry for the detection of reducing monosaccharides. In this connection, it was used to show the difference in the chemical reaction between starch paste and hydrolytic products obtained from starch paste by boiling with dilute hydrochloric acid. It was found that when starch could no longer be detected with iodine it was impossible to obtain the typical glucose reaction with Barfoed's reagent, but that a greenish white precipitate was formed. All mineral acid had been removed from the solution of the hydrolytic products by neutralization with sodium hydroxide and the solution was subsequently made very faintly acid with acetic acid. These experiments seemed to indicate that sodium chloride was the substance that interfered with the reaction. In order to ascertain whether this

¹ Preliminary communication; *Proc. Am. Soc. Biol. Chem.*, 1909, p. 180. Read before the Medical Research Club, University of Illinois, Chicago, Ill., June 9, 1915.