XXIII. THE FORMATION OF HUMIC BODIES FROM ORGANIC SUBSTANCES.

By WILLIAM BEECROFT BOTTOMLEY.

From the Botanical Laboratories, King's College, London.

(Received May 10th, 1915.)

The true nature of the dark brown and black substances formed by the decomposition of organic matter in the soil, and grouped together under the collective name of humus, has long been a problem to both chemists and biologists, owing to the fact that the composition of these substances varies not only with the nature of the humus-producing materials, but also with the temperature, moisture and pressure relations, and the methods of extraction.

A study of the extensive literature which deals with humus shows that the widely divergent opinions expressed and the inconsistent results obtained group themselves around two chief views regarding the nature of humus.

The first view is that humus has a simple composition and consists of one or a few definite chemical substances. De Saussure [1804] describes it as a "brown combustible powder soluble in alkalies and ammonia compounds." Mulder [1849] says that it consists of seven different organic substances—crenic acid, apocrenic acid, geic acid, humic acid, humin, ulmic acid and ulmin. He considered these substances to be intimately related, and five at least were distinct steps in the decay of organic matter in the soil.

This view of the simple composition of humus was the dominant one amongst the earlier writers on the subject; and we find it existing even to-day in a slightly modified form in most agricultural text-books. In these books humus is regarded either as a more or less definite substance which can be obtained by extracting the soil with a dilute alkali after first treating with a dilute acid to remove the lime which renders the humus insoluble, or as being synonymous with the so-called humic acid which can be precipitated from alkaline solutions of the soil.

The second view, that humus is of a very complex nature, was first advanced by Hermann [1842]. He laid special stress on "the presence of a great number of various kinds of humus-substances," and divided these various substances into three groups: *humus extract*, soluble in water; *humic acid*, soluble in alkalies and precipitated by acids; *humin*, insoluble in water and alkalies.

Hoppe-Seyler [1889] added another group which he calls the *phlobaphene* group, comprising those substances dissolved out by alcohols from the acid precipitates of the humic acid group.

Still more recently Schreiner and Shorey [1910] have given a somewhat similar grouping for the various compounds they have isolated from the organic matter of soils. They, however, include another group—the *crenic acid* group—comprising the substances isolated from the acid filtrate obtained when an alkaline solution of the soil is treated with mineral acid. Their grouping may be illustrated as follows:

Schreiner and Shorey have isolated and identified 20 distinct compounds from the organic matter of soils, only one of which—a pentosan—is a carbohydrate. These substances have all been obtained from the crenic acid and alcohol-soluble humic acid groups, and nothing is known as to the identity of the constituents of the humin and alcohol-insoluble humic acid groups. Yet they state that these last two groups, the components of which are unknown, contain 39.8 per cent. of the total organic carbon found in soils.

When one considers that carbohydrates, represented by sugars, starch and celluloses, make up by far the greater portion of the organic substance of plants and so form the greater part of the material from which soil humus is derived, it appears strange that so far only one carbohydrate has been isolated from soil, and the suggestion naturally arises that there may be some connection between carbohydrates and these unknown components of the humic acid and humin groups.

The probability of such a connection was indicated during a research as to the value of certain kinds of so-called "humates" as a nutrient for the nitrogen-fixing organism *Azotobacter*, and the present paper describes the results of an investigation of the relationship between these substances.

It has been pointed out that the terms humic acid and humin are used with varying connotation by different writers. For the purpose of this investigation it has been assumed that the terms refer to two groups of humic substances: *humic acid*—substances thrown down as brown colloidal precipitates by mineral acids from the water or alkaline extracts of humus; *humin*—substances insoluble in water and alkalies, but rendered soluble by fusing with caustic soda or potash, from the solution of which humic acid can again be precipitated.

HUMIC BODIES FROM SUGARS BY THE ACTION OF MINERAL ACIDS.

The discovery by Malaguti [1858] that humic acid and humin are formed when sugar is boiled with either hydrochloric or sulphuric acid was important as indicating a possible origin for these substances in the soil, but no attempt was made to trace their inter-relationship.

Later, Conrad and Guthzeit [1885] stated that on boiling raw sugar with dilute hydrochloric acid it is first changed into dextrose and laevulose, and that then the dextrose forms humic acid and the laevulose humin. As these authors give no experimental proof of the accuracy of their statement, an experiment was made to test the effect of boiling sucrose, dextrose and laevulose separately with hydrochloric acid. Five grams of each were boiled with a 7·5 per cent. solution of hydrochloric acid under a reflux condenser for 30 minutes. All three yielded a brown deposit which on testing proved, in each case, to be a mixture of humic acid and humin. The relative proportions, however, of these two substances in the three mixtures showed considerable variation, and the proportion of humic acid to humin always diminished as the boiling continued. It was found that with a strong solution of hydrochloric acid the conversion of humic acid into humin took place rapidly. In order therefore to trace in detail the changes taking place, a weak solution of the acid was employed. Five g. of sucrose, dextrose and laevulose respectively were boiled with a 3 per cent. solution of hydrochloric acid under a reflux condenser for varying periods of time. As is well known, the colourless solutions on heating pass through a series of colour changes: yellow, orange, red, brown, black, and with the brown colour the formation of a deposit is first noticed. Laevulose and sucrose pass through these changes fairly rapidly, but with dextrose the rate of change is much slower.

Laevulose:	Yellow	1 minute;	red	5 minutes;	brown	8 minutes.
Sucrose:	"	2 minutes	"	8	"	12 "
Dextrose:	"	11	"	60	"	90 "

The first deposit obtained in each case was found to be humic acid without a trace of humin. On further boiling the humic acid becomes changed into humin. The proportions obtained by boiling for periods of 0.25, 0.5, 1, 2 and 3 hours are given in the following table:

	15 mins.	30 mins	1 hr.	2 hrs.	3 hrs.
5 g. sucrose; total ppt.	0.106	0.077	0.140	0.254	0.405
„ humic acid	0.007	0.040	0.101	0.148	0.157
„ humin (by diff.)	0.009	0.037	0.039	0.106	0.248
5 g. dextrose; total ppt.	} No deposit	} No deposit	} No deposit	0.003	0.011
„ humic acid				0.002	0.005
„ humin (by diff.)				0.001	0.006
5 g. laevulose; total ppt.	0.028	0.156	0.244	0.492	0.685
„ humic acid	0.010	0.103	0.104	0.184	0.165
„ humin (by diff.)	0.018	0.053	0.140	0.308	0.520

From the above results it is seen that as the boiling is prolonged the proportion of humic acid to humin decreases, although the total amount of the deposit increases. This suggests that humic acid is an intermediate stage in the conversion of sugars into humin. That humic acid is directly convertible into humin was proved by boiling freshly precipitated humic acids, obtained from peat and laevulose, with a 7.5 per cent. solution of hydrochloric acid for four hours, when it was found that 3.5 per cent. of the peat acid and 98 per cent. of the laevulose acid had been converted into humin.

The varying proportions of humic acid and humin in these deposits from sugar probably account for the diverse analytical results which have been obtained by various investigators.

A comparison of the so-called „artificial” humic acid from sugar with the “natural” product from soil or peat shows that in appearance, solubility and behaviour towards alkalis they are almost identical. Yet when analysed a considerable difference is seen to exist in their composition.

Robertson, Irvine and Dobson [1907] state that the average composition of different specimens of artificial and natural humic acids obtained by an ammonium salt was as follows:

	C	H	O
Artificial (sugar) humic acid	64.74	4.69	29.81
Natural (peat) „	54.29	4.94	38.16

This variation in composition leads these authors to consider that a structural difference exists between the artificial and natural acids.

Recent researches by Baumann [1909] have shown that natural humic acid freshly precipitated possesses colloidal properties, especially the power of forming adsorption compounds.

Considering that natural humic acid is formed in association with various decomposition products, it is probable that the true humic acid substances formed from the carbohydrates of the soil are admixed with various other products. As already indicated, some of these bodies have been extracted by means of alcohol from natural humic acid, and it was considered probable that, if the theory is correct that carbohydrates are the primary source of humic acid in the soil, the composition of the natural humic acid after purification by alcohol would approximate more closely to that of artificial humic acid.

This hypothesis was confirmed as follows: a specimen of "natural" humic acid was obtained by treating finely divided peat with a 4 % solution of hydrochloric acid until all soluble salts were extracted and then digesting the well-washed residue with an excess of 5 % ammonium hydrate solution. The dark brown liquid obtained after filtering the mass on a Buchner funnel was acidified with strong hydrochloric acid, and the flocculent brown precipitate thus obtained was separated by filtration and well washed. It was then redissolved in ammonia and reprecipitated by hydrochloric acid, the precipitate being washed with distilled water until free from chlorides. The residue was divided into two portions, one of which was dried in the steam-oven at 100°, while the other was boiled for one hour under a reflux condenser with absolute alcohol. The alcohol was filtered off, the residue washed with fresh alcohol, and dried. The average results of a series of combustion analyses of these two products were as follow:

	C	H	O (by diff.)
Natural humic acid	48.64	4.55	46.81
Natural humic acid after extraction with alcohol	60.37	5.39	34.24

These results indicate that when natural humic acid has been purified by means of alcohol its carbon content approximates much more closely to that of the artificial acid, the alcohol having removed most of the adsorbed bodies. It is well known that the carbon content of artificial humic acid varies with the method of its preparation, and no definite formula can be assigned to it, but it was found that the above analysis of purified natural humic acid showed a remarkable similarity with that of one of the artificial humic acids we obtained by boiling dextrose with hydrochloric acid. This is shown by the following comparison:

	C	H	O (by diff.)
Artificial humic acid	60.74	5.13	34.13
Natural humic acid (purified)	60.37	5.39	34.24

HUMIC BODIES FROM SUGARS BY THE ACTION OF ORGANIC ACIDS.

It is well known that the humic acid and humin groups of substances are formed during the decomposition of organic matter in the soil. It is also known that in the process of this decomposition various organic acids are produced by bacterial activities. Experiments were therefore made to determine the effect on sugars of certain organic acids known to exist in the soil.

It was found that lactic, acetic, propionic, butyric, citric, tartaric and oxalic acids when boiled with either sucrose, dextrose or laevulose yield humic acid and humin, after producing the characteristic series of colour changes obtained by the action of mineral acids on sugars. The rapidity of the action varies with the kind and concentration of acid used, as shown by the following tables:

The action of 12.5 % acids on sucrose.

Acid	Time for yellow colour	Time for red colour	Time for first deposit	Wt. of total ppt. in 24 hrs.	Wt. of re-ppd. humic acid in 24 hrs.
Lactic	1 hr. 15 mins.	2 hrs.	5 hrs. 10 mins.	0.123 gm.	0.082 gm.
Acetic	2 hrs.	7 hrs. 10 mins.	12 hrs. 10 mins.	0.050 gm.	0.025 gm.
Propionic	3 hrs.	9 hrs. 10 mins.	15 hrs. 30 mins.	0.016 gm.	0.009 gm.
Butyric	3 hrs. 10 mins.	12 hrs. 10 mins.	20 hrs.	0.024 gm.	0.004 gm.

The action of 36 % acids on sucrose.

Acid	Time for yellow colour	Time for red colour	Time for first deposit	Wt. of total ppt. in 24 hrs.	Wt. of re-ppd. humic acid in 24 hrs.
Lactic	Immediate	2 mins.	5 mins.	0.715 gm.	0.695 gm.
Acetic	5 mins.	35 mins.	10 hrs.	0.211 gm.	0.200 gm.
Propionic	Immediate	12 mins.	16 hrs.	0.365 gm.	0.164 gm.
Butyric	55 mins.	4 hrs. 40 mins.	18 hrs.	0.040 gm.	0.008 gm.
Citric	20 mins.	1 hr.	2 hrs. 40 mins.	0.042 gm.	0.024 gm.
Tartaric	10 mins.	15 mins.	30 mins.	0.009 gm.	0.009 gm.
Oxalic	Immediate	5 mins.	25 mins.	0.160 gm.	0.093 gm.

HUMIC BODIES FROM SUGARS BY THE ACTION OF HEAT.

In the course of the present investigation it was found that the action of heat alone on sucrose, dextrose and laevulose will produce bodies very similar to humic acid and humin.

It is well known that sucrose fuses at 160°, and is converted into a mixture of dextrose and laevulosan; on further heating to about 190° it yields caramel and at higher temperatures gradually becomes carbonised.

In a preliminary experiment it was found that the black mass produced by heating sucrose at 220° for two hours contained at least four distinct substances: (1) water-soluble caramelan; (2) a water-soluble substance precipitated as fine particles by hydrochloric acid; (3) an alkali-soluble substance giving the typical flocculent precipitate of humic acid when treated with hydrochloric acid; (4) a black residue insoluble in water and alkalies giving the characteristic humin reactions when fused with caustic soda or potash.

Further experiments showed a great difference between dextrose and laevulose in their heat reactions, laevulose forming a black mass at a much lower temperature than dextrose. Dextrose on heating for four hours at 180° forms a light brown toffee-like mass completely soluble in water, but giving no precipitate with hydrochloric acid. On further heating for two hours at 220° it changes to a dark brown colour. Very little of this is water-soluble. The residue is almost entirely soluble in a 5 per cent. soda solution, and gives no trace of a precipitate with hydrochloric acid. On heating for three hours more at 280° it is converted into a black brittle mass, insoluble in both water and alkalies, but yielding humic acid on fusing with caustic soda and precipitating with hydrochloric acid. This black mass is evidently very similar to humin.

Laevulose even at the low temperature of 120° forms water-soluble substances which give a granular precipitate with hydrochloric acid if the heating be continued long enough, about six hours. When heated for two hours at 150° it gives a dark chocolate-brown mass all of which is soluble in water, giving a typical flocculent humic acid precipitate. Heated for two hours more at 180° it forms chiefly alkali-soluble humic acid, and further heating at 200° for two hours converts a large proportion of this into humin.

Here again, as in the acid-formed substances, humic acid is antecedent to humin. One important point of difference, however, must be noted between the acid-formed and heat-formed humic acid. The heat-formed humic acids after being dried in a water bath at 100° are no longer soluble in dilute alkali solutions; only on fusing with caustic potash or soda are they rendered soluble.

HUMIC BODIES FROM PROTEINS.

Proteins are said on hydrolysis to yield "an insoluble brownish black residue of humin." A careful examination of this black residue shows that it is chiefly composed of humic acid and contains very little humin. That

the formation of this humic acid depends on the amount of carbohydrate present in the protein is seen by comparing the amount of humic acid obtained from proteins with varying content of carbohydrate. Mucin and ordinary egg white are rich in carbohydrates; caseinogen and purified egg white are practically free from carbohydrates. One gram of each of these substances, the dry weight of the ordinary egg white being calculated by a moisture determination, was boiled for eight hours under a reflux condenser with 33 cc. of a 7·5 per cent. solution of hydrochloric acid. The contents of each flask were filtered, the residue extracted with a 5 per cent. solution of caustic soda, and the extract acidified with hydrochloric acid. The precipitate of humic acid was filtered, washed, dried and weighed, with the following results:

Mucin	0·021 g.	Caseinogen	0·001 g.
Ordinary egg white	0·035 g.	Purified egg white	no precipitate.

A similar experiment with tyrosine and asparagine gave no trace of humic acid after eight hours' boiling.

CONCLUSIONS.

The general result of the investigation so far has been to indicate that carbohydrates generally and certain sugars in particular pass through a regular series of changes when submitted to the reactions described. It was also found that air-dried *sphagnum* moss is readily acted on by acids, with the formation of a brown peat-like mass from which humic acid can be extracted.

One hundred grams of air-dried *sphagnum* moss when boiled for 24 hours with 5 per cent. solutions of hydrochloric, oxalic and lactic acids respectively yielded the following results:

Hydrochloric acid	11·7 g. humic acid
Oxalic acid	2·3 g. „
Lactic acid	1·8 g. „

The humic substances comprising the humic acid and humin groups probably pass through a series of changes characterised by a progressive increase of their carbon content. In ordinary cultivated soils these changes cannot be traced owing to the constant addition of fresh organic matter, and cultural operations, but in peat beds, where the deposits remain undisturbed for many years, the stages are indicated by the increasing carbon content of the humus from varying depths. This was shown by Detmer [1871], who gives the following figures:

	C	H	O
Light brown peat, surface	52·14	7·03	40·19
Brown peat, 1 foot	57·75	5·43	36·02
Dark peat, 7 feet	62·02	5·21	30·67
Black peat, 14 feet	64·07	5·01	26·87

Thus in peat bogs the carbohydrates of decaying organic matter may possibly pass through changes very similar to those observed when sugar is heated to a high temperature: sugar, caramelan, humic acid, humin, and finally carbonisation (peat coal). The fact established by Van Bemmelen [1900] that humic acid is a colloid body which has the property of uniting with certain radicles explains the various empirical formulae ascribed to humic acid, and the complex nature of humus in general. But underlying this complexity there is the possibility of finding in the series of sugar changes described above two fairly definite groups of substances which serve as a basis for humus formation, the humic group gradually merging into the humin group.

SUMMARY.

1. Sugars on boiling with hydrochloric acid yield a mixture of humic acid and humin bodies, varying in proportion with the different sugars used.
2. The composition of "natural" humic acid from soil or peat, after purification by alcohol, approximates very closely to that of "artificial" (sugar) humic acid.
3. Humic acid and humin are also produced from sugars by the action of various organic acids: lactic, acetic, propionic, butyric, etc.
4. The action of heat alone on sugars produces humic acid and humin bodies, these substances being stages in the process of carbonisation.
5. Humic bodies are not obtainable from proteins free from carbohydrates.
6. The two groups of humic bodies—humic acid and humin—obtained artificially from carbohydrates, indicate a basis for the natural processes of humus formation.

REFERENCES.

- Baumann (1909). *Mitt. k. bayr. Moorkulturst.*, Heft 3, 103.
 Bemmelen (1900). *Zeitsch. anorg. Chem.*, 23, 321.
 Conrad and Guthzeit (1885). *Ber.*, 18, 443.
 Detmer (1871). *Landwirtsch Versuchs-Stat.*, 14, 248.
 Hermann (1842). *J. pr. Chem.*, 25, 189; 27, 165.
 Hoppe-Seyler (1889). *Zeitsch. physiol. Chem.*, 13, 62-120.
 Malaguti (1858). *Ann. Chim. Phys.*, 54, 407.
 Mulder (1849). *The Chemistry of Vegetable and Animal Physiology*. Trans. by Fromberg.
 Robertson, Irvine and Dobson (1907). *Biochem. J.*, 2, 458.
 Saussure (1804). *Recherches chim. sur la végétation*.
 Schreiner and Shorey (1910). *U.S. Dept. Agric. Bur. of Soils, Bulletin* 47.