and further work will be necessary to elucidate the exact mechanism of all the processes occurring.

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- ¹ G. Cario and J. Franck, Zt. Physik., 11, 161 (1922).
- ² Dickinson, Proc. Nat. Acad. Sci., 10, 409 (1924).
- ³ Cario, Zt. Physik., 10, 185 (1922).
- ⁴ K. Donat, Zt. Physik., 29, 345 (1924).
- ⁵ Herbert Stuart, Zt. f. Physik., 32, 262 (1925).
- ⁶ Loria, Phys. Rev., 25, 212 (1925).
- ⁷ Compton and Turner, Phil. Mag., 48, 360 (1924).

WHAT IS HUMUS?1

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Several theories have been proposed at various times to explain the origin of the black-colored organic substances, which go to make up the bulk of the soil organic matter and which are commonly known as "humus." None of these theories, however, sufficiently explained processes involved in the formation of the complex mass of soil organic matter, to be universally accepted. The very nature of the chemical constituents of this "humus" is little understood, although an extensive literature concerning some of these constituents, namely the "humic acids," is available. number of methods have been suggested for determining quantitatively this soil "humus" and "humic acid," since this was usually considered to be the available portion of the soil organic matter. However, none of the methods proved satisfactory; the results obtained for the same soil by different methods varied greatly, and we are still in the dark concerning the nature of the soil organic matter, its formation, and physical and chemical properties. Schreiner and Shorey, in summing up in 1909 the survey of the literature on this subject, came to the conclusion that "the most conspicuous feature is the lack of specific knowledge concerning the organic matter of the soil." Since then, new theories and new methods have been proposed, but even at present "the complete lack of knowledge concerning the nature of soil organic matter, the indefinite limit between the humus and the rest of the organic matter remains the weakest spot in soil science both in classification of soils as well as in a number of other questions" (Gedroiz).

This lack of specific knowledge concerning the most important constituent of normal soils is largely due to the fact that very few attempts have been made to study just how "humus" is formed in the soil. When organic matter, in the form of green manure, plant stubble, straw, leaves, roots, etc., is added to the soil, decomposition sets in immediately, as can be conveniently demonstrated by an increase in the evolution of carbon dioxide. The rate and nature of decomposition depend upon the organisms concerned and soil environmental conditions. Sooner or later the rate of decomposition becomes more or less uniform, after the easily available ingredients of the organic matter are decomposed; the residual organic matter then becomes a part of the soil, is converted into "humus" or is said to be "humified." This "humus" decomposes only very slowly and will persist in normal soils for considerable time. Why does this take place, why does a part of the organic matter decompose rapidly and a part only very slowly? This question is of great economic importance, since most/ of the nitrogen and minerals introduced into the soil with the natural organic materials remain bound up in this soil "humus." How does that take place? Is "humus" formed because the organic substances are decomposed so far and no further, or because certain constituents of the original organic matter are decomposed and others not, or because certain resistant substances have been synthesized by the microorganisms active in the decomposition of the original organic matter?

The chemist has analyzed the soil organic matter by ignition, by extracting with alkalies, by treating the soil with different reagents. He considered the soil organic matter to be in a static condition, and has sometimes been led to conclude (Gortner) that "humus" is not formed in the soil, but that similar extracts can be obtained from natural organic substances. The biologist has limited himself to the general statements that the various groups of soil microörganisms take an active part in the formation of the soil "humus," but how and to what extent remained unknown. It has even been pointed out (Falck) that the nature of the soil organic matter, resulting from the decomposition of the natural organic substances added to the soil, depends entirely upon the character of the organisms taking part in the decomposition; the latter are in their turn influenced to a large extent by environmental conditions. However, experimental facts, which would establish definitely what constituents of the natural organic matter contribute to the formation of "humus" or "soil organic matter," and by what chemical reactions this takes place, are lacking; this accounts largely for the lack of knowledge concerning the nature of the soil "humus" and its various constituents, such as the "humic acids."

Among the various theories suggested to explain the formation of "humic acids" that of Fischer and Schrader deserves careful study. They have demonstrated that soil "humus," peat, and soft coal are probably derived from the lignins, which form a considerable part of most of the natural organic substances. The lignins are most resistant to decomposition; they are not acted upon at all, or only to a very limited extent, by the great majority of soil microörganisms. Fischer suggested, therefore, that the celluloses, pentosans, and other carbohydrates, proteins and their derivatives, and various other cell constituents are decomposed sooner or later by different soil microörganisms, while the lignins and fats remain undecomposed. The accumulation of these substances results in the formation of the soil "humus" as well as of peat soils and coal. This theory may account for the formation of coal, but it does not fully account for the formation of the soil "humus," due to several reasons: (1) It does not explain the origin of the presence of a considerable amount of nitrogen in the soil "humus," which is a highly important constituent from the point of view of soil fertility. (2) It does not consider the fact that in normal soils the ratio of the carbon to the nitrogen is always constant, namely about 10 to 1. (3) It does not allow for the rôle played in the formation of organic matter in the soil by the numerous microörganisms which inhabit the soil; as much as 20-40 per cent of the organic matter decomposed as a source of available energy may be resynthesized into microbial proto-(4) It does not consider the fact that even the resistant soil "humus" is gradually decomposed, as can readily be demonstrated, when the soil is kept under optimum conditions, without the addition of fresh organic matter; constant additions of organic substances to cultivated soil may be just sufficient to keep up the balance of the soil organic matter. (5) There is undisputed proof (Gortner, Schreiner and Shorey, S. Oden) that the soil organic matter consists of several fractions: (a) one insoluble in water and alkalies (so-called "ulmin" and "humin"), (b) one soluble in alkalies, but insoluble in alcohol ("humic acid," according to S. Oden), (c) one soluble in alkalies and in alcohol ("hymetomelanic acid" of Hoppe-Seyler), (d) one soluble in water ("fulvic acid" of S. Oden). Gortner found a dark and light fraction in the portion soluble in alkalies, depending on the nature of the alkali used for extraction.

In approaching this question, an attempt has first been made to learn not what "humus" is, but how it is formed in the soil; what chemical substances and what microbiological agencies, as influenced by environmental conditions, contribute to its formation. The chemical and physicochemical nature of the "humus" would then become better understood. These studies will be published in detail at a later date. It is sufficient to call attention here to a few results which tend to throw some light upon this complex subject.

The soil organic matter or "humus" can be conveniently divided (similar to S. Oden's classification) into three general groups, on the basis of its solubility in alkalies and in water: (a) The part insoluble in alkalies, as 5 per cent NaOH; this includes most of the undecomposed plant residues, such as celluloses, pentosans, some proteins, etc., and certain synthesized substances, such as chitin. (b) The part soluble in alkalies and precipitated by acids; this comprises 60 to 70 per cent of the organic matter of normal soils and includes the various "humic acids." (c) The part soluble in alkalies and not precipitated by acids; this part includes the water soluble portion of the soil organic matter and the part made soluble by the hydrolytic action of the NaOH. The second part is the most important and most interesting. It is usually determined either directly in the alkaline extract (in that case the third portion of the soil organic matter was also included) or it is precipitated with acids and then determined either gravimetrically or colorimetrically. When the alkaline extract of normal mineral soils is carefully neutralized by hydrochloric acid, a heavy precipitate is formed; a part of this precipitate is redissolved when an excess of acid is added. The soil organic matter dissolved by the alkali treatment can thus be conveniently divided into two definite fractions; a lack of recognition of these fractions led often to a great deal of confusion in the results obtained both gantitatively and qualitatively. The two fractions which can be separated from the portion of the soil organic matter soluble in alkali and precipitated by acid, are (a) fraction α , soluble in dilute alkalies and insoluble in dilute acids and (b) fraction β , soluble both in dilute alkalies and in dilute acids and precipitated at a more or less definite isoelectric point, which lies close to pH 4.8.

The method as used at present is carried out as follows: 50 gm. of fresh soil (the moisture content of which is known) are treated with 100 cc. of 5% sodium hydroxide solution (for 48 hours in the cold or for 30 minutes at 15 pounds pressure). If the soil bases are to be first removed, it should be done not by washing with dilute hydrochloric acid, since this removes a part of the β fraction, but by washing with a normal or half-normal solution of KCl, then washing with water; a somewhat stronger NaOH solution is then added to allow for the water left in the soil. The alkaline extract is filtered through ordinary filter paper and the soil is washed 2 or 3 times with distilled water. The residual moist soil is then again extracted in the same manner, by adding 25 cc. of 5% NaOH solution to the moist The second filtrate and washings are added to the first. An excess of hydrochloric acid (1:1) is then added to the black extract; a brown precipitate is formed which becomes darker on further addition of acid; an excess of acid is used for the purpose of redissolving the β fraction. mixture is well shaken and the precipitate is filtered through a weighed Gooch crucible or a weighed filter paper; it is washed with dilute hydrochloric acid, then several times with distilled water and dried to constant weight, giving the α fraction. This fraction contains practically no ash (only about 0.5 to 3 per cent); it is comparable to the "humic acid" obtained by several methods, and it accounts largely for the black color of the original solution; it is comparable to the "humus" which would result according to Fischer's theory, since it is extracted by a method similar to that used for extracting lignin; however, it contains about 2.5 to 3.5 per cent nitrogen, independent of the soil from which it has thus far been obtained, unless it is a soil with a large amount of fresh undecomposed organic material. This fraction α dissolves completely in alkalies and is reprecipitated quantitatively by acids, without any marked change in its composition.

When the brown solution, obtained on filtering off the α fraction, is neutralized with sodium hydroxide, another precipitate is formed; this precipitate (fraction β) redissolves both in an excess of alkali and in an excess of acid; it comes down quantitatively at a certain hydrogen-ion concentration, near to pH 4.8, the precipitation beginning at both sides of this point. This is also filtered off through a Gooch crucible or weighed filter paper; the filtrate should be of a light straw color and only seldom, as in the case of soils very rich in organic matter such peat soils, yellow to brown colored; if the solution is yellow, the addition of either acid or alkali to adjust the reaction to pH 4.8 will result in the formation of a further precipitate which should be added to the rest of fraction β . The combined precipitate is washed with distilled water until free from soluble salts. It is then dried, weighed and ignited. Fraction β is found to contain 30 to 50 per cent ash and a small amount of nitrogen (about 1 per cent).

These two fractions were found to be derived in the soil by different processes and probably play in the soil different functions. The α -fraction or the "humic acid," which gives the black color to the soil, is derived largely from the lignins of the natural organic substances and from the cells of the microorganisms in the soil; it contains the largest amount of nitrogen. It is the dominant and often the only fraction in soil in which organic matter accumulates in large quantities, as in peat soils and forest mold, and probably accounts for the non-availability of the large store of the soil nitrogen. The β fraction gives to the soil its buffering properties; it accounts to a large extent for the various phenomena usually ascribed to the indefinite "organic soil colloids;" it combines with the acids and alkalies added to, or formed in the soil preventing them from becoming too injurious to the growth of plants and microörganisms. This fraction is very changeable in quantity, increasing or decreasing according to soil conditions. On redissolving the β -fraction in an alkali solution, a small amount of inorganic material may remain insoluble; on filtering the solution and reprecipitating the β -fraction by adjusting with acid to pH 4.8, a straw-colored solution is

obtained; the precipitate is washed with water, dried, weighed and ignited. The organic matter is here also recovered practically quantitatively.

The results given in table 1 have been obtained from three differently treated plots of a Sassafras soil and one peat soil.

TABLE 1
"Humus" Content of the Soil

	Treatment	α-FRACTION			β-FRACTION			
NO.		TOTAL NITRO- GEN	TOTAL, IN 100 GM. OF SOIL	NITRO- GEN CON- TENT	ASH CON- TENT	TOTAL IN 100 GM. OF SOIL	NITRO- GEN CON- TENT	ASH CON- TENT
		PER CENT	MGM.	PER CENT	Per Cent	MGM.	PER CENT	Per Cent
5A	Manured every year but unlimed	0.134	725	3.34	1.2	1.717	1.34	47.1
7A	Unmanured, unlimed	0.074	368	3.57	1.1	1.841	1.00	48.5
5B	Manured and limed	0.128	631	3.20	1.4	1.820		44.9
	Florida muck soil		31,500	3.95	1.4	Trace		

The α -fraction represents a part of organic matter which accumulates in the soil, as a result of constant addition of fresh organic materials, such as plant roots and stubble, green manures, and stable manure; it can be decomposed, but only very slowly and by a very limited number of soil microörganisms, largely actinomycetes and a few non-spore forming bacteria. The origin of the β -fraction is still unknown. The fact that peat soils contain largely the α -fraction and mineral soils contain, in addition to the α , also the β fraction, often in even larger amounts, point to the distinct differences in the nature of organic matter in peat and mineral soils, a fact overlooked by most investigators on "humic acids."

A knowledge of these two fractions (α and β) of the soil organic matter, which accounts quantitatively for the larger part of the organic matter and the nitrogen in the soil and which probably play distinct rôles in soil processes, and a better understanding of the origin of these two fractions in the soil, seem to point to a clearer understanding of soil phenomena.

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Schreiner, O., and Shorey, E. C. The Isolation of Harmful Organic Substances from Soils. Bull. 53, Bur. of Soils, U. S. Dept. Agr. (1909).

Gedroiz, K. K. Soil Absorbing Complex and Absorbed Soil Cations as a Basis of Genetic Classification of Soils. Leningrad. (1925.)

Gortner, R. The Organic Matter of the Soil. I. Some Data on Humus, Humus Carbon and Humus Nitrogen. Soil Sci., 1, 395-442 (1916).

Falck, R. Mykologische Untersuchungen und Berichte. Gebr. Gotthelft, Cassel. vol. 2 (1923).

Fischer, H., and Schrader, H. Über die Entstehung und die chemische Struktur der Kohle. *Brennstoff. Chem.*, **2**, 37–45 (1921).

Fischer, H. Was lehrt die Chemie über die Entstehung und die chemische Struktur der Kohle. *The Naturwiss.*, **9**, 958-965 (1921).

Oden, S. Die Huminsäuren. Kolloidchem. Beihefte., 11, 75-260 (1919).