either in gross or percentage amount. In the value of the rest nitrogen individual peculiarity is an important factor.

There appears to be no relation between the amount of meat in the diet and the indican excretion. To some extent this excretion is an individual peculiarity.

A rather close relation seems to exist between creatinine and neutral sulfur. Both vary in percentage amount in essentially the same manner with the change from low to high protein in the diet. It is possible that a considerable part of the neutral sulfur, like the creatinine, may be of endogenous origin.

Tables are given showing the relation of the acid to the basic ions, expressed on the hydrogen scale, and a comparison is made between the apparent acidity and the titration acidity.

NORTHWESTERN UNIVERSITY MEDICAL SCHOOL,
June, 1912.

[CONTRIBUTION FROM LABORATORY OF SOIL FERTILITY INVESTIGATIONS.]

THE CHEMISTRY OF STEAM HEATED SOILS.[1]

BY OSWALD SCHREINER AND ELBERT C. LATHROP.

Received July 1, 1912.

The effect which heat has upon soils is a subject rather prominently before agricultural investigators at the present time. This subject has received increasing attention during the past decade, prominently from the point of view of sterilization and partial sterilization. There has been much valuable information obtained along the biological lines concerned. The biological factors cannot and should not be ignored, but it must be remembered that they are only of significance or interest in the light of the biochemical changes which they produce in the soil. Moreover, any chemical changes produced in the soil through other means, be they cultural or by the addition of manures and fertilizers, or by the influence of steam heating, in turn affect the biological activities. The chemistry, therefore, of the soil both before and after heating in sterilization work becomes of the greatest significance to the biological worker.

Since heat activates the changes going on normally in soils, it is obvious that a study of heated soils also throws light upon the biochemical changes taking place in soils under field conditions. The present paper is a contribution to the knowledge concerning soil organic matter and the changes which it undergoes. Notwithstanding the fact that some of the results obtained by some of the investigators on this subject[2] are contradictory,

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[1] Published by permission of the Secretary of Agriculture.

[2] In this connection see: Franke, *Ber. botan. Ges.*, 6, 89 (1888); Liebscher, *Deut. Landw. Presse*, 20, 976 (1893); Schmoeger, *Ber.*, 26, 386 (1893); Pfeiffer and Franke, *Landw. Vers.-Sta.*, 46, 117 (1896); Dehérain and Demoussy, *Ann. agron.*, 22, 305 (1896); Richter, *Landw. Vers.-Sta.*, 47, 269 (1896); Schulze, *Jahresber. Ver. Vertreter angew.*

especially in regard to the effects of steam sterilization on crops, they seem to warrant the following conclusions:

By steam heating the physical, chemical and physiological properties of the soils are more or less altered. The chemical changes consist in the increase in soluble matter in the heated soils. This is partially of an inorganic nature, potash and phosphoric acid being mainly determined, but the largest increase is in the organic matter rendered soluble. Ammonia is formed by the reduction of the nitrates to nitrites and ammonia, but especially from the decomposition of organic matter. Large amounts of nitrogenous matter are made soluble and apparently more available for plant use, and carbon dioxide is produced in large quantities.

By partial sterilization the larger phagocytic microörganisms which are said to limit the activity, in normal soils, of ammonifying bacteria, are killed. The nitrifying bacteria do not grow on the freshly sterilized soils, there apparently being compounds formed which are of an inhibitory nature, but which disappear after a time. By complete sterilization all life in the soil is killed and reinoculation must be resorted to.

With regard to crop production, in general, the sterilized soils seem to contain some substance which is decidedly injurious both to germination and to plant growth; this is particularly so if calcium carbonate is not present in excess during the heating to prevent the formation of acids. After standing for some time, and especially after planting, these injurious effects weaken and finally disappear, with the result that the growth on the heated soil is greater than that on the normal soil, the plants showing large increases in dry weight and in the amounts of plant nutrients absorbed by them.

The nature of the chemical changes observed by these investigators is not made clear, although a number of suggestions or hypotheses have been put forward. These concern themselves partly with the explanation of the increase in mineral constituents, due to altered absorption qualities

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Bot., **1**, 37 (1903); *Centr. Bakt.*, **2**, Abt. II, 716 (1903); Hiltner and Störmer, *Arbeit. Biol. Abt. Landw. Forstrwirtschaft*, **5**, Heft 3 (1903); Dietrich, *Jahresber. Landw. Vers. Marburg* (1901–02), p. 16; *Bied. Centr.*, **32**, 68 (1903); König, Coppenrath, *et al.*, *Landw. Vers.-Sta.*, **63**, 471 (1906); **66**, 401 (1907); Kassaroff, *Arb. Kais. Biol. Anstalt. Landw. Forstrwirtschaft*, **5**, 126 (1906); Darbishire and Russell, *J. Agric. Sci.*, **2**, 305 (1907); Loew, *Porto Rico Agric. Exp. Sta. Cir.*, 11, 3 (1906–08); Loew and Aso, *Bull. Col. Agric. Tokio*, **7**, nos. 3, 5 (1907); Pickering, *J. Agric. Sci.*, **2**, 411 (1908); **3**, 32 (1908); Russell and Hutchinson, *ibid.*, **3**, 111 (1909); Russell, *Sci. Progress*, **4**, 353 (1910); Seaver, *Mycologia*, **1**, 131 (1909); Seaver and Clark, *ibid.*, **2**, 109 (1910); Lyon and Bizzel, *J. Soc. Chem. Ind.*, **28**, 721 (1909); *Cornell Agric. Exp. Sta. Bull.*, 275 (1910); Rudd, *Am. Florist*, **9**, 171 (1893); Stuart, *Vermont Agric. Exp. Sta. Rep.* (1905), p. 297; Selby, *Ohio Agric. Exp. Sta.*, *Bull.* **73**, 231 (1896); May, *Am. Florist*, **13**, 721 (1898); *Farmer's Bull.*, **259**, U. S. Dept. Agric.; Stone and Smith, *Mass. Agric. Exp. Sta. Rep.* (1901), p. 57; *ibid.*, (1902), p. 38; Stone and Monohan, *ibid.* (1904).

for these constituents brought about by heat and by the greater hydrolysis or solubility of the minerals under the conditions of higher temperature. Nearly all have particularly noticed the greater increase in soluble nitrogen and coincident with this the increase in soluble organic matter. It is these latter observations that have been the source of most of the speculations to explain the increase and decrease, as the case may be, of plant growth on soils thus heated. These views are usually coupled with the biological idea which furnished the motive for the experiment.

In no case has any definit compound been obtained, either as an end or intermediary product of the changes brought about by heat, and the subject is left in the same state of indefinit generalizations in which soil organic matter or humus found itself in the course of the past half century. In view of this lack of knowledge concerning the chemistry of soil organic matter itself, and its transformations under ordinary conditions, it is not to be expected that changes like the above could have been clearly defined. As a consequence of this lack of knowledge concerning soil organic matter, these investigators had to confine themselves to the use of simple analytical methods, such as the determination of ammonia formed, the determination of the quantity of organic matter in water or dilute acids, and the amount of nitrogen in organic combination contained therein. In addition to the above, there was also usually made a determination of total soluble solids, and of phosphoric acid and potash, the other principal plant nutrients, besides the nitrogen.

The investigations of the past few years have brought with them an increasing knowledge of the nature of organic matter, especially with regard to the isolation and identification of specific chemical compounds from soils, so that the biochemical changes taking place in soil are becoming clear. In connection with these investigations, methods for the recognition or isolation of specific chemical compounds have been developed. With such knowledge at hand, it seemed possible to go one step farther and show the changes produced in soil organic matter in the process of sterilization by heat, and thus be able to interpret the nature of the changes.

The Soils Studied.

The soils used in this investigation are two types of the Coastal Plains Soil Province known as Elkton silt loam and Sassafras silt loam. These two soils were collected from the same farm, about four miles east of Easton, Md., April, 1911. The samples taken were from several spots within a radius of 40 feet in each case and were collected in adjoining fields within 150 yards of each other.

The Elkton soil was in clover, following wheat the year before, which had followed corn. The corn had yielded about 15 bushels to the acre, wheat about 20 bushels and the clover was very poor. The soil was very moist and plastic and was collected from several spots upon which nothing was growing. The field was full of such spots. The Sassafras soil was collected in the next field, which had been plowed but not harrowed, and the soil was in excellent condition. Corn last year yielded 50 bushels, and the year before wheat had given about 20 bushels to the acre.

Although these soils have the same geological origin, they are rather widely different in physical and chemical characteristics, and in fertility. The differentiation is apparently due to drainage conditions. While the two soils are very distinct in color, and in general agricultural value, the change from one to the other is frequently so gradual that it is difficult to draw an exact boundary. The samples taken are strictly typical of the types.

Analysis shows that these two samples of the soils contain practically the same amount of organic matter, the Elkton 2.25% and the Sassafras 2.24%, the former contains 0.13% of carbonates, expressed as carbon dioxide, and the latter only 0.03%. The two soils are both acid to litmus paper, the Sassafras being slightly the more acid of the two. In regard to their oxidizing and their catalyzing power, that is, the decomposition of hydrogen peroxide, they are both very weak, the Elkton showing none at all and the Sassafras only a little. They both contain practically the same percentage of total nitrogen. When the soils are boiled with hydrochloric acid and the different forms of nitrogen determined according to the method of Osborne and Harris,[1] which was applied to soils by Shorey,[2] the soils show only trifling differences. From the table it is apparent that the only difference worthy of mention is the fact that the Sassafras soil contains slightly more ammonia than does the Elkton soil, but this is much too small to account for the differences in fertility.

TABLE I.—DIFFERENT FORMS OF NITROGEN IN ELKTON SILT LOAM AND SASSAFRAS SILT LOAM. RESULTS EXPRESSED IN PER CENT. OF THE TOTAL NITROGEN IN OVEN DRIED SOIL.

Form of nitrogen.	Elkton.	Sassafras.
Per cent. of total N in soils	0.0859	0.0869
Total N in solution	91.83	91.08
N insoluble in HCl	8.17	8.92
N as NH_3	0.83	2.27
N as amides	12.06	12.45
N as "humin" N	18.96	19.63
N as diamino acids	5.53	7.17
N as monoamino acids	54.45	49.56

From the foregoing analytical data it is apparent that, in a general way, these two soils are very similar in their organic composition, and that no methods of analysis, excepting the actual isolation of the various

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[1] THIS JOURNAL, **25**, 323 (1903).

[2] Agr. Invest. Hawaii in 1905, *Rep. Chemist*, p. 34; see also Lathrop and Brown, *J. Ind. Eng. Chem.*, **3**, 657 (1911).

definit compounds which compose the organic matter, will show wherein this difference in their organic composition lies.

The methods of farm practice have been identical with these soils, and each has received the same kind of debris, manures, plant residues, etc., which by processes of decomposition go to form the organic matter of soils. If therefore differences in the organic matter of these soils are shown to occur, they must be the result of different processes of decomposition in the soils.

The Method of Heating.

For the purpose of heating the soils an autoclave such as is in general use was employed. The autoclave is of copper, so constructed that it may be automatically kept at 30 lbs. steam pressure for a given length of time. The soils were heated in one-gallon stoneware jars placed in the autoclave in a metallic rack, the jars standing one above the other, the lower one being about 4 to 5 inches above the bottom of the autoclave. These jars held approximately 10 lbs. of soil. The soils were heated at 30 lbs. pressure for 3 hours, at a temperature of about 135°. The soils were treated with alkali just as soon as they had cooled to room temperature, so that only the changes caused by the immediate heating were under observation.

Method of Extraction.

Owing to the small amounts of organic matter in these soils and also to the fact that not all of this organic matter can be extracted, large amounts of soil were worked upon. Generally 40 to 50 lbs. of soil, both heated and unheated, were extracted with 2% sodium hydroxide solution. No attempt was made to study the humus precipitate. The chief interest in the work centered about the nitrogen compounds, especially those rendered soluble by the heating of the soil. Such compounds would appear chiefly in the alkalin extract, and later in the acid filtrate from the humus precipitate.

Experimental Results.

In harmony with the observations of other investigators, the total amount of water soluble solids was increased by the heating. The soils by the bridge method showed the following content of total soluble solids: The normal Elkton soil, 184; the heated Elkton soil, 336; the normal Sassafras soil, 151; the heated Sassafras soil, 270 parts per million of dry soil. It will be noticed that the poorer soil showed the higher content in both heated and unheated conditions. No further examination of the saline material was made.

By heating, the acidity of both soils was increased toward litmus paper. It should be noted, however, that ammonia and amines were also formed in the process of heating, as was indicated by the fishy odor upon adding the alkali in the process of extraction.

The results obtained in isolating and identifying the organic constituents extracted from the heated and unheated soils are concisely given in Table II.[1]

TABLE II.—ORGANIC COMPOUNDS ISOLATED FROM HEATED AND UNHEATED SOILS.

Elkton silt loam.		Sassafras silt loam.	
Unheated.	Heated.	Unheated.	Heated.
Nucleic acid	Nucleic acid	Nucleic acid	Nucleic acid
Xanthine	Xanthine	......	Xanthine
......	Hypoxanthine	Hypoxanthine	Hypoxanthine
Adenine	Adenine	......	......
......	......	......	Guanine
......	Cytosine	......	Cytosine
Pentose sugar	Pentose sugar	Pentose sugar	Pentose sugar
Pentosans[2]	Pentosans	Pentosans	Pentosans
Histidine	Histidine	Histidine	Histidine
......	......	......	Arginine
Creatinine	Creatinine	Creatinine	Creatinine
Dihydroxystearic acid	Dihydroxystearic acid	......	Dihydroxystearic acid
......	Unidentified aldehyde	......	Unidentified aldehyde

The results in Table II show only whether the organic compound was present or not. While no quantitative methods for the separation of these compounds from soils exist, owing to a great variety of difficulties which will not be discussed here, still, in the work it was possible to distinguish between very small amounts and larger ones. As seen from the table, the nucleic acid was found in both soils, in the heated as well as in the unheated samples.

Xanthine was found in both heated and unheated Elkton soil, but in larger quantity in the heated soil. In the case of the Sassafras soil it was found only after heating.

Hypoxanthine was not found in the Elkton soil, but after heating a small amount was obtained. The Sassafras soil contained this constituent, but its quantity was much increased by heating the soil. In both cases the heating increased the amount which could be obtained.

Adenine was found in small amounts in the Elkton soil, but was not obtained from the Sassafras. Heating effected no change in either case.

Guanine was found only in the Sassafras soil after heating, and is of

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[1] For the methods of isolating and identifying the compounds dealt with in this paper, see "The Isolation of Harmful Organic Substances from Soils," by Oswald Schreiner and Edmund C. Shorey, *Bull.* 53, Bur. Soils, U. S. Dept. Agric. (1909); "The Chemical Nature of Soil Organic Matter," by Oswald Schreiner and Edmund C. Shorey, *Bull.* 74, *ibid.* (1910), and "Examination of Soils for Organic Constituents, Especially Dihydroxystearic Acid," by Oswald Schreiner and Elbert C Lathrop, *Bull.*, 80, *ibid.* (1911).

[2] From the pentosans of this soil, xylose was obtained, showing that it was, at least in part, xylan.

especial interest since this is the first time it has been encountered in soil.

Cytosine is likewise a decomposition product of nucleic acids, and it is interesting to note that it was found only in the heated samples of both soils. It was obtained in much larger amounts in the case of the heated Sassafras soil than in the heated Elkton soil.

Pentose sugar was indicated in both soils, heated and unheated. Pentose sugars have been found, however, in every soil tested in this laboratory, which is also the case with pentosans.

Histidine occurs in both soils and a slight increase was noticed as the effect of the heat.

Arginine, on the other hand, was found only in the heated Sassafras soil.

Creatinine was present in all cases.

The unidentified aldehyde in the above table contains a benzene ring and is possibly salicylic aldehyde. It was found in small amounts, but only in the heated soils, and may, therefore, be assumed to be formed during the process of heating.

Dihydroxystearic acid occurs in the Elkton soil but not in the Sassafras soil, as has been reported in a special study of the distribution of this compound in soils.[1] The present samples of the two soils, from the same field, but collected two years later, show this same relationship, indicating that the compound is a rather constant constituent of the Elkton soil under the field conditions to which it has been subjected.

The heated Elkton soil showed an increase in the amount of dihydroxystearic acid. While no dihydroxystearic acid exists in the sassafras soil under normal conditions, it is significant that upon heating a very considerable amount of dihydroxystearic acid was isolated, an amount even larger than that found in the unheated Elkton soil, and fully as large as in the heated Elkton soil.

Nucleic Acid Degradation.

While it is not the purpose to discuss here fully all the changes which are involved in protein decomposition, it is, however, necessary to partially discuss certain phases of this subject related to this investigation, so that a clear conception of the changes observed in the course of the investigation may be obtained.

There get into the soil continually such complex organic compounds as proteins, lecithins, nucleic acid, nucleoproteins. While the final products of decomposition of these are no doubt such simple combinations or elements as carbon dioxide, marsh gas, ammonia, nitrogen, hydrogen, these products are arrived at by certain definit and fairly well understood stages of degradation. Depending on the factors present under various

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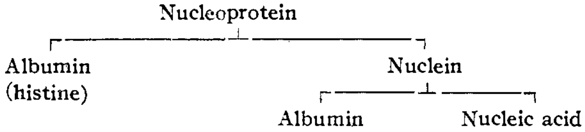
[1] Schreiner and Lathrop, THIS JOURNAL, 33, 1412 (1911).

soil conditions, this process may be a rapid one or a slow one, or one entirely arrested at certain stages. This may be due to the fact that some of the factors necessary to accomplish the next change in the process of degradation are absent.

Nucleoproteins are perhaps the most complex of the compounds which enter the soil. They are common constituents of plants, animals, bacteria and molds, and occur, therefore, wherever these occur. It will be well to trace such a complex compound through the chemical changes which it suffers by decomposition. Lilienfeld[1] has expressed the decomposition of the nucleoprotein by the following schematic representation:



The products then are albumin or protein and nucleic acid. Protein material has not been isolated as such from soils, but that it is present is indicated by the production of its decomposition products, as well as by the occurrence of these products in soils under normal conditions. The isolation of nucleic acid from soils, on the other hand, has been accomplished.

The nucleic acid is still a very complex compound and on decomposition yields a large number of compounds of somewhat less complicated nature. The name nucleic acid is a class name, covering a large number of compounds composed of carbon, hydrogen, oxygen, nitrogen and phosphorus. It occurs in plant and animal cells and has been isolated from soils.[2]

The proportion of the above elements varies, according to the source of the nucleic acid, due to the fact that the unit substances composing it are different. Thus, for instance, the nucleic acid from yeast, according to Herlant,[3] has the composition $C_{36}H_{48}O_{30}N_{14}P_4$, and that from salmon, according to Schmiedeberg,[4] the composition $C_{40}H_{52}O_{25}N_{14}P_4$, that from wheat, according to Osborne and Harris, the composition[5] $C_{41}H_{61}O_{31}N_{16}P_4$.

The constitution of these complex nucleic acids is unknown, but the constitution of most of the dissociation products is known. As above mentioned, these dissociation products vary according to the source of the nucleic acid. These degradation products are: Phosphoric acid, which is always found; carbohydrates, always found; levulinic acid, sometimes found; pyrimidine derivatives, always found; purine bases,

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[1] *Arch. Anat. Physiol.*, 1892, p. 128.

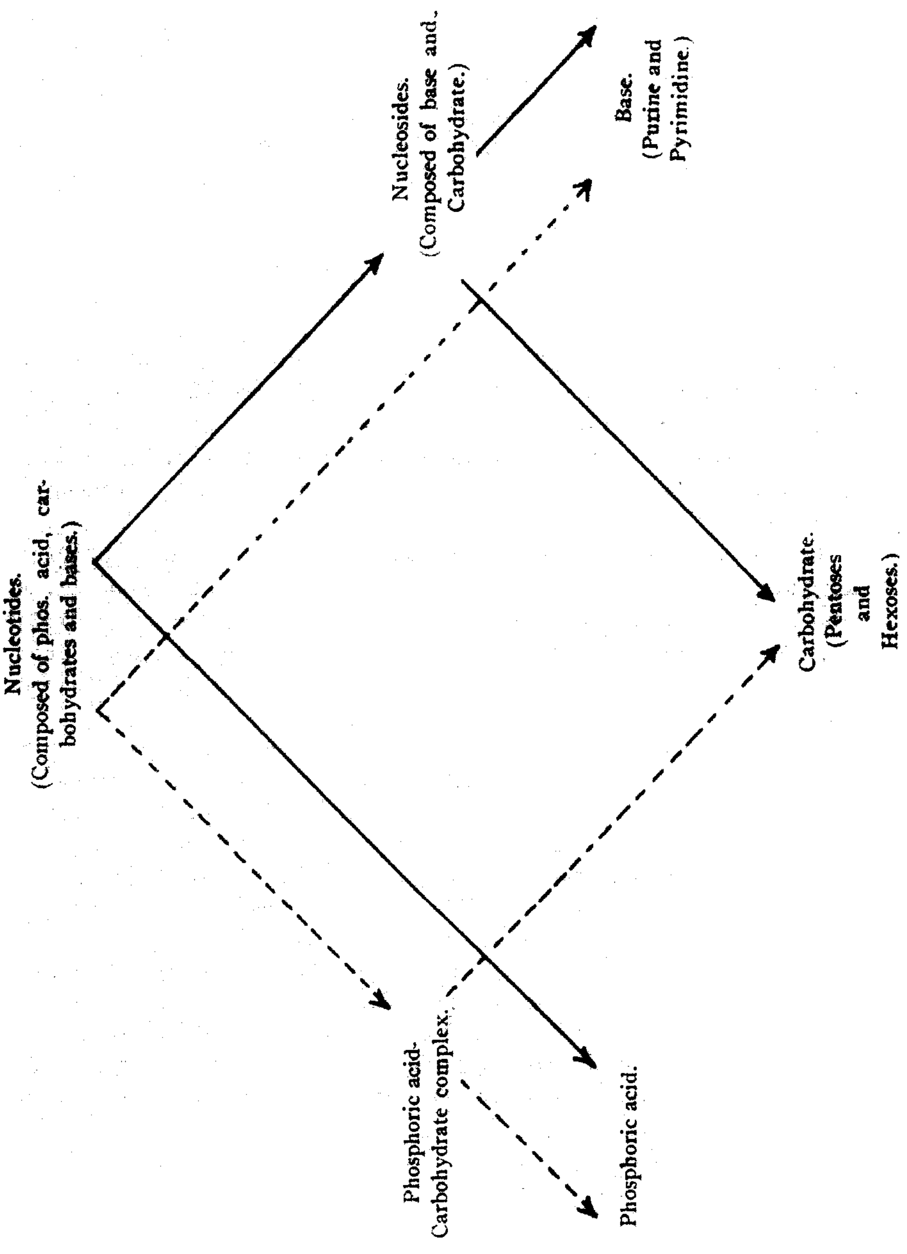
[2] Shorey, *Science*, **35**, 390 (1912).

[3] *Arch. exper. Path. Pharm.*, **44**, 148 (1900).

[4] *Ibid.*, **43**, 57 (1899).

[5] *Z. physiol. Chem.*, 36, 85 (1902).

always found. According to which pyrimidine derivative, thymine, cytosine or uracil, is present, and according to which purine base, guanine, adenine, xanthine or hypoxanthine, is present, the composition of the



acid varies. Several of these compounds are even present at the same time, so that a great variety of combination is possible.

While the simpler end products of nucleic acid are as above mentioned, this dissolution of the nucleic acid molecule does not take place in one step. There are several intermediary products or combinations of the above mentioned final products which have definit chemical and physical identity. The researches of Levene[1] and his school have served to clear up, to a large extent, the nature of these intermediary products. The steps in the disintegration of the plant and animal nucleic acids can perhaps be best made clear by the following schematic representation.

The complex zoo- or phyto-nucleic acids, for instance, those from wheat, yeast, salmon, are composed of simpler nucleic acids which Levene calls nucleotides. The nucleotides are composed of phosphoric acid, carbohydrates and bases, either purine or pyrimidine. Several of these nucleotides have been isolated, for instance, inosinic acid, guanylic acid, cytidinphosphoric acid, uridinphosphoric acid, thymohexonephosphoric acid. On further decomposition the nucleotide can part with its phosphoric acid on the one hand and yield a complex consisting of the base and the carbohydrate, designated as nucleoside in the diagram, and on the other hand it can part with the base and leave a complex consisting of the phosphoric acid and the carbohydrate. Of the nucleosides, a considerable number are known, inosin, guanosin, adenosin, xanthosin, which are respectively hypoxanthine, guanine, adenine, and xanthine combined with the carbohydrate. Further, from the nucleotides cytidin and uridinphosphoric acids, the nucleosides cytidin and uridin are obtained which consist respectively of combinations of cytosine and uracil with carbohydrate. Several of these compounds are well defined crystallin bodies and form salts of the same type as the base contained in them. A definit carbohydrate phosphoric acid complex has also been isolated and a crystallin barium salt obtained. This is the *d*-ribosephosphoric acid obtained by Levene. All of these various products can be broken down into the phosphoric acid, carbohydrate, and base, purine or pyrimidine.

The purine and also the pyrimidine derivatives are capable of being changed one into the other, by both chemical and biochemical agencies. Nitrous acid has been used to effect such changes. In this manner, Fischer[2] changed guanine into xanthine, Kossel[3] changed adenine into hypoxanthine, and Kossel and Steudel[4] changed cytosine into uracil.

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[1] Levene, *Abderhalden Biochem. Arbeitsm.*, **2**, 605 (1910); **5**, 489 (1911); Levene and Jacobs, *Ber.*, **41**, 2703 (1908); **44**, 746 (1911); *Biochem. Zeit.*, **28**, 127 (1910).

[2] *Ann. Chem. u. Pharm.*, **215**, 253 (1882).

[3] *Z. physiol. Chem.*, 10, 258 (1886).

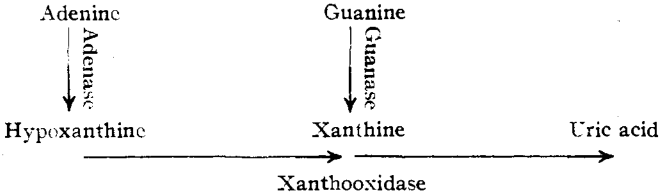
[4] *Ibid.*, **38**, 54 (1903).

Similarly Levene[1] changed the complexes guanosin, adenosin and cytidin into xanthosin, inosin, and uridin, respectively.

The changes produced by strictly chemical means are paralleled by the biochemical changes produced by bacteria and enzymes. It was shown by Schittenhelm and Schröter[2] that putrefactive bacteria, especially colon bacillus, were able to convert adenine and guanine into hypoxanthine and xanthine. They also show that the bacteria have the power of splitting down the nucleic acid itself. This change of nucleic acid is also brought about by certain enzymes which have been called nucleases, such as erepsin.[3]

Schittenhelm[4] has shown the presence of an amidase in the pressed juice of lupine seeds capable of converting guanine into xanthine.

Burian[5] showed that enzymes were capable of oxidizing xanthine and hypoxanthine to uric acid. The existence in animal tissues of amidases capable of causing the conversion of the amino purines (guanine and adenine) into the oxypurines (xanthine and hypoxanthine) has been repeatedly demonstrated. These changes produced by the various ferments are perhaps best shown by the diagram given by Jones and Austrian.[6]



The above exposition of changes involved in the degradation of nucleic acid makes clear the fact that this operation occurs in many stages. Also that some of these intermediary as well as final products are capable of being transformed one into the other. This degradation and transformation can be brought about in many different ways; by chemical means, such as the action of acids, alkalies, and steam under pressure, by microörganisms and by enzymes. The fact that such changes have been worked out makes it possible to interpret the changes observed in soils when heated, as well as to explain differences between soils not heated.

Applying these principles to the interpretation of the results obtained

[1] Abderhalden, *Handb. Biochem. Arbeitsm.*, **5**, 489 (1911).

[2] *Z. physiol. Chem.*, **41**, 284 (1904).

[3] Nakayama, *Z. physiol. Chem.*, 41, 348 (1904); Iwanoff, *Ibid.*, **39**, 31 (1903); Sachs, "Ist die Nuclease mit dem Trypsin identisch," Diss. Heidelberg (1905).

[4] *Z. physiol. Chem.*, **63**, 289 (1909).

[5] *Ibid.*, **43**, 497 (1905).

[6] *J. Biol. Chem.*, **3**, 228 (1907).

by heating the Elkton and Sassafras soils, it becomes evident at once that hydrolysis of the nucleic acids of these soils has taken place. This is evidenced by the fact that in each case there was a diminution in the amount of nucleic acid obtained from the soils, and by the increase both in quantity and in number of the nucleic acid bases.

In the Elkton soil unheated, xanthine and adenine were found; on heating, the amount of xanthine increased, adenine was found as before, and in addition hypoxanthine appeared. The hypoxanthine had been formed from a nucleoside containing this base. The fact that it did not appear in the unheated soil may be due to an arrested decomposition of its antecedents or to the fact that it is changed by enzymes or microorganisms into xanthine or lower nitrogenous compounds. Similarly, the appearance of cytosine in the heated soil is undoubtedly due to the same cause, namely, the action of the heating on antecedents containing it.

In the Sassafras soil the changes are even more definit. In the unheated soil the only nucleic acid base found was hypoxanthine, but after heating there appeared xanthine, guanine and cytosine, as well as a larger amount of hypoxanthine. The absence of xanthine and guanine in the unheated soil would seem to indicate that their antecedents are not subject to further decomposition under the existing soil conditions or else they are rapidly changed to uric acid, urea, ammonia, etc.

These two soils, as already mentioned, have had the same general field treatment, and have received the same kind of organic matter in the form of manures and crop debris, so that the nucleoproteins, nucleins, nucleic acids, etc., which have entered into these soils, have been practically the same. The different results in the heated as well as in the unheated soil can only show that the decompositions occurring normally under the two different soil environments are biochemically different. It has been repeatedly emphasized in the publications from this laboratory that the organic matter of different soils is different, and in the present instance not only is this clearly shown, but it is also pointed out that this difference is brought about by biochemical factors.

One difference between the two soils is found in the fact that the Elkton soil contains no antecedent compound which will yield guanine by heating. This fact may be explained by the assumption that the guanine antecedent, possibly guanisin, is changed to an xanthine antecedent in the soil. Color is lent to this assumption by the fact that xanthine is found in both the unheated and heated soil, while guanine, as well as xanthine, is found in the heated Sassafras soil.

Another difference is in the adenine. This is found in Elkton soil and not in Sassafras soil, and any antecedent bodies seem also absent from the latter soil. Hypoxanthine on the other hand, into which adenine or its antecedents may change, is absent from Elkton, but present in Sassafras.

Cytosine antecedents are present in both soils. The fact that cytosine itself is not found in the unheated soils may again be due to permanency of the antecedent under both soil conditions, or else to the presence of factors, either chemical or biochemical, which transform it into uracil. Whether uracil exists in these soils, or, in fact, any other soil, cannot be experimentally determined at present, owing to lack of characteristic derivatives or properties of uracil, although it seems probable that it must exist at least under certain conditions.

Protein Degradation Products.

The nucleic acid decomposition has been followed in the preceding sections from its combination in a nucleoprotein to its final end products. The albumins or proteins which split off in the early stages, as well as proteins which exist as such in plant and animal tissues, are even more complex than the nucleic acid, and the intermediate products between protein on the one hand, and the final products of complete hydrolysis on the other, are less clearly defined than in the case of the nucleic acid products. The final products, however, are well known and their properties clearly defined.

Two of these final products of protein hydrolysis appear in the present investigation. These are arginine and histidine. The latter was found in both soils, unheated and heated, but was found in greater amounts in the heated soil. Arginine was found only in the heated Sassafras soil. There is present, therefore, in the Sassafras soil a protein complex which acts as the antecedent of arginine. The question then remains, why is arginine absent from the unheated soil, and also, why is this antecedent body absent from the Elkton soil? Arginine, although a very common dissociation product of proteins, has, nevertheless, been found in only two other soils. This is rather peculiar in view of the fact that its close associate, histidine, has been found very often, a fact which is best explained on the supposition that arginine is very susceptible to changes through biochemical influences. For instance, Kiesel[1] found that the arginine in the juice of the green sprouts of lupine disappeared in the course of a four weeks' autolysis. It seems probable that this is the reason of its non-appearance in the normal Sassafras soil. In regard to the second part of the question as to the absence of any direct antecedent body, no very definit statement can be made, but it seems reasonable since there is protein material still in the soil, that the change has not proceeded to such a stage that arginine could be produced by such a procedure as was here employed. In other words, the biological factors in the Sassafras soil were such as to furnish an intermediary product

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[1] *Z. physiol. Chem.*, 60, 460 (1909).

which was dissociable by heat, and for this very reason, in a more readily decomposable form in the soil. From this it would again appear that the biochemical factors in the two soils are somewhat dissimilar under field conditions.

Concerning creatinine, nothing definit is known as to its antecedent compounds and no special lesson can be learned from the occurrence of this constituent in these soils. The same may be said of pentose sugars and pentosans.

Effect on Plant Growth.

The compounds considered thus far, taken as a whole, show an increase in the heated soil, both in amount and in number. This is in harmony with the often observed analytical fact that the heating produces an increase in soluble nitrogenous material. These results show the nature of the compounds thus made soluble. It remains to discuss the effect of such compounds on plant growth. Nearly all of these soil constituents have been studied in regard to their effect on plant growth and the results reported.[1] Among those tested were nucleic acid, hypoxanthine, xanthine, guanine, histidine, arginine and creatinine. All of these were shown to be decidedly beneficial to the growth of wheat seedlings. Take, for instance, hypoxanthine. The effect of this soil constituent was studied in solutions containing many different ratios of fertilizer elements. The effect of the hypoxanthine was very marked in those cultures containing phosphate and potash, but no nitrate. The average increase in growth in these cultures was 41%, due to the presence of 100 parts per million of hypoxanthine. When nitrate was also present the increase due to hypoxanthine was not so great, but it was shown that in the presence of hypoxanthine less nitrate was consumed by the plants. All the other compounds mentioned above behaved in this same general way.

In the absence of nitrate, nucleic acid gave an increase of 74%, histidine gave an increase of 30%, arginine an increase of 33%, creatinine an increase of 36%. Guanine and xanthine were not tested in this same comprehensive manner, but the results show conclusively that they too are beneficial.

In view of these facts, the cause of the increased productiveness of heated soils would seem to be explained by an increase in beneficial, readily and directly assimilable organic soil constituents. But what of the undoubted fact that some soils when heated show a harmful rather than beneficial effect on plant growth, especially on germination and the early stages of growth?

The heated and unheated soils, as well as water extracts from them, were tested for their effects on plant growth. The soil test was made in

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[1] "Nitrogenous Soil Constituents and their Bearing on Soil Fertility," by Oswald Schreiner and J. J. Skinner, *Bull.* **87**, Bur. Soils, U. S. Dept. Agric. (1912).

paraffined wire pots in the usual manner. The wheat seedlings grew from July 6th to July 21, 1911. The results of this test were as follows:

TABLE III.—PLANT GROWTH ON HEATED AND UNHEATED SOILS.

Soil and treatment.	Green weight. Grams.	Relative growth.
Sassafras silt loam	2.525	100
Sassafras silt loam, heated	1.070	42
Elkton silt loam	2.043	81
Elkton silt loam, heated	1.820	72

In harmony with the field experience on these soils, the Sassafras is the better soil, the Elkton giving in this case only 81% of the growth obtained in the Sassafras soil. The heating, however, had a bad effect on both soils, by far the greatest depression occurring in the normally better soil. All of this is of course surprising, in view of the fact that more nitrogen compounds, shown to be in themselves beneficial, are liberated by the heat in both soils, and particularly in the Sassafras soil. It is obvious that the chemical changes so far discussed do not explain this observed fact in regard to plant growth, and it is further obvious that some harmful compounds are produced in both cases, and apparently to the greatest extent in the Sassafras soil, in addition to the beneficial compounds produced as already discussed. The depressed plant growth was also shown by the water extracts from the heated soils as compared with the unheated, although, as might be expected, the effect is not so marked. The proportion of water and soil used was 4 of water to 1 of soil and the solution culture made in the manner usual to this laboratory. The wheat seedlings grew from April 9th to April 19, 1912. The results are as follows:

TABLE IV.—PLANT GROWTH IN WATER EXTRACTS FROM HEATED AND UNHEATED SOILS.

Soil extract.	Green weight. Grams.	Relative growth.
Sassafras silt loam	5.100	100
Sassafras silt loam, heated	4.200	82
Elkton silt loam	4.800	94
Elkton silt loam, heated	4.600	90

The effects of the soil extracts are in the same order as the soils in the preceding table, the heated Sassafras being again the poorest medium for plant growth.

Presence of Dihydroxystearic Acid in the Elkton Soil.

The lower productivity of the Elkton soil in the field and in laboratory experiments has been repeatedly noticed during several investigations conducted by this Bureau. According to the latest publication on this subject,[1] this lower productivity may be explained, in part at least, by the presence in this soil of dihydroxystearic acid. This compound has been

[1] *Bull.* 80, *loc. cit.*

isolated from a number of unproductive soils and its relation to infertility has thus been strongly indicated. Experiments with the compound itself has shown it to be very toxic to wheat seedlings, causing a diminution in growth, stunted root development with darkened root tips, often bent into hook like forms, and diminishing very markedly the absorption of nutrients by the plants.

As before, the dihydroxystearic acid was found in this sample of Elkton soil and was absent from the Sassafras soil. Dihydroxystearic acid is fairly easily destroyed or altered by oxidation. The influence of nitrate, which stimulates root oxidation,[1] and lime, which has the same effect on root oxidation, and probably also in the soil itself, since organic matter always disappears under its influence, have been shown to diminish the toxicity of dihydroxystearic acid.[2]

The Influence of Heating the Soil on the Dihydroxystearic Acid.

Since this compound is apparently easily attacked, it was thought that heating the soil might cause a diminution of the amount present, or perhaps the entire disappearance of the compound. It soon became evident, however, that on heating the Elkton soil the amount of this compound increased rather than decreased. The plant tests in the water solution bore out this fact of an increase in the harmful constituent. The water solution of the heated Sassafras soil showed even greater toxicity than the extract from the heated or unheated Elkton soil.

This as stated above, points to the generation of a toxic compound by the process of heating. Dihydroxystearic acid appears normally in the Elkton soil which, as mentioned, has had the same sort of organic matter introduced into it as had the Sassafras soil, but has been subject to different biochemical changes. The toxic substances generated by heat in the Sassafras soil should at least bear some relation chemically to that found normally in the Elkton soil. Examination of the heated Sassafras soil showed the toxic compound to be identical with that in the Elkton soil, namely, dihydroxystearic acid; furthermore, this compound was obtained in even larger amount from the heated Sassafras than was obtained from the Elkton soil, either heated or unheated.

The production of this harmful compound, although this may not be the only one produced, on heating, explains in itself the decrease in the fertility of the heated soil.

What the antecedent body of this compound in the Sassafras soil under field conditions may be, is not known. Certain it is, however, that dihydroxystearic acid as such is not found in the Sassafras soil until after

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[1] Schreiner and Reed, *Bot. Gaz.*, **47**, 355 (1909).

[2] Schreiner and Skinner, *Ibid.*, **50**, 161 (1910).

heating. The antecedent is probably the same in both soils, but under the influence of the different biochemical factors existing in the Elkton soil, this antecedent compound is so changed that the harmful influence of the dihydroxystearic acid shows itself.

In the foregoing it has been shown that beneficial compounds have been increased by heating, but simultaneously with this increase there has been a production of harmful compounds. The effect on plant growth is naturally the mean of these two influences, and the full beneficial effects cannot show themselves until the harmful compound has been eliminated by oxidation, reduction, cultural or other means. Similarly the full harmful effect of a compound is not attained in the presence of a beneficial compound. This state of affairs is, however, not an unusual one confined to heated soils, but it is found in soils under normal field conditions. The soils under consideration are an excellent illustration of this point. The Elkton soil contains beneficial constituents, but also contains dihydroxystearic acid, a harmful constituent. The plant growth upon it depends upon the balance of these two opposing factors, when one is decreased the other gains the ascendancy. In the other soil, that is, the Sassafras, this harmful compound is absent or at a minimum under field conditions, and the beneficial compounds exert their full effect. Any change in soil condition may bring about a change in this balance of the opposing forces so that the soil becomes temporarily or permanently more fertile or less fertile as the case may be. The heating of the soil brings about a very rapid or sudden change in this respect, with the result that, even though beneficial compounds are liberated in this fertile soil, the production of the harmful compound more than overbalances them, with the result that the plant growth drops to 42% of the normal. In the case of the Elkton soil, which already contained this compound, the effect of the heating was also to increase the beneficial compounds, and only slightly increase the harmful compound, so that the net result was that crop growth was only depressed to 89% of the normal. In other words, the balance was more disturbed in the case of the Sassafras soil than in the case of the Elkton. The addition of fertilizers to the two soils is to alter this balance, and to alter it most in the case of the Elkton soil. Especially is this true in regard to the effect of lime and of nitrates in the two soils. Cultural methods or crop rotation can, in the same way, influence the biochemical factors in soils and through them effect this balance of good and harmful properties.

When the dihydroxystearic acid has disappeared from the heated soil, through oxidation, cropping, liming or use of nitrates, the full effect of the nitrogenous nutrients will manifest themselves and plant growth will be much better on the heated than on the unheated soil.

In this manner is explained, for these soils at least, why on heating the soils, although increasing the nitrogen-soluble organic matter, the first effects are a decreased fertility, the latter effects an increased fertility.

Summary.

This paper deals with the chemical changes involved when soils are steam heated as in the process of sterilization. The results may be summarized as follows:

(1) There was an increase in water-soluble constituents and an increase in acidity, even though at the same time ammonia and amines were formed.

(2) By the process of heating there was an increase in nearly all of the constituents isolated from the unheated soil except perhaps nucleic acid.

(3) Both beneficial and harmful compounds were produced by heating the soils and were isolated.

(4) By the process of heating there were formed xanthine, hypoxanthine, guanine, cytosine, and arginine, when not previously existing. These compounds are decomposition products of nucleic acid and protein material and are all beneficial to plant growth.

(5) Dihydroxystearic acid was increased when present, and was produced by the heating process when not previously present. This compound is harmful to plant growth.

(6) Cultural tests in the soils and their extracts showed that the heated soils gave a poorer plant growth.

(7) Although the majority of the compounds formed must be classed as beneficial, the harmful compounds formed more than overbalances their effects. Not until this harmful compound is eliminated or diminished can the full beneficial effects of heating be shown.

(8) In soils there is a balance of beneficial and harmful factors, soil fertility or infertility being the resultant of the two groups. As one or the other group of factors gains the ascendancy, the fertility is raised or lowered as the case may be. This balance is influenced by cultural treatment, fertilizers, liming, crop growth or crop rotation, etc., as well as by steaming.

(9) The results show that although the soils studied have received the same kind of organic matter and have received the same farm treatment, they have been subject to different biochemical factors resulting in differences in their organic matter and in differences in their fertility.

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