INVESTIGATIONS ON THE RÔLE OF ORGANIC MATTER IN PLANT NUTRITION.

Part I. Some Chemical Aspects of the Oxidation of Soil Organic Matter.

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The accumulated farming experience of the past several centuries and the more systematic scientific researches of recent years bear evidence to show that the organic matter of the soil plays a far more important part in crop production than has generally been realized. Not only does it improve the tilth and increase the absorptive and water-holding capacity, but it also alters, under certain conditions, even the ultimate physical structure of the soil (Sreenivasan and Subrahmanyan, 1934[1]). It is well known that the organic matter, particularly that which is added in the form of manure, undergoes decomposition yielding mineral nutrients in available form. More recent studies also show that the decomposition products include certain water-soluble constituents capable of stimulating plant reproduction (Subrahmanyan and Siddappa, 1933[1]) ; that the carbon dioxide which is one of the major products of oxidation enriches the surrounding atmosphere and facilitates increased assimilation by plants (Lundegårdh, 1927, 1928) ; that at any rate, in the early stages, the carbon dioxide thus produced is sufficiently large in quantity to maintain plant life independent of the gas present in the atmosphere (Subrahmanyan and Siddappa, 1933[2]). Evidence has also been adduced to show that the decomposition of organic matter which proceeds slowly under natural conditions can be hastened by treating soil with even mild oxidising agents resulting in the release of larger quantities of plant nutrients and bigger yield of crop (Subrahmanyan, Harihara Iyer and Rajagopalan, 1934). The foregoing observations having suggested that the organic matter of the soil is far less resistant than has hitherto been believed, the present enquiry which relates to certain aspects of oxidation by chemical reagents was undertaken. The results have thrown much useful light on the nature and behaviour of carbon compounds present in the soil and their relation to the nitrogenous constituents known to be associated with them.

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The literature on the chemical oxidation of soil organic matter is comparatively limited. G. W. Robinson and Jones (1925) observed that the organic matter is either oxidised or brought into solution on treatment with 6 per cent. hydrogen peroxide. W. O. Robinson (1927) noted, on the other hand, that certain forms of organic matter known to be present in or associated with the soil are not attacked by hydrogen peroxide and that the treatment is not applicable to soils which are rich in lime, manganese or chromium oxide. McLean (1930) observed that whereas cellulose is normally resistant to the action of 6 per cent. hydrogen peroxide, it is oxidised by that reagent in presence of soil. He also investigated the nature of the complexes attacked by different concentrations of hydrogen peroxide with special reference to their carbon and nitrogen contents (1931). Working with chernozem soils, Gedroiz (1930) noted that, on treatment with 30 per cent. hydrogen peroxide, the major part of the nitrogen of the soil is converted into ammonia together with traces of nitrate. On addition of increasing quantities of hydrogen peroxide, the soil tends to become acid suggesting the formation of free organic acids (Dojarenko, 1911). These observations though of considerable scientific interest do not yet throw much light on the manner and extent of oxidation to carbon dioxide which is of importance from the point of view of plant assimilation. Further information is also required regarding the influence of various factors on the efficiency of oxidation ; on the behaviour of soil organic matter as compared with other carbon compounds ; and on the comparative efficiency of oxidation under dry and swamp soil conditions. These and certain related aspects of the problem have been studied and are discussed in the present paper.

Experimental.

Estimation of Organic Matter.—The improved method of Walkley and Black (1934) yielded fairly concordant estimates of carbon when parallel specimens of the same soil were examined. When extended to pure organic substances, the method did not yield satisfactory results. Sucrose was an exception and when calculated without applying the correction factor, yielded approximately the theoretical value. (Expected, 42·1 per cent., Found, 44·2 per cent.). Urea, on the other hand, yielded the same value as the blank, thereby showing that it did not react with the oxidising agent under the conditions of the experiment.

With a view to determining whether the method can be applied to the study of organic matter in the swamp soil, minute quantities of different substances known to be formed under such conditions (Subrahmanyan, 1929[1] ; Robinson, 1930) were added to samples of a local soil and the total organic carbon in the mixtures estimated in the manner recommended

by those authors (Table I). Since some of the added materials were in the liquid form, small quantities (5 c.c.) of water were added so as to make the volume of the reaction mixture the same in all the cases, including the control.

TABLE I.

Material oxidised	Carbon per cent.	
	Expected	Found
Soil alone (control)	..	0·78
,, +Lactic acid ..	1·42	1·19
,, +Acetic acid ..	1·77	0·78
,, +Propionic acid ..	2·12	1·28
,, +Ferrous sulphate ..	0·78	0·84

It may be seen from the above that the added organic matter was either not oxidised at all or only partially so. Ferrous sulphate being itself a reducing agent led to an exaggerated value being obtained.

The Degtjareff Method (1930).—As already observed by Walkley and Black (*loc. cit.*) this method yielded exaggerated values though the errors were not so considerable as envisaged by those authors. In the case of one soil (peaty specimen from Travancore), at any rate, the Degtjareff method yielded a very much lower value than that obtained according to Walkley and Black (Table II).

TABLE II.

Description of Soil	Carbon per cent. according to the method of	
	Degtjareff	Walkley and Black
Red sandy loam—Bangalore ..	1·12	0·74
Peaty soil—Travancore ..	9·19	18·72
Black cotton soil—Nagpur ..	1·22	0·81
Alkali (*Kalar*) soil—Sindh ..	0·89	0·41

The abnormally high value obtained for the peaty soil according to the method of Walkley and Black is due to the presence of large quantity of ferrous iron (Pillai and Subrahmanyan, 1931) which reacted with the chromic acid thus leading to an exaggerated estimate of carbon. According to the Degtjareff method, on the other hand, hydrogen peroxide, which is first added, would appear to have oxidised the ferrous iron, thus saving a part of the chromic acid so that the resulting value was lower than that obtained according to the other method.

Heating the soil suspension with the Degtjareff reagent for varying periods of time resulted in higher and more discordant values being obtained. In the case of one specimen which yielded 1·12 per cent. before heating, the following percentages were obtained after boiling for 1 minute—1·88, 1·70, 1·90 and 1·66. Control experiments with the oxidising agent showed that this was largely due to uneven decomposition of chromic acid under such conditions.

Experiments with different other organic substances also led to discordant results. Thus, in the case of cane molasses, ten parallel trials yielded values ranging from 31·8 to 38·3 per cent. Urea did not react with the Degtjareff reagent.

Reaction between Soil and Chromic Acid under Different Conditions.—With a view to determining the conditions under which the indirect method of oxidation can be made more quantitative, a number of trials were carried out with aqueous or alkaline suspensions of soils.

A specimen (5 g.) of local soil ground to pass the 100-mesh sieve was extracted with normal alkali and the volume made up with further quantities of alkali to 200 c.c. Aliquots (10 c.c.) of the extract were treated with 10 c.c. of chromic acid prepared according to Degtjareff and 10 c.c. of syrupy phosphoric acid (Sp. Gr. 1·75). The mixtures were heated over the water bath for varying periods of time and the unused chromic acid determined by titration against standard thiosulphate. Control experiments were also carried out without adding chromic acid and correction made for the iron present in solution. The estimates of carbon obtained at different stages were, however, highly discordant, thereby showing that the oxidation did not proceed quantitatively under such conditions. Even prolonged heating did not lead to any constant value being attained. Some experiments were also carried out neutralising the alkali extract with dilute sulphuric acid and then adding mixtures of chromic and phosphoric acids. The results thus obtained were discordant as may be seen from the following —carbon as estimated before heating, 0·30–0·36 per cent.; after heating for 30 minutes on the water bath, 0·57–0·65 per cent.

Experiments conducted with and without phosphoric acid showed that the acid, though slightly helpful in hastening the reaction, was not, however, quite essential. Nor did its presence have any influence on the spontaneous decomposition of chromic acid observed on heating the reaction mixture.

Further trials with alkali suspensions as well as alkali extracts showed that, even under identical conditions, parallel samples did not yield concordant results. The observations suggested that there were certain factors other

than soil organic matter which determined the reduction of chromic acid under different conditions. Furthermore, prolonged heating, especially in the case of soils containing chlorides, led to the evolution of chlorine. Since the latter was formed through reduction of chromic acid, a further error in the estimate of carbon was thus introduced.

Oxidations conducted in aqueous solution with sucrose or molasses, yielded fairly satisfactory results on applying correction for the decomposition of chromic acid on heating. The reactions were, however, generally slow. In the case of molasses, the mixture had to be heated for 45 mins. before constant values could be obtained.

When extended to aqueous or alkaline suspensions of soil which had been treated with molasses as fertiliser, the method proved a failure. A large number of trials conducted at different concentrations and heating for varying periods of time showed that the reaction with chromic acid was not quantitative ; that owing to the interference of other factors, the values obtained from the reaction would not truly represent the organic carbon present in the soil system. The following results obtained in one of the experiments would illustrate the above :—The material used was alkali extract (1 per cent.) of soil containing 1 per cent. molasses. Aliquots (10 c.c.) were oxidised with chromic acid in the usual way. Back titration values (in terms of 0·216 N thiosulphate) obtained for parallel samples—whole suspensions, 9·8, 11·0, 9·5 and 8·7 c.c. respectively ; alkali extract, 18·0, 16·8, 20·1 and 17·5 c.c. respectively.

In view of the above and other difficulties, it was concluded that none of the indirect methods would be suitable for the accurate study of the transformations of carbon in aqueous or alkaline suspensions of soil ; that only a direct method of estimation involving quantitative oxidation of all forms of carbon into carbon dioxide would prove useful under such conditions.

Some of the Factors influencing the Oxidation of Organic Matter in Dry as well as Swamp Soils.—Although a variety of methods can be adopted in the case of dry soils, the conditions prevalent in the swamp soil, as also the nature of the products formed therein, impose certain restrictions on the choice of technique for the study of the latter. Thus, dry combustion methods would be unsuitable because the swamp soil cannot be dried without either losing certain of the volatile constituents or otherwise undergoing undesirable chemical or biological changes. It would be necessary, therefore, to follow a method of wet oxidation, the efficiency of which may be influenced by (*a*) the quantity of soil, (*b*) the proportion of water to soil and, if the specimen is coarse or sticky, the mode of dispersion, (*c*) the nature and quantity of oxidising agent used, (*d*) the proportion of sulphuric acid, if

any be used, to those of soil, water and oxidiser, (e) the order in which the reagents are added, and the precautions observed in heating the reaction mixture, and (f) the mode of absorption of carbon dioxide and the procedure for estimating unused alkali. Some of the conditions have been partly investigated by previous workers, but the others have yet to be studied.

Perhaps the most important factor determining the accuracy of the study of organic matter is the efficiency of absorption of the resulting carbon dioxide. Many previous workers have also laid stress on this aspect and have suggested the use of absorption towers with perforated discs or glass bead traps containing alkali. In all cases, initial cooling of the vapours in a condenser followed by displacement of the gases with a current of CO_2-free air has been suggested. This method of absorption is generally efficient but it proves unsatisfactory when the evolution of gas is vigorous as in the case of soils which are rich in carbonates. Sudden rush of gas combined with the force of suction (however gentle) would render even a tall column of alkali inefficient. If, on the other hand, the reaction mixture is heated very gently and the evolution of gas slow, the experiment will have to be continued over a long period of time. Even then the absorption may often be incomplete and lead to low estimates being obtained.

Into a Kjeldahl flask (capacity, 300 c.c.) fitted with a tap funnel and a condenser, 50 c.c. of a 1 per cent. solution of potassium carbonate was introduced. Sulphuric acid (5 c.c., 1 : 1) was then let in through the funnel and the reaction mixture heated with a low flame. A stream of CO_2-free air was drawn through the apparatus and the gases, after passing through the condenser, were drawn downward through a vertical column into an absorption vessel containing large excess of standard alkali. The rate of absorption of CO_2 was followed at intervals as follows:—A tap funnel was fitted to the absorption vessel in such a manner that its leg was dipping into the alkali. At the appointed time, a portion of the alkali was sucked into the funnel. From this an aliquot was pipetted out while the rest was allowed to flow back into the absorption vessel. The free alkali present in the aliquot was determined by titration against standard acid after adding excess of barium chloride. From the titre values and the volume of liquid left in the absorption vessel, the percentage of total carbon absorbed upto each stage was calculated and the results plotted as a curve. The experiment was repeated a number of times varying the rates at which CO_2-free air was drawn through the apparatus.

The influence of the rate of suction on the efficiency of absorption was similarly followed during wet combustion of sucrose. The oxidation was by chromic acid as recommended by previous workers.

It was observed that the values obtained from both the sets of experiments were discordant. In no case did the equilibrium stage correspond to complete absorption of the resulting carbon dioxide. The highest values attained were generally 5–10 per cent. less than the expected ones. Further observations showed that the low values obtained on drawing a swift current of air was due to incomplete absorption of carbon dioxide. The results obtained with a slow stream were first inexplicable because there was no escape of the gas from the absorption vessel. On repeating the experiment, however, with stronger heating and without allowing any water to flow through the condenser, correct values were obtained. This observation suggested that some of the CO_2 was retained in the cool spaces of the condenser and was not displaced by a slow stream of air. With a view to eliminating this defect, the position of the condenser was altered by having it immediately above the absorption flask in such a manner that its stem was actually dipping in the alkali. The hot vapours issuing from the Kjeldahl flask were first passed through a trap containing glass beads moistened with a mixture of chromic acid (with sulphuric acid) and silver sulphate and then drawn downward through a straight condenser. Some carbon dioxide was still retained in the condenser and since it was observed that the efficiency of absorption was greatly reduced by sending in vapours without first cooling them, it was considered desirable to use the condenser itself as a part of the absorption system. This was effected by filling it with glass beads and sending in a gentle stream of alkali from above.

It was further observed that drawing a current of air to displace carbon dioxide was not only unnecessary but also often inconvenient. Even gentle heating of the reaction mixture is quite sufficient to drive out the major part of that gas into the absorption system. Stronger heating leads to the evolution of steam which displaces the CO_2 quantitatively and carries it down the condenser. Condensation of moisture in the trap leads to immediate rise in temperature (due to dilution of sulphuric acid) which also helps to drive out the gas. In this manner, complete displacement of carbon dioxide into the absorption system can be ensured.

Some previous workers have drawn attention to the inadequacy of using dilute alkali, however large in volume, for absorbing CO_2. Trials with alkali of different concentrations showed that this was due to the poor solubility of the gas in very dilute solutions. Alkali of at least 2–4 N strength, and equivalent to about 10 times the CO_2 evolved was necessary to ensure complete absorption. Caustic potash was found to be more efficient than caustic soda of the same strength.

Arnold and Page (1930) have emphasised the need for breaking up big

bubbles before they escape through the alkali. Such bubbles contain mostly air and, in the later stages, only oxygen. When the oxidation is most vigorous, however, the bubbles contain small quantities of CO_2 which may thus escape absorption. After experimenting with a number of devices, it was found that a convenient arrangement is to tie a close-meshed copper gauze around the bottom of the condenser so that all the gases will have to pass through it before escaping into the alkali. The alkali trickling from above also forms a thin layer around the gauze and thus helps to remove a further quantity of CO_2.

With an absorption system of the type mentioned above it was found that supplementary traps could be dispensed with. The major part of the CO_2 was absorbed by the stream of alkali trickling over the large surface presented by the glass beads and the little quantity that was left over was taken up by the alkali in the bottom vessel.

Kinetics of Oxidation of Soil Organic Matter : Preliminary Experiments with Pure Substances.—It was observed that many organic substances were readily oxidised by chromic acid while others, including some apparently simple substances, resisted action until a late stage. Since, in a complex system like the swamp soil, carbon is present in a variety of forms, it was considered necessary to have an idea of the range of conditions under which the oxidation will have to be conducted. Some experiments were carried out therefore following the kinetics of oxidation in a few representative cases (Fig. 1).
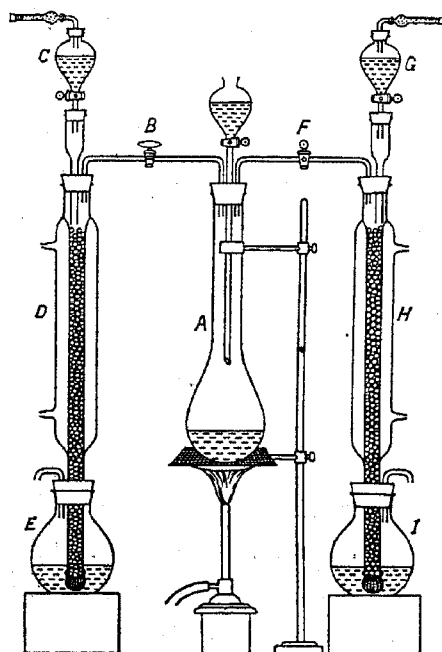

FIG. 1.—Apparatus used for following the Kinetics of Oxidation.

A Kjeldahl flask A (capacity, 800 c.c.) was fitted with a three-holed stopper through the centre of which passed a tap funnel while the two ends connected through taps B and F with CO_2 absorption systems of the type previously described. The material to be oxidised was introduced into the Kjeldahl flask in aqueous solution or suspension together with sufficient quantity of solid potassium dichromate to correspond to at least 10 times the approximate amount of oxygen required to complete the oxidation. Tap B was then opened (while F remained closed) and concentrated sulphuric acid introduced through the central tap funnel in small quantities at a time, the acid being blown in if the pressure inside was rather high. During this period, the alkali was kept trickling down the absorption system CDE, so that any CO_2 that was evolved immediately after addition of acid was absorbed. Tap B was then closed and F opened. The reaction mixture was then heated with a low flame and the CO_2 evolved during the next 5 mins. absorbed in the system GHI. In the meantime, the contents of funnel C were rapidly discharged through D and the traces of alkali adhering to the sides of the funnel or to the glass beads in the condenser washed into E which was then removed and a flask of similar capacity fitted in its place. Fresh alkali was introduced into C and again kept trickling down D. At the end of 5 mins. the vapours were again passed through B while F was closed. In this manner the CO_2 evolved at each stage was separately absorbed and estimated by titration against standard acid after adding excess of barium chloride. From these values the percentages of total carbon oxidised at different intervals were calculated.

In one experiment, 10 c.c. of a 1 per cent. solution of sucrose was oxidised with a mixture of 10 g. of dichromate and 30 c.c. of concentrated sulphuric acid. The carbon dioxide evolved at 5 minute intervals was absorbed in 20 c.c. lots of 3·45 N alkali and the unused alkali titrated almost upto the end-point against 4·208 N acid. In the final stages, 0·4 N acid was used for the titration. In another experiment, 20 c.c. of a 1 per cent. solution of urea were oxidised with 10 g. of dichromate and 50 c.c. of acid. The other details were the same as in the case of sucrose except that 25 c.c. lots of alkali were used for absorption. The results have been presented in Fig 2.

It may be noted that in the case of sucrose, the action was almost instantaneous, nearly 20 per cent. of the total carbon being oxidised to carbon dioxide even before the commencement of heating. By the time the reaction mixture came to boiling, more than 90 per cent. of the CO_2 had passed over and a few minutes later the entire amount had been absorbed by the alkali. Reverse was the experience with urea. There was no reaction with chromic acid for the first 20 minutes. It was only after a part of
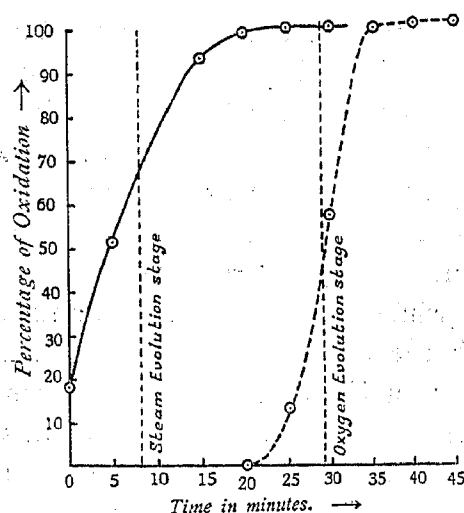
FIG. 2.—Oxidation of Sucrose and Urea.

o——o Sucrose.
o---o Urea.

the water had distilled over and the acid had become somewhat concentrated that any of the carbon was oxidised. The subsequent reaction was, however, very rapid, the entire oxidation being complete in about 10 minutes.

Oxidation of Biological Products.—The foregoing observations having suggested that the oxidation of even apparently simple substances may proceed under different conditions, further experiments were carried out with a number of substances of biological interest studying their mode of oxidation. The substances were introduced as aqueous solutions or suspensions (10–20 c.c.) and treated with mixtures of dichromate (10 g.) and concentrated sulphuric acid (30–60 c.c.). No special precautions were adopted with regard to starting the reaction, but in the case of volatile substances like acetone, ethyl alcohol, or acetic acid, the flasks containing the reaction mixture were cooled with ice during addition of sulphuric acid. The mixtures were then heated, first with a low flame and then more vigorously, and the carbon dioxide absorbed in the usual way.

It was observed that in the case of polysaccharides like cellulose or starch, the oxidation proceeded as rapidly as in that of sucrose. Acetone, ethyl alcohol or acetic acid could not be oxidised completely as long as the proportion of sulphuric acid to water was under 2 to 1. These observations are in agreement with those of Friedmann and Kendall (1929). With increasing quantities of acid, the reaction proceeded with greater rapidity and in all cases, the oxidation was complete if the temperature was maintained just sufficiently high to allow the reaction (which is accompanied by evolution of gas and change in colour) to proceed slowly.

Vigorous heating, especially in the early stages, was accompanied by partial loss, by volatilization, of the material to be oxidised. Citric and lactic acids were oxidised with comparative ease. Uric acid, casein or tyrosine offered no special difficulties when the usual precautions were adopted and the heating continued until chromic acid began to decompose evolving oxygen. The following results (Table III) would show that, allowing for small experimental errors, the oxidation proceeded quantitatively in all the cases.

TABLE III.

Material	Carbon in Milligrams	
	Expected	Found
Cellulose	45·4	45·4
Starch (A. R. soluble)	33·6	33·6
Sucrose	21·1	21·1
Ethyl alcohol	23·1	22·8
Acetone	49·5	49·5
Citric acid	21·9	21·9
Lactic acid	13·1	12·9
Acetic acid	10·1	10·1
Urea	25·0	24·9
Uric acid	34·7	34·7
Casein	..	42·4
Tyrosine	59·6	59·5

It may be mentioned that the "expected" values, cited above, were as far as possible, checked by independent methods. Thus, the purity of starch was verified by reduction methods (after hydrolysis), ethyl alcohol and lactic acid by oxidation to acetic acid (Subrahmanyan, 1929[2]) and uric acid by estimation of nitrogen.

Influence of the weight of soil on the efficiency of oxidation.—A number of experiments with representative Indian soils (which generally contain under 2 per cent. of carbon) showed that quantities under 10 g. can be completely oxidised and the CO_2 efficiently absorbed with an apparatus of the type used in previous experiments. In the case of poor gravelly soils (containing 0·5 per cent. or less), such as are found in many parts of the Deccan plateau, even larger quantities (15–20 g.) can be handled accurately. If, on the other hand, the soil is rich in carbon, the quantities taken for oxidation should be so adjusted that the resulting CO_2 does not exceed 600–800 mg. Much caution is needed in the addition of acid to soils which contain large quantities of carbonates because there is a tendency for the gas to rush out

suddenly and thus partially escape absorption. A preliminary trial adding dilute mineral acid to small samples of soil would be helpful in determining the rate at which the acid is to be added. In such cases, heating in the early stages will also have to be carried out with a low flame.

Effect of increasing the proportion of water to soil.—Ames and Gaither (1914) observed that disolution of sulphuric acid led to incomplete oxidation of soil organic matter. With a view to determining whether mere addition of increasing quantities of water had any adverse effect on the progress of oxidation, the following experiments were carried out. To 10 g. lots of three different soils, varying quantities of water (10, 20, 30, or 40 c.c.) were added and the suspensions oxidised with 10 g. of dichromate and 30 c.c. of acid in each case. The distillations were continued upto the stage at which chromic acid began to decompose evolving oxygen.

It was observed that although increasing quantities of water prolonged the time of distillation, the oxidations proceeded to completion in all the cases. The gravelly soil of Bangalore yielded a constant value of $0 \cdot 72$ per cent.; the alkaline (*Kalar*) soil of Sindh, a value of $1 \cdot 15$ per cent. and the peaty (*Kari*) soil of Travancore, $12 \cdot 4$ per cent. It would thus be seen that if the total quantity of acid is sufficiently large and the distillation continued upto a constant point, the oxidation proceeds quantitatively irrespective of the amount (within the range that was tried) of water present in the medium.

Comparative study of the efficiencies of different oxidising agents.—It was observed that in presence of concentrated sulphuric acid the reaction with potassium permanganate was very violent, resulting in sudden rise in temperature and such rapid evolution of CO_2 that the gas could not be effectively absorbed. A part of the permanganate also passed over as such and was absorbed by the alkali. In spite of the early vigour the oxidation was generally incomplete because the permanganate decomposed spontaneously within the first few minutes leaving only the less active manganese dioxide behind. In view of these and the difficulty in controlling the reaction, the use of that oxidising agent was given up as unsatisfactory. Chromic acid was tried as aqueous or acid solution at different concentrations. That acid being sparingly soluble in sulphuric acid, large volumes of the solution had to be used for oxidising even minute quantities. The use of aqueous solutions, though otherwise satisfactory, involve certain defects— (1) even a saturated solution involved the introduction of an extra quantity of water which, under certain conditions, was not desirable; (2) if the same tap funnel is used for delivering chromic and sulphuric acids in succession, there is the immediate precipitation of chromium trioxide on adding the

second acid so that the funnel gets choked. Washing with water is inadvisable because of the danger of cracking on adding sulphuric acid. A number of experiments with solid potassium dichromate showed, on the other hand, that in addition to being as efficient as chromic acid (the mechanism of oxidation being the same in both the cases), its use is free from the above-mentioned defects. Moreover, it is stable and can be handled more easily than the hygroscopic chromium trioxide. It has no action, by itself, on most carbon compounds so that the rate of oxidation can be largely regulated by addition of sulphuric acid alone. In view of these advantages, solid dichromate was preferred as an oxidising agent to an aqueous solution of chromic acid. A convenient arrangement for adding the dichromate would be to standardise the volume of powder corresponding to a given weight in a spoon or glass tube so that the required quantity can be easily measured out without having to weigh on each occasion.

With a view to determining the minimum quantity of dichromate to be used in practice, 10 g. lots of the Bangalore and Kalar soils used in the previous experiment and 1 g. lots of the peaty soil were suspended in 25 c.c. each of water and treated with varying quantities (1–15 g.) of dichromate. Sulphuric acid (30 c.c.) was then added and the resulting CO_2 absorbed in the usual way.

It was observed that when the quantity of dichromate was 1 or 1·5 g., the Bangalore soil yielded relatively low values (0·49–0·54 per cent.) for carbon while with 2 g. and above, the correct value (0·72 per cent.) was obtained. In a like manner, the peaty soil yielded accurate estimates (12·4 per cent.) only when oxidised with 3 g. and above. The Kalar soil, on the other hand, yielded correct values (1·15 per cent.) even with 1 g. of dichromate. This observation first appeared rather inexplicable, but subsequent examination showed that the Kalar soil contained practically no organic matter and that the bulk of the total carbon was derived from the carbonates present in it.

It is generally reckoned that carbon is the chief constituent of soil organic matter undergoing oxidation on treatment with chromic acid. In fact, many of the methods of estimating soil organic matter are based on this assumption. A computation based on the true carbon content would show that in the case of Bangalore soil, the dichromate required for complete oxidation would be 1·2 g. and that for the peaty soil, 2·1 g. The quantity actually required was, however, about 50 per cent. more in each case.

Influence of the quantity of sulphuric acid on the efficiency of oxidation.— Aqueous suspensions (10 g. in 25 c.c.) of the three soils used in the previous experiment were treated with 10 g. each of dichromate and varying quantities

(12·5, 25, 50, 75 or 100 c.c.) of sulphuric acid and the oxidations conducted in the usual way.

It was observed that with increasing quantities of acid, the end-point (oxygen evolution stage) was very rapidly attained. In those cases, the heating had also to be done carefully as the acid tended to pass over as fine spray.

The results showed that except in the case of the Bangalore specimen treated with 12·5 c.c. of acid (and which yielded a low value of 0·49 per cent.), there was complete oxidation in all the others. A study of the conditions that led to the single low value showed that although the acid was sufficient for the oxidation of organic matter, more was yet required for reaction with the minerals, especially iron and aluminium, in which that soil was rich. On the other hand, the Sindh soil was mostly sandy and did not take up much sulphuric acid.* The quantity of peaty soil taken was only 1 g., so it required very little acid. It may thus be seen that although the oxidation may ordinarily proceed to completion even with small quantities of sulphuric acid, there may yet be certain special cases which may require very much larger quantities. It would be desirable therefore to allow for such possibilities and use at least 5–6 times the weight of sulphuric acid to that of soil taken.

Influence of the mode of dispersion on efficiency of oxidation.—Some experiments were carried out to determine whether fineness of division in grinding or the mode of dispersion of the particles had any influence on the oxidation of soil organic matter.

It was observed that coarse powders passing 20–30 or 30–40 mesh sieves did not yield concordant results. As advocated by some previous workers, samples passing the 100-mesh were tried and found to yield satisfactory results.

Dispersion with water or alkali had no influence on the progress of oxidation. In the case of some soils (particularly those which are rich in minerals), however, aqueous suspensions did not yield homogeneous samples while alkali (NaOH, 4 per cent.) did. It was further observed that in addition to dispersing the soil, the alkali also helps to arrest microbial action and can therefore be used with advantage in the study of carbon transformations in the soil. On standing for more than 24 hrs., even the alkali suspension tends to flocculate so that accurate sampling again becomes difficult. As the result of this, one set of experiments yielded values ranging from 0·44 to 0·55 per cent. of carbon for parallel samples taken during a period of 120 hours. The alkali extract (which contained the bulk of the organic

matter) was, on the other hand, quite reliable and yielded the same value (0·23 per cent.) throughout the period of observation.

Kinetics of Oxidation of Soil Organic Matter.—It was expected that this study would provide much valuable information regarding the nature and behaviour of the different forms of organic matter present in the soil. The experiments were carried out with the four soils used in the previous studies. The gravelly (Bangalore) and the black cotton (Nagpur) soils were weighed out in 10 g. lots together with 10 g. of dichromate and 25 c.c. of water. The oxidation was initiated by adding 30 c.c. of concentrated sulphuric acid and heating with a low flame. The CO_2 evolved at 5 minute intervals was absorbed in an apparatus similar to that sketched in Fig. 1. In the case of the alkali soil 5 g. lots were taken for oxidation and in that of the peaty soil which is very rich in organic matter, only 1 g. was taken. The details relating to the oxidation were otherwise the same in all the experiments. The results have been presented in Fig. 3.
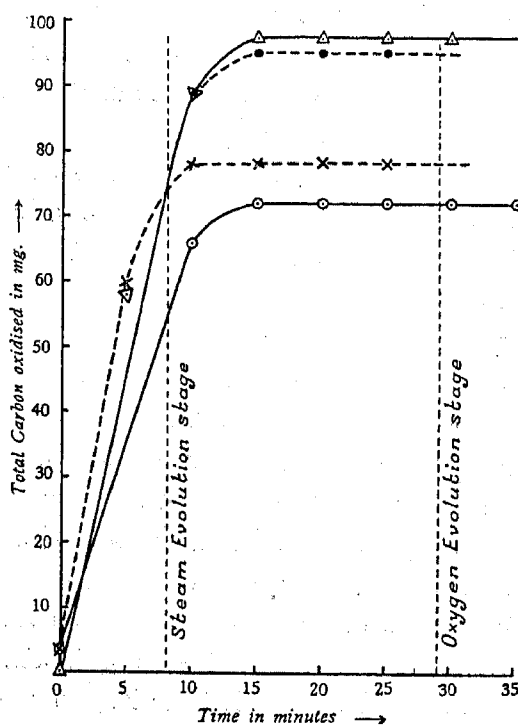
FIG. 3.—Oxidation of Soil Organic Matter.
o——o Gravelly Soil (Bangalore).
x----x Alkali (Kalar) Soil (Sindh).
•----• Peaty (Kari) Soil (Travancore).
△——△ Black Cotton Soil (Nagpur).

It may be noted that in all the cases the major part of the CO_2 passed over within the first 15 mins. In the case of the Bangalore and the black

cotton soils, the rate of evolution was as vigorous as in the *Kalar* soil the major part of the carbon of which was present as carbonate. In the case of the peaty soil, the reaction was apparently a little slower than in the others but even in that case all the carbon was completely oxidised before the 20th minute. These observations combined with the previous ones would show that at any rate the major part of the soil organic matter is of readily oxidisable character ; that irrespective of the chemical nature of the various materials combining to form soil humus, the ultimate composition is such that it can be oxidised nearly as easily as sucrose. This conclusion is further strengthened by the observations of Harihara Iyer and Rajagopalan (unpublished data) who find that even mild oxidising agents like manganese dioxide or ferric oxide can oxidise a considerable portion of the soil organic matter to CO_2 at ordinary temperatures (25-30°), within a few weeks. Further work on this aspect of the problem is now in progress and will form the subject of a later communication.

The results presented in Fig. 3 only represent the quantities of CO_2 that passed over from the Kjeldahl flask into the absorption system. The heating was carried out with a low flame so that it is hardly probable that all the CO_2 was carried over immediately after it was formed. It was, in fact, observed that even in the case of carbonate solutions from which the liberation of CO_2 is instantaneous, it takes 10–15 minutes for all the gas to be displaced by steam. It has to be inferred, therefore, that in all the cases, the reaction between soil and chromic acid was very rapid and that the oxidation was complete in under 10 minutes.

The results of the foregoing experiment provide useful information that will be of assistance not only in controlling the rate of absorption of CO_2 but also in determining when the distillation is to be stopped. The most rapid evolution of CO_2 is in the early stages, just before and for some time after steam begins to condense in the absorption system. If at this stage the heating is carried out carefully with a low flame, then the CO_2 is slowly displaced so that the stream of alkali absorbs all the gas that passes into the condenser. On the other hand, strong heating, especially in the case of a carbonate soil, would cause all the CO_2 to rush suddenly into the condenser and thus partially escape absorption. It is obvious therefore that careful heating is needed in the early stages.

After the steam has condensed for about 5 mins., the flame can be raised and the heating continued vigorously for a further period. The minute quantities of CO_2 which then pass over can be easily absorbed by the alkali. Moreover, condensation of the steam causes a fall in pressure in the absorption system so that the alkali actually rises to some extent in the condenser thus

making an effective seal through which no gas can escape. The distillation can ordinarily be stopped about 15 mins. after the steam begins to issue, but a time limit of that type would be only arbitrary and not always a safe criterion to follow. The efficiency of displacement of CO_2 depends largely on the rate at which steam issues from the Kjeldahl flask : the latter is determined by a number of factors such as the height of flame, volume of water in the flask and such like, so it would be desirable to choose a more definite stage for stopping the distillation.

Attention has already been directed to the fact that after the major part of the water has distilled over, there follows the rapid decomposition of chromic acid accompanied by the formation of chromium sulphate and evolution of oxygen. This is an unmistakable stage. Not only is the colour change prominent but the rapid bubbling of gas through alkali after a quiet interval is also distinctly noticeable. These changes continue over several minutes so that at any time during the interval the distillation can be stopped. Continuing the distillation beyond the gas production stage is not only unnecessary but also undesirable because there is the danger of acid vapours then passing over.

Oxidation of organic matter in swamp soils.—As already mentioned, the conditions in the wet soil are more complicated than those prevalent in the dry soil. Not only is the former more inconvenient to handle but it also includes a variety of fermentation products such as alcohols, esters and acids many of which are difficult to oxidise (Subrahmanyan, *loc. cit.*). In view, however, of the relative ease with which the humic matter of the soil can be oxidised, some experiments were carried out to determine whether in presence of soil minerals, the entire organic matter of the swamp soil can be oxidised under conditions similar to those observed in the case of the dry soil. To 10 g. lots of black cotton soil, different quantities of 1 per cent. acetic acid together with 30 c.c. of water were added and the mixtures oxidised with 10 g. of dichromate and 30 c.c. of concentrated sulphuric acid in the usual way.

It was observed that although the reaction proceeded apparently smoothly, the alkali in the absorption system turned rapidly blue suggesting that a substance (probably acid) capable of attacking the copper gauze at the bottom of the condenser was passing into the distillate. This suspicion was later confirmed by orientation and other tests which showed that acetic acid was being carried over, as such, by the steam. Examination of the liquid suspension in the Kjeldahl flask also showed that a considerable part of the added acetic acid remained unattacked. The following were the results obtained (Table IV).

5

TABLE IV.

Carbon in milligrams		
Present in the soil	Added as acetic acid	Found in the mixture
98·0	76·0	86·9
,,	32·1	70·7
,,	,,	98·5

The foregoing observations having shown that the oxidation of acetic acid is not facilitated by the presence of soil, experiments were next carried out reducing the proportion of acetic acid and increasing those of dichromate and sulphuric acid.

It was observed that increasing the quantity of dichromate above 7–10 g. for every 10 g. lots of soil containing 20 c.c. of 1 per cent. acetic acid was not only unnecessary but also, in some respects, inconvenient. Reducing the volume of acetic acid solution (including that of water added to the soil) or increasing the quantity of sulphuric acid led to increased efficiency in oxidation. The lowest proportion at which the oxidation proceeded to completion was 2 of acid (by volume) to one of solution, while with higher proportions of acid, the reaction commenced very much earlier and proceeded more rapidly. These observations are similar to those made in the case of acetic acid alone, so it may be inferred that the mineral matter of soil has no influence on the chemical oxidation of some of the more resistant forms of organic matter known to be formed under swamp soil conditions.

Effect of addition of dehydrants on the efficiency of oxidation.—Since the efficiency of oxidation of certain biological products is lowered by the presence of excessive amounts of water and since, under certain conditions, the presence of rather large quantities is unavoidable, some experiments were carried out to determine whether addition of certain inorganic dehydrants would reduce the effective concentration of water and thus lead to better results. Varying quantities of anhydrous sodium or copper sulphate were added to suspensions of Bangalore soil (10 g.) in 20 c.c. of 0·085 N acetic acid and the mixtures oxidised in the usual way with 10 g. of dichromate and 30 c.c. of concentrated sulphuric acid.

It was observed that although the anhydrous salts (especially copper sulphate) produced the desired effect by turning the aqueous suspension into semi-solid mass, the reaction mixture became liquid again on addition of

concentrated sulphuric acid. Even on heating with a low flame, the mixture soon came to boil without either gas evolution or change of colour which generally indicates the commencement of oxidation. As previously observed in cases of incomplete oxidation, the alkali in the absorption vessel turned light blue indicating that acetic acid was passing over as such into the distillate. This was subsequently confirmed by the results which yielded a consistently low estimate of 56·6 mgms. while the correct value was 94·0 mgms. The carbon present in the soil was 53·3 mgms., so the quantity of acetic acid that had oxidised or passed over as such, corresponded to only 3·3 mgms., out of a total of 40·7 mgms. Similar low estimates were obtained whether 10 or 25 g. of anhydrous sodium or copper sulphate was used. These observations would show that those salts had no effect on the progress of oxidation.

Subsequent observations showed that the anhydrous salts were ineffective because of the presence of sulphuric acid which was even more powerful as a dehydrant and withdrew the water even after combination with the salts. Addition of phosphorus pentoxide would, no doubt, effectively reduce the concentration of water, but it was felt that both on account of expense and the increased quantity of acid, it would be no improvement on the addition of excess of sulphuric acid alone.

Influence of mineral catalysts on the oxidation of organic matter in swamp soils.—Some experiments were carried out to determine whether the oxidation of more resistant forms of organic matter can be catalysed by addition of certain metallic salts or other substances which would react with chromic acid causing the liberation of oxygen. To suspensions containing 10 g. lots of Bangalore soil and 20 c.c. of 1 per cent. acetic acid or urea, 10 g. of dichromate and varying quantities of different peroxides or salts were added. Sulphuric acid (30 c.c.) was then added through the tap funnel and the oxidation conducted in the usual way.

It was observed that in the case of hydrogen peroxide (20 c.c., 3 per cent.) the reaction was instantaneous. There was vigorous evolution of oxygen accompanied by reduction of dichromate. The reaction with barium peroxide was comparatively mild. There was fairly rapid evolution of oxygen, but there was only a slight reduction of dichromate. In the case of manganese dioxide, the reduction was still milder; there was some evolution of oxygen on heating but there was no perceptible reduction of dichromate. Ferrous sulphate reduced the dichromate but did not start any further action. In presence of salts of heavy metals, the reaction proceeded in the same manner as in dilute aqueous suspensions; there was neither evolution of gas nor any other apparent improvement on the rate

of oxidation. The results showed that in spite of the promising indications observed in some of the cases, the oxidation of organic matter did not proceed to completion in presence of any one of the several catalysts that were tried. Total carbon expected in mg., $97 \cdot 7$ (soil, $57 \cdot 0 +$ acetic acid, $40 \cdot 7$). Found— Hydrogen peroxide (20 c.c., 3 per cent.), $59 \cdot 0$; barium peroxide (5 g.), $56 \cdot 6$; barium peroxide (10 g.), $51 \cdot 0$; manganese dioxide (3 g.), $61 \cdot 8$; copper sulphate (hydrated, 3 g.), $61 \cdot 8$; mercuric sulphate (3 g.), $66 \cdot 9$.

Further observations showed that although the addition of hydrogen peroxide or a solution of barium peroxide in dilute phosphoric acid led to the momentary formation of perchromic acid, there was absolutely no reaction with the organic matter of the soil. In presence of concentrated sulphuric acid there was only decomposition of the dichromate, the oxygen produced as the result of such action having no effect on the oxidation of organic matter present in the medium. Even in presence of very dilute mineral acids the reaction proceeds in the same way, the two oxidising agents acting preferentially on each other rather than on the organic matter present in the medium. In neutral medium, only hydrogen peroxide acts partially on soil organic matter. The most resistant forms are left unattacked. If barium peroxide is added by itself to a soil suspension containing dichromate and sulphuric acid, hydrogen peroxide is produced only in traces while there is copious evolution of oxygen. Ferrous sulphate as also the other mineral salts which were tried as catalysts were only partially effective. It was concluded therefore that in the case of the swamp soil containing different products of fermentation, addition of large excess of sulphuric acid is quite necessary to ensure complete oxidation.

Manganese dioxide or Barium peroxide as supplement to chromic acid in the oxidation of soil organic matter.—Estimates of nitrogen in the residue left after oxidation of carbon showed that the large quantities of chromium sulphate left in the medium interfered with the progress of subsequent digestion according to the Kjeldahl method. Other observations (which will be described in a subsequent communication) also showed that chromium tended to combine with the soil matter and thus prevented the digestion from proceeding to completion. With a view to eliminating or, at any rate, minimising this defect, some experiments were carried out using only small quantities (1 g.) of dichromate for oxidation and supplementing it by addition of varying quantities of barium peroxide or manganese dioxide. To 10 g. lots of soil suspended in 30 c.c. of water, the different oxidising mixtures together with 30 c.c. of concentrated sulphuric acid were added, and the oxidations conducted in the usual way. The following were the results: Carbon (per cent.) expected, 0.72. Found.—Manganese dioxide (3 g.),

0·54; manganese dioxide (10 g.), 0·62; barium peroxide (3 g.), 0·49; barium peroxide (3 g.), 0·52; barium peroxide (10 g.), 0·40.

It may be inferred from the above that neither manganese dioxide nor barium peroxide can usefully supplement chromic acid in the oxidation of soil organic matter.

Proportion of sulphuric acid to water and temperature of oxidation.—In the course of some previous experiments it was observed that as the proportion of sulphuric acid to water was increased, the reaction with organic matter appeared to commence correspondingly more rapidly. This suggested that the proportion of sulphuric acid had some influence on the temperature of oxidation. Some systematic experiments were carried out, therefore, adding minute quantities of urea (solid) or acetic acid (Sp. Gr. 1·075) together with solid dichromate to mixtures (50–60 c.c.) of sulphuric acid and water in different proportions and noting the temperatures at which (*a*) the oxidation as indicated by production of carbon dioxide and (*b*) the boiling of the reaction mixture commenced in each case. The temperature of the vapour distilling over was also noted in each case (Table V).

TABLE V.

Proportion of sulphuric acid to water	Oxidation of Urea			Oxidation of Acetic Acid		
	Temp. at which oxidation commences	Temp. at which the reaction mixture boils	Temp. of vapour which distils over	Temp. at which oxidation commences	Temp. at which the reaction mixture boils	Temp. of vapour which distils over
6 : 1	60–62	190*	97	64–65	199–200	97
4 : 1	66–68	170	97	68–70	168–170	97
2 : 1	70–72	150	97	73–75	160	97

It is of interest to note that although the two substances are widely different in character, the temperature at which the oxidation commences is very nearly the same for a given proportion of acid to water. Even the slight difference that was observed is presumably due to the water (about 20 per cent.) in the solution of acetic acid. As may be naturally expected, the temperatures at which the mixtures commenced boiling were determined by the proportion of sulphuric acid to water. The temperatures were not constant but tended to rise with the elimination of water. The temperature of the issuing vapour corresponds to the boiling point of water at Bangalore.

The precise nature of the conditions that determine the temperature at which oxidation commences is still obscure. Further work on this aspect of the problem is in progress.

Discussion.

The results of the present investigation lend much useful support to the previous observations (Subrahmanyan, Harihara Iyer and Rajagopalan, *loc. cit.*) regarding the behaviour of soil organic matter towards chemical oxidisers. The kinetics of oxidation show that at any rate the major part of soil organic matter is as easily oxidised as sucrose or any other carbohydrate material. The reaction is quantitative and proceeds even in presence of considerable quantities of water. On the other hand, acetic acid which is one of the products formed under swamp soil conditions, offers considerable resistance to chemical oxidation.

It is well known that the humic matter of the soil is highly resistant to the action of micro-organisms, while substances like urea are so rapidly ammonified even under swamp soil conditions that there is danger of considerable loss of nitrogen by volatilization (Subrahmanyan and Sreenivasan, 1934[2]). Acetic acid is also readily attacked by a number of insects, fungi and bacteria associated with the soil. These observations are the very reverse of those made during chemical oxidation. In the latter case, the humic matter is readily attacked while urea and acetic acid offer considerable resistance. Since under field conditions the release of plant nutrients is, to a large extent, dependent on the decomposition of organic matter, it would appear that the chemical and biological methods of oxidation can be advantageously combined in practice. As indicated elsewhere, there are several mild oxidising agents which could act on humic matter while the micro-organisms act on others so that the combined activity would result in increased benefit to the crop.

In addition to the useful information regarding the oxidation of soil organic matter, the present study has also provided a mass of data that has been of much assistance in the development of a simple and accurate method of estimating carbon in soils and biological media. This will be the subject of a later communication.

Further work on the mode of oxidation of soil organic matter by different other oxidising agents—particularly those of the milder type—is in progress. The associated nitrogen and mineral transformations are also being studied.

Summary.

1. Attention has been drawn to the relative ease with which the organic matter of the soil can be oxidised by chemical agents and the possible application of the treatment to obtain increased crop production.

2. Indirect methods of estimating soil organic matter yield comparative estimates when a number of specimens are examined, but are not sufficiently reliable for the study of carbon transformations, especially under swamp soil conditions.

3. The various factors influencing the oxidation of organic matter in dry as well as swamp soils have been discussed.

4. The usual practice of displacing carbon dioxide by a stream of (CO_2-free) air is not only inconvenient but also unnecessary. The steam generated during the heating of the reaction mixture is itself sufficient to quantitatively displace all the carbon dioxide produced.

5. Among the several agents that were tried, chromic acid was the most satisfactory in regard to speed and efficiency of oxidation. The conditions relating to the addition of reagents, distillation and condensation have been standardised.

6. The most efficient way of absorbing the carbon dioxide resulting from the reaction was found to be that of using the condenser packed with glass beads as a part of the absorption system. A slow stream of 3–4 N alkali trickling from above was found to be most suitable for the purpose.

7. The kinetics of oxidation of soil organic matter has been followed and compared with those of other carbon compounds. It has been found that in presence of chromic acid the major part of soil organic matter is almost instantaneously oxidised ; that within a few minutes after steam begins to condense, the oxidation is complete. Among the other carbon compounds only the carbohydrates are so easily oxidised while others like alcohol, acetone, acetic acid or urea offer more resistance.

8. Oxidation of soil organic matter proceeds quantitatively even in presence of excess of water provided the total quantity of sulphuric acid is at least twice the weight of the soil and the distillation is continued upto the oxygen evolution stage. Addition of increasing quantities of either dichromate or sulphuric acid above a minimum does not improve the efficiency of oxidation.

9. Some of the products formed under swamp soil conditions are highly resistant to oxidation by chromic acid. Quantitative oxidation is possible only when the proportion of sulphuric acid is at least 2 to 1 by volume.

10. Addition of dehydrants does not improve the efficiency of oxidation of the more resistant forms of organic matter. The reaction is not catalysed by addition of peroxides or salts of heavy metals.

11. Application of the results to the standardisation of an elegant and accurate method of estimating carbon in soils and biological media is indicated.

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