whether the state of the gut favourable to the formation of coprosterol persisted when the next diet (oatmeal) was tried, as, owing to an accident, the whole of the ethereal extract was lost. In the subsequent experiment with horseflesh, the gut had recovered its normal condition.

Whether any of the cholesterol of the food is actually absorbed along with that of the bile in the intestine, these experiments do not show, but others are in progress, which we hope will throw light on this point, and this we hope to make the subject of a communication in the near future.

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Bacteria as Agents in the Oxidation of Amorphous Carbon.

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The problem which presented itself to my mind in commencing the following investigation was primarily one connected with agriculture. When considering the application of such insoluble substances as charcoal, cinders, soot, etc., to the land, an explanation was sought of their ultimate fate in the soil. What becomes of the carbon? Is it oxidised into CO_2, and if so by what agency?

Little is known at present as to the means whereby amorphous carbon is rendered available for plant life, except through its union with oxygen in the process of combustion, and further investigation upon this point offered an important field of enquiry. It is well established that carbon readily absorbs oxygen, and, in the case of coal, that carbonic acid is given off, but the cause of the latter phenomenon is still obscure, and in the theories advanced to account for it no consideration is ever given to the possible action of micro-organisms.

My investigations have shown that under the action of certain bacteria a slow oxidation of amorphous carbon takes place, CO_2 is slowly evolved, and the carbon can thus be at once utilised for the nutrition of green plants.

This led to a wider consideration with regard to the action of bacteria upon certain carbon compounds such as coal and peat, and opened up the question as to whether it was possible that the vast supplies of carbon locked up in these formations could be utilised for plant life without the intervention of direct combustion. That coal undergoes considerable wastage when exposed to the air is a fact very generally known, and in the case of large storages of coal the depreciation may reach a very high percentage. The conditions often preclude this loss being entirely attributed to weathering, and the question arises: are there then any other agents, such as bacteria, concerned in the process of disintegration?

In experiments dealing with substances of the nature of coal, peat, and charcoal, quite peculiar care had to be taken for the purpose of sterilisation, which proved to be a very difficult matter; while the chemical changes which take place during heating these substances to such a degree as was necessary, and the probable conservation of gases, were all points requiring due consideration for the elimination of possible sources of error. Further, the highly sensitive character of the electrical apparatus employed rendered special precautions necessary to guard against extraneous influences. It will therefore be needful to give in detail, though as shortly as possible, the methods employed in conducting my research.

The problem was attacked by three distinct methods, briefly stated as follows:—

1. By passing a stream of air, freed from all trace of CO_2, over the material subject to investigation and determining the presence of CO_2 by titration with standard oxalic and hydrochloric acids.

2. By determining the rise of temperature due to oxidation by means of a thermopile and galvanometer.

3. By detecting, in the case of charcoal, the presence of calcium carbonate in the flasks inoculated with the bacteria.

Charcoal.

Pieces of ordinary wood-charcoal were pounded and passed through a double sieve, the first having a mesh of 1/10 inch, the second of 1/20 inch, so that in this way small fragments of fairly uniform size were retained between the two. A quantity of this charcoal was next heated by means of a metallurgical furnace to a white heat (about 1200°), in a crucible protected from any access of atmospheric oxygen. Heating to this point was a necessary treatment, as commercial charcoal is seldom sufficiently charred to drive o all volatile compounds. The entangled oxygen would thus, in the presence o[illegible] excess of carbon, be mainly converted into CO, and the calcium salts

naturally contained in the woody tissue into the oxide. This process was also important as an efficient means of sterilisation, and for the destruction of any organic matter in the shape of dust with which the material might be contaminated.

Determination of CO_2 by Titration.

The *apparatus* employed was in main outline that used by Sachs in his classical experiment on Respiration, with modifications to suit the special difficulties in this case. After some preliminary trials the form of apparatus finally adopted was as follows :—

Air drawn by means of an aspirator was passed first through a Reiset absorption apparatus containing 100 c.c. of a strong solution of caustic soda, next through a similar apparatus containing an equal volume of baryta water, then through the flask containing the material under investigation (known henceforth as the research-flask), and finally through a Reiset containing 100 c.c. of baryta water.

To avoid corks and the consequent difficulty of ensuring their perfect sterilisation, specially constructed research-flasks (Cloëz flask) were employed, in which the delivery tube, reaching nearly to the bottom, and the exit tube were fused into the neck of the flask. The flasks were thoroughly cleaned and subjected to the vapour of boiling nitric acid for some hours, a necessary precaution to remove any trace of organic matter, as such substances might give rise to CO_2 under bacterial action. All trace of nitric acid was then removed by water condensed in the flasks, this water being derived from the steam of distilled water. Such treatment rendered the flasks perfectly clean, and precluded the slightest fear of any contamination. Into such a flask about 5 grammes of charcoal, freshly heated as previously described, were introduced by means of a clean platinum spatula, the entrance and exit tubes were then plugged with asbestos heated to redness and short pieces of rubber tube fitted to them. The entrance tube was next connected with a flask containing distilled water, and steam from it blown through the research-flask. After enough water had condensed to cover the charcoal and all the air had been expelled from the flask, the apertures were securely closed by clamps while the steam was still passing through. The flask was then allowed to remain for 24 hours, during which time the charcoal would be exposed to a partial vacuum. After this interval air was allowed to enter the flask, the air being passed first through a Mohr's bulb containing strong potash, and then through a short glass tube which was plugged with asbestos freshly heated. The entrance and exit tubes, after disconnection, were again heated to redness, steam passed through, and the flask closed as before. This

operation was repeated daily for at least three days. In this manner any gases entangled in the charcoal would be removed, and at the same time complete sterilisation would be effected. Finally the excess of water was removed by evaporation.

Asbestos plugs, previously heated to redness, were always employed in place of cotton wool, as the latter might give rise to CO_2, and thus become a source of error.

Several sets of apparatus, as described, were set up, and into some of the research-flasks bacteria were introduced, while the others were used as controls.

The *bacterium* was obtained from the soil. For the purpose of isolation a number of test-tubes were partially filled with the reheated charcoal moistened in distilled water and sterilised in a steamer. A small quantity of garden soil was shaken up with water, and, after allowing the coarser particles to settle, about 1 c.c. of this water was introduced into one of the test-tubes, which was then placed in an incubator at 20° C. After two days a similar test-tube was inoculated from the first by means of a loop of platinum wire, the process was repeated in a third test-tube, and so on. Those bacteria which could not live on charcoal were thus gradually eliminated, and finally, by constant inspection, a *Diplococcus*, diameter 1 μ, was obtained in pure culture, which was employed for this research. (There is, however, no reason to suppose that this species alone is capable of oxidising carbon, probably it is a property possessed by many other species.)

The bacteria were introduced into some of the research-flasks by removing the asbestos plug and pouring in a little distilled water containing the *Diplococcus*. The aperture was then closed as quickly as possible, the asbestos replaced, and the whole end of the tube heated to dull redness in the Bunsen flame. The inoculated flasks and the controls were then treated in a precisely similar manner. The apertures were closed with rubber tubes and glass stoppers, and all the flasks placed in an incubator at 20° C.; at intervals of a week a stream of air—about 5 litres—was drawn through the apparatus, and titrations were made of the baryta water, great care being taken to prevent the latter from absorbing any CO_2 from the air during this process.

For the first week no CO_2 could be detected in the air from either the controls or the inoculated flasks. This result was not encouraging, and at this stage of the proceedings it appeared as though the investigation might prove fruitless. However, after nearly another week the air contained in the inoculated flasks gave a distinct indication of the presence of CO_2. The amount detected in this way was never large, only amounting to 7 milligrammes

per week, but it was measurable and, continuing to be demonstrable while the controls exhibited no trace of this gas, it was sufficient to encourage the further prosecution of the research and the endeavour to confirm the results by other means.

A parallel series of experiments with charcoal in which 5 litres of CO_2-freed air was drawn each morning through the research- and control-flasks gave similar results. In this arrangement the baryta water was contained in Pettenkofer tubes and titrated every seventh day. The control-flasks showed no trace of CO_2, while from the research-flasks, after the first week, an average of 8 milligrammes per week was obtained during a period of one month.

An explanation of this delayed result may be found in the fact that calcium salts are contained in the plant cells from which the charcoal was derived; the calcium oxide therefore present in the charcoal would combine with the CO_2 as soon as formed, and until the process of neutralisation was completed no CO_2 would be present in the stream of air. Also a sufficient time was required for the growth and multiplication of the bacteria.

Determination of Calcium Carbonate.

If the explanation given above were true, calcium carbonate should be present in the inoculated flasks but absent from the controls. Therefore the next step taken was an endeavour to detect the presence of calcium carbonate among the charcoal fragments.

A small portion of charcoal was removed from the inoculated flask and mounted as a microscopic slide; a weak solution of acetic acid was then run under the cover-slip and an evolution of bubbles was immediately seen to take place, while no such evolution could be observed in the charcoal from the controls.

A further confirmation was found in the fact that when freshly-heated charcoal was moistened with water and treated with acetic acid no bubbles appeared, but after exposure for some hours to an atmosphere containing CO_2, a similar treatment with acetic acid resulted in a vigorous evolution of gas.

An objection might be raised that these bubbles were due to the displacement of gases included in the charcoal, and proof is wanting that they were in reality CO_2. This proof was supplied by treating the charcoal in one of the inoculated flasks with weak hydrochloric acid, and then passing a stream of air-free CO_2 through it and then through baryta water. When this was done, barium carbonate was precipitated, and titration showed that 33 milligrammes of CO_2 had been evolved.

A further test in confirmation of the above was afforded by an artificial imitation of the conditions. About 5 grammes of freshly-heated charcoal was exposed to a partial vacuum in the manner before described, in two Cloëz flasks. One of these was filled with CO_2 and the other with air free from CO_2. After standing for 24 hours a stream of air freed from CO_2 was drawn through each of the flasks, and all traces of CO_2 would thus be effectively removed. About 5 c.c. of weak hydrochloric acid was then introduced into each and connections made as speedily as possible with the Reiset absorption apparatus. After the lapse of 24 hours the first flask showed that 34 milligrammes of CO_2 had been given off from the charcoal, and in the second flask only 2 milligrammes, this small amount being possibly due to some residual carbonates which had escaped reduction in the charcoal.

As the analysis of ordinary wood-charcoal gives about 3 per cent. of ash, and lime is one of the chief constituents, the above readings are in agreement with the amount of lime normally present in the charcoal and also with the amount of CO_2 given off from the research-flasks (inoculated with bacteria) when treated with weak hydrochloric acid. This further proves the truth of the supposition that CO_2 is only evolved after the calcium oxide has been converted into the carbonate.

Control experiments made with distilled water inoculated with bacteria, without any charcoal, etc., gave no evolution of CO_2, thus disposing of any criticism which might suppose the carbonic acid to be derived from the bacteria themselves, and not necessarily from their action upon the amorphous carbon. Moreover, the evolution of CO_2 is not confined to the duration of the first experiment, and subsequent titrations made from the same material, which had been returned to the incubators for a further period, invariably demonstrated a further production of CO_2, thus indicating a continuous process of evolution.

Electrical Method of Determining a Rise of Temperature.

Since the phenomenon of oxidation is accompanied by an evolution of heat, it follows that if charcoal were undergoing oxidation it should be possible to detect any rise of temperature due to this process. The amount of CO_2 evolved, however, being sm[illegible]ll during the period of observation, the rise of temperature to be expected wo[illegible]ld at most be only a fraction of a degree.

In order to discover whether t[illegible]ere was any difference in temperature between the sterile and inoculated charcoal, the apparatus shown in the figure was designed. It consisted of two specially constructed flasks connected with a thermopile and placed in an incubator, with leads passing through a perforation in the side of the incubator to a galvanometer.

Measurement by means of the galvanometer of the E.M.F. produced by two thermo-elements at different temperatures, one placed in a sterile and the other in an inoculated flask, served to determine the difference of temperature between the two.

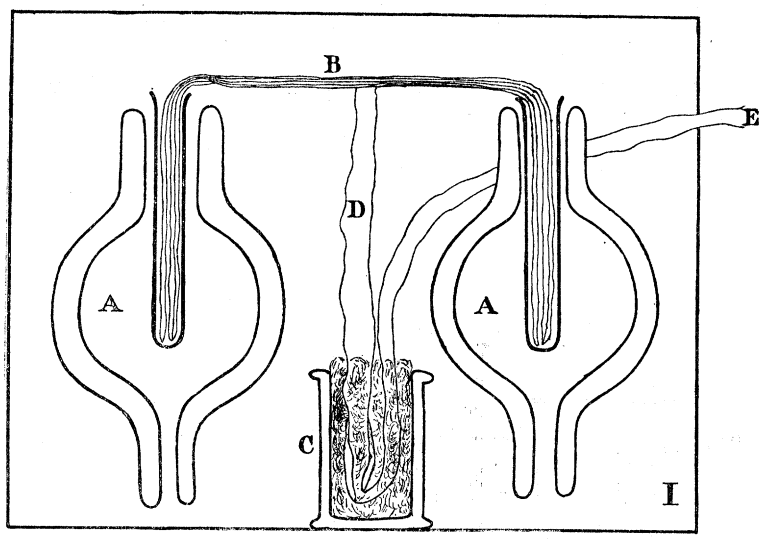


Diagram of Interior of Incubator.

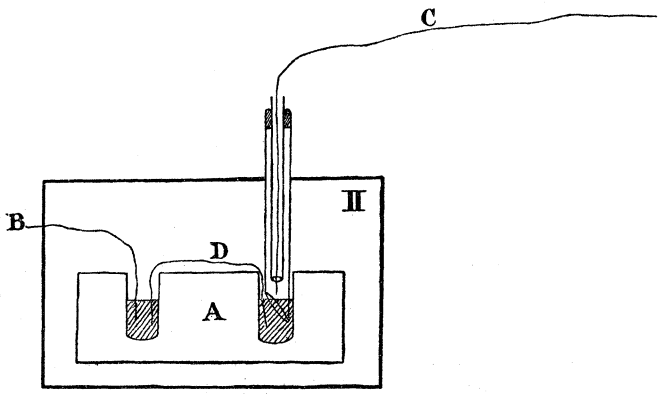
AA, Double-walled vacuum flasks.
B, Thermopile, with terminals inserted in test-tube inside these flasks.
C, Jar packed with cotton-wool, containing junctions of thermopile wires D and galvanometer leads E.

A thermopile with 20 junctions of the iron-nickel combination was employed and formed a highly sensitive instrument. The wires, silk-covered, 32 gauge, had the junctions carefully brazed together and coated with shellac, and the wires themselves were enclosed in thin rubber to give protection against moisture.

The mirror galvanometer used was of the Broca type with a resistance of 50 ohms, and the electromotive force produced by a difference in temperature of 1/200° C. between the terminals registered a deflection of one scale-division. In an experiment of this nature, extreme precautions must be taken with all the junctions of different metals to avoid any thermal effects.

To ensure that the junctions of the thermopile wires and leads to the galvanometer were maintained at the same temperature, these were connected with binding screws, insulated with thin rubber, and packed with cotton wool in a glass jar placed inside the incubator. The galvanometer leads passed through a small perforation in the side of the incubator to a key of special construction and finally to the galvanometer.

A solid block of paraffin formed the basis of the key. Into this block four holes were bored to serve as mercury cups and these were connected in pairs by a metal bridge. One cup of each pair received the wires from the galvanometer and into the others were fixed short glass tubes. Through these tubes the ends of the wires from the incubator could be raised or lowered to break or make a contact. The key was packed with dried cotton wool and enclosed in a box, the two glass tubes slightly projecting through perforations in the lid.



Section of Special Key.

A, Paraffin block with two mercury cups.
B, Lead to galvanometer.
C, Lead from incubator.
D, Connecting bridge of copper wire.

All the junctions of the different metals were thus carefully protected from light or changes of temperature which might set up an electric current, however small, and thus give rise to experimental error.

The accuracy of the measurements of temperature, as recorded by means of the thermopile and galvanometer, is shown by the fact that the calculated differences of temperature were found to correspond with those registered by standard mercurial thermometers under the same conditions.

The Hearson's incubator was first regulated for 20° C., but as this apparatus was heated from one side, it was necessary to ascertain whether the temperature inside was uniform. The terminals of the thermopile were packed with dry cotton wool in glass jars, and placed in different positions inside the incubator, and by this delicate method a difference of temperature of 0°·09 C. was detected between the two sides. On discontinuing the heat and allowing the incubator to assume the laboratory temperature, 14° C., this difference gradually disappeared, and I was able to determine, by

renewing the tests, that the air contained within the incubator was then maintained at a strictly uniform temperature. The galvanometer leads passing through the incubator wall obviated the necessity of opening and closing the door, and permitted the research-flasks to remain quite undisturbed.

The glass flasks designed for this experiment were constructed with double walls. The inner flask, with a capacity of about 300 c.c., was prolonged into two tubes diametrically opposite to each other, the upper one serving for the introduction of the charcoal and the lower for the escape of any CO_2 that might be formed. This inner flask was surrounded by a similar larger flask to provide a vacuum, the intermediate space being exhausted of all air in order to prevent any radiation of heat.

Two similar vacuum-flasks were employed. After washing with fuming nitric acid, to remove any organic matter and for the purpose of sterilisation, they were filled with freshly-heated charcoal, which was moistened with sterile distilled water until it had absorbed all the water possible, the surplus being allowed to escape by the lower tube. It is important to note that the flasks were filled from the same supply of distilled water and with the same quantity. Into each of these flasks there was then inserted a thin test-tube previously washed externally in nitric acid and sterilised over a Bunsen flame, and the flasks were plugged with sterile asbestos wool. Finally, the iron-nickel thermopile was introduced into the test-tubes.

The flasks were then placed in the incubator, the connections made with the galvanometer, and the apparatus was complete. Observations were taken at frequent intervals. When first arranged, the unavoidable handling of the metallic junctions necessarily produced thermo-electric currents, but these died away when the apparatus was left undisturbed for two or three days. When on depressing the key no movement of the spot of light could be observed, the whole arrangement was considered to be perfectly reliable, and the experiment could be proceeded with.

One of the flasks was then inoculated with the bacterium, and thereafter readings with the galvanometer were repeatedly taken. At first no deflection was apparent on depressing the key, but after two days a movement of the spot of light could be observed, and by depressing the key synchronically with the swing of the mirror, a deflection of several divisions of the galvanometric scale could be registered. The thermo-electric current gradually increased until after an interval of six days the maximum deflection of 38 divisions was attained, proving a definite rise of temperature in the inoculated flask, while the sterile flask indicated no increase.

To ascertain with certainty that the movement of the mirror was a correct

measure of the electromotive force, and in reality due to the difference of temperature between the two flasks, the terminals of the thermopile were interchanged, that is, the terminal in the inoculated flask was placed in the sterile flask and *vice versâ*. The galvanometer then indicated an equal current in the reverse direction (after a sufficient time had elapsed to counteract the effects due to handling), thus proving that the deflection was due to the difference of temperature and not to any accidental error in the apparatus.

By measurement, the maximum deflection indicated by 38 scale-divisions corresponded to a rise in temperature of 0°·19 C., and this temperature was maintained for the further period of a week, when the apparatus was taken down. *This conclusively establishes the fact that a measurable rise of temperature takes place in charcoal, owing to oxidation through the action of bacteria.*

Lamp-black.

Experiments were also undertaken with commercial lamp-black as another source of amorphous carbon, the results of which may be briefly stated. This substance in the first instance was heated to a white heat in the metallurgical furnace; afterwards it was soaked in aqua-regia for nine days, then carefully washed in distilled water, and any excess of acid still remaining neutralised with metallic sodium cut from the centre of a block to ensure the absence of any naphtha. The lamp-black so prepared was treated in the Cloëz flasks in the manner described for charcoal, and some were inoculated with bacteria, while the rest were kept as controls. For a period of 24 days the flasks inoculated with bacteria showed, upon titration, the evolution of a small quantity of CO_2 (17 milligrammes) as compared with the non-inoculated flasks.

The result of the titrations agreeing so closely with those obtained in the case of charcoal, it was not deemed necessary to determine the rise of temperature by the thermo-electric method.

Peat.

A set of experiments upon peat, corresponding to those already described for charcoal, was carried out in an exactly similar manner, except that the peat was not calcined; it will not, therefore, be necessary to do more than briefly state the results. The peat was obtained from the Solway district, and was of the ordinary dried kind such as is used for fuel, *Sphagnum* being the chief constituent.

First, as regards the evolution of CO_2. Several flasks were prepared containing small fragments of the dried peat, soaked with water, and sterilised by discontinuous boiling. A stream of air, carefully freed from all trace of CO_2, drawn through the research-flasks and then through baryta water, failed to exhibit any trace of this gas, even after many days. When this result was well established, some of the research-flasks were inoculated with bacteria, and within a day the cloudy precipitate appearing in the baryta showed that a considerable amount of CO_2 had been evolved as the result of bacterial action, and a copious precipitate continuing to be deposited indicated that a somewhat vigorous oxidation was taking place.

Secondly, to measure any rise of temperature due to oxidation, the experiments with the double-walled vacuum-flasks, the thermopile and galvanometer, were again set in operation, substituting only peat for charcoal. The results were completely in accordance with those noted for the charcoal, except that, as might be expected from the nature of the substance and the consequent greater evolution of CO_2, the oxidation was more vigorous and a greater rise of temperature was recorded. It was found that the inoculated flask maintained a temperature of 1°·05 C. above the incubator for a considerable time.

These experiments clearly show that when peat is exposed to damp air and to the action of suitable organisms it decays rapidly, with the evolution of CO_2 *accompanied by a rise in temperature.*

Coal.

The investigation of ordinary household coal presented many difficulties, chiefly on account of problems connected with sterilisation and the presence of occluded gases, and I have not been able to entirely overcome some special difficulties of the case. Obviously the inflammable gases contained within the coal and its combustible nature rendered sterilising by dry heat an impossibility.

For the purpose of experiment the coal was taken from the centre of a large piece to avoid contamination with foreign matter; it was pounded and passed through sieves similar to those used for the charcoal, and then sterilised in the same manner by passing steam through the Cloëz research-flasks and subjecting the coal to a partial vacuum. The titrations showed that the flasks which were inoculated with bacteria gave off some 10 milligrammes of CO_2 in excess of the non-inoculated flasks during the course of three weeks, but even with the strictest precautions traces of CO_2 were found in the non-inoculated flasks, owing to the escape of this gas from the occluded state. It was this difficulty which first suggested that the measure-

ment of any rise of temperature was a better means for testing oxidation than the collection and determination of the carbonic anhydride.

The thermopile was therefore relied upon in order to determine whether coal sterilised by discontinuous boiling gives out any heat, and whether any thermal changes occur consequent upon the addition of bacteria. Small fragments of coal obtained as described were placed in a flask and immersed in distilled water, the aperture being covered with a small beaker, as cotton wool was inadmissible as a plug. The flask was steamed for five hours on two consecutive days, and on the third day the excess of water was partially driven off by boiling. The coal so treated was then introduced into a sterilised double-walled vacuum-flask, and placed in the incubator, a thermopile was inserted as before, and the connections made with the galvanometer according to the method previously recounted for charcoal. Immediately after setting up the apparatus, the thermopile indicated a temperature of the coal considerably above that of the incubator, this being due to its retaining some heat after boiling; but after 26 hours the research-flask had cooled down to the temperature of the incubator. Following this interval, the temperature of the coal gradually descended, which may be explained by the evaporation continuously taking place from the damp fragments of coal. Then for some three days it remained at 0°·2 below that of the incubator, and it was quite clear that the coal thus sterilised generated no heat.

This point being determined, the coal was inoculated with bacteria by pouring in distilled water containing these organisms, care being taken that the temperature of this added water was below that of the research-flask. The reduction of temperature was at once indicated by the thermopile, the spot of light moving off the galvanometric scale in the direction opposite to the movement when the warm coal was first put in. Twenty-four hours after inoculation the temperature of the research-flask had not only risen to that of the incubator, but it had increased to 0°·08 C. above it, and afterwards a marked rise of temperature amounting to 0°·18 C. was steadily registered for 11 days.

In the experiment just described, one terminal of the thermopile was inserted in the research-flask, and the other in a glass jar packed with cotton wool placed in the incubator, and in this manner any difference of temperature between the research-flask and the incubator could be measured. But the experiment would not be complete without a control, and a second double-walled vacuum-flask, containing fragments of coal from the same source and treated in a precisely similar manner, was also placed in the incubator with a second thermopile, one terminal in each flask. The coal, however, in this

second flask was not inoculated with bacteria. Throughout the whole duration of the experiment, the control always maintained a lower temperature (0°·38 C.), than that of the inoculated flask, and by means of a third thermopile it was proved to possess a temperature 0°·19 C. lower than that of the incubator.

For a period of 11 days the temperature of the control flask exhibited no upward tendency, while that of the inoculated flask rose from a point much below that of the incubator to nearly 0°·4 C. above it, and steadily maintained that degree of heat. *It is thus clearly demonstrated that bacteria have a decided action upon coal, resulting in a distinct rise of temperature, and that this increase of temperature does not occur when the coal is preserved from bacterial action.*

Some further experiments with the thermopile and galvanometer upon moist and dry coal emphasise these conclusions. At a temperature of 40° C. the coal moistened with distilled water and inoculated showed a difference of temperature of 1°·25 C. above that of similar coal dried at 100° C. It was also found that at a temperature of 4° C., when the activity of the bacteria would be reduced almost to a minimum, the difference between sterile and non-sterile charcoal was inappreciable, amounting to only 0°·03 C.; while at 14° C. the amount registered was 0°·19 C., which shows that the difference of temperature increases as the thermal conditions become more favourable to bacterial life.*

Important additional evidence that the CO_2 production from the carbon is undoubtedly due to bacterial action would be afforded if it could be shown that the CO_2 is only evolved under conditions consistent with the life of these organisms. I owe to Dr. F. F. Blackman the suggestion that it would be a critical test to show if the CO_2 production increased with a rise of temperature, and whether it goes up or down with causes that have the corresponding effects upon bacterial activity.

With the object of elucidating this point, a further series of experiments was undertaken. Cultures of coal and charcoal were prepared under varying

* In this investigation the actual measurements given must not be understood as representing a quantitative analysis, which would be impossible under the conditions of treatment necessary to preserve the charcoal from contamination. The figures must be regarded only in a qualitative sense, and all that is claimed is that the titration method shows definitely that CO_2 is given off from the research material only when bacteria are present.

From the equation $C+O_2 = CO_2+97650$ calories we learn that 1 milligramme of CO_2 is produced by oxidation, with the evolution of 2·22 calories, and hence the rise of temperature measured by the thermopyle is of the same order as the heat derived from oxidation. But as it is impracticable to suddenly destroy all bacterial action in a vacuum-flask and determine its rate of cooling, an exact equation cannot be obtained.

conditions of treatment, and these were subjected to different temperatures of 20°, 30°, 40°, and 100° C. The first three were maintained by Hearson's incubators and the last by a steamer continuously boiling. Cloëz flasks were employed as before, each containing 5 grammes of the research material.

The conditions selected for the purpose of experiment were:—

(a) *The Provision of Moisture suitable for the Growth of Micro-organisms.*—The charcoal or coal in each flask, after inoculation, was moistened with distilled water, and the flasks then sealed. One of each kind was placed in the steamer, and the others in the incubators at the different temperatures.

(b) *Absolute Dryness which would inhibit Bacterial Life.*—The flasks, after washing, were placed in a drying oven at 100° C., and then heated with the Bunsen flame while a stream of air dried by H_2SO_4 was drawn through them, this operation being repeated several times. The coal and charcoal were also dried for some hours at 100° C., and, while still hot, inserted in the flasks. To remove any trace of CO_2, air was again drawn through them, first passing through a Reiset with a strong solution of caustic soda and then through H_2SO_4. The flasks were sealed as speedily as possible and placed in the incubators.

(c) *Treatment with Antiseptics.*—A 2-per-cent. solution of corrosive sublimate and a solution of iodine in potassic iodide, 95 c.c. of water with 5 c.c. of solution of iodine (12·59 grammes I + 18 grammes KI + 1000 c.c. H_2O) were the antiseptics preferred, and an excess of chloroform vapour was also employed in deference to the prevalent idea that this substance can be relied upon as an antiseptic. The use of any carbon-compounds, however, such as chloroform or prussic acid, etc., was unsatisfactory, as a special investigation might be required to determine whether they were themselves responsible for any CO_2 production.

The whole series of flasks remained in the steamer and the various incubators for a definite period, after which a stream of air-free CO_2 was drawn through the flasks, then through baryta solution, and titrations made.

The results obtained with uncalcined charcoal are given in the table for the sake of comparison.

The results as set forth in the above table show that under the conditions suitable for the growth of bacteria increasingly higher temperatures indicated a corresponding increase in the amount of CO_2 given off, the production at 40° C. being greatly in excess of that at 20° C.; while at the temperature of 100° C., at which active bacterial life would be impossible, there was no evolution of CO_2. Also, under dry conditions which prevented bacterial growth, no CO_2 was evolved at any of the temperatures tried.

The treatment with antiseptics proved to be untrustworthy and it was evident that this method must be abandoned and the results discarded as

valueless for the purposes of the present investigation. There is a danger of chemical reactions taking place, and directly any antiseptic is employed the research becomes complicated by the introduction of an entirely fresh set of problems which need special investigation.

Table of Results of Titrations.

Duration of Experiment 20 Days. 5 grammes of Material used in each case.

Temperatures	Milligrammes of CO_2.			
	20° C.	30° C.	40° C.	100° C.
Coal—				
Moist inoculated	2·0	3·1	4·6	0·0
Moist sterilised by boiling	0·0	0·0	0·0	0·0
Dry	0·0	0·0	0·0	0·0
Charcoal—				
Moist inoculated	0·77	1·1	2·5	0·0
Moist sterilised by boiling	0·0	0·0	0·0	0·0
Dry	0·0	0·0	0·0	0·0
Charcoal uncalcined—				
Moist inoculated	5·4	8·0	22·8	—

Neither corrosive sublimate nor chloroform were found to be effective as antiseptics. In the flasks treated with both these substances a microscopic examination at the conclusion of the experiment showed the presence of motile bacteria (not Brownian movement), which stained with gentian violet and grew feebly as stab-cultures on gelatine. In the flasks treated with iodine no movement of the bacteria could be observed and they appeared to have been entirely destroyed. It should be mentioned that a weaker solution of iodine proved to be less efficient, owing possibly to the smaller margin allowed for its reduction.

I was not prepared to find the bacteria able to resist a 2-per-cent. solution of corrosive sublimate, though chloroform I well knew to be of little use. The value of antiseptics, however, in securing absolutely sterile conditions, is often doubtful, and as the author has previously pointed out, even very strong percentages of such poisons may be quite ineffective in destroying micro-organisms (8 and 9).

The recent work of Adrian J. Brown (2) upon "The Existence of a Semi-permeable Membrane enclosing the Seeds of certain Gramineæ" throws considerable light upon the action of antiseptics. Brown has shown that when these seeds are soaked for three days in a 5-per-cent. solution of cupric sulphate, silver nitrate, and potassium ferrocyanide, no trace of these substances penetrated to the interior of the grain, though water was freely

absorbed. After this treatment the vitality of the seeds was not impaired nor their power of germination. Further, the semi-permeable covering enclosing these seeds permits the absorption of water from weak solutions of acids and alkalies, while excluding the latter compounds; but it does not prevent the passage of iodine.

An important question raised by these investigations is: to what extent are plants in general protected by this means, and may it be that some bacteria may possess a cell-wall of a semi-permeable nature which acts as a protective envelope and so accounts for the immunity from injury which many species possess? Some observations I have made on the behaviour of certain bacteria in solutions of mercuric chloride seem to indicate that this might be so. Plainly, they have some means of protection against a strong solution of such a poison, but it proved too difficult a piece of microscopy to determine whether the sublimate was actually excluded by the enclosing membrane.

The well-known oligodynamic theory of Nägeli (5) may also be cited as bearing upon the effect of antiseptics in certain cases. His observations show that the toxic action of poisons is reduced in the presence of certain insoluble substances such as graphite, etc. True and Oglevee (13) have more recently extended the work in this direction and entirely in favour of Nägeli's conclusions. Their results establish the fact that the presence of certain insoluble substances tends to decrease the toxic activity of solutions of strongly toxic compounds; and their experiments with mercuric chloride prove that the presence of insoluble bodies modifies very markedly the toxic influence of this salt upon the roots of seedlings. The theory advanced is the attraction of the ions, or molecules, of the dissolved substances for the solids and their absorption by the latter.

An explanation is thus afforded of the inefficiency of a high percentage of corrosive sublimate and the behaviour of iodine as an antiseptic, in the presence of finely-divided coal and charcoal. The insoluble nature of the research material, and the possibility of a semi-permeable or selective property of the bacterial cell-wall, appear to offer an intelligible interpretation of the uncertain effect of the antiseptics employed in this research.

The whole question of the dependence upon antiseptics requires consideration, and it is evident that the efficiency of any one cannot be taken for granted, but must be tested for the special conditions under which it is employed.

Lately, Stoklasa (11) has published a preliminary note upon the oxidation of coal and charcoal, and concludes from comparative researches upon "sterile and non-sterile" coal that the evolution of CO_2 is due to: (1) Auto-oxidation, (2) the action of an enzyme. The theory of auto-oxidation is not

in accordance with my own experiments, which show that while the amount of CO_2 produced increases with a rise of temperature, CO_2 is not given off at the supra-vital temperature of 100° C., nor under other conditions which prevent the growth of bacteria.

In this preliminary note it is not clear what means Stoklasa employed for sterilisation. If he trusted to corrosive sublimate, the acid reaction commonly found in this salt would have to be taken into account as a source of CO_2 in the presence of any carbonates, as well as other causes affecting its reliability. In some previous experiments Stoklasa (12) himself notes that, notwithstanding all care, active bacteria appeared on the roots of sugar beet which had been steeped for 25 minutes in a 0·5-per-cent. solution of mercuric chloride, and this he attributes to the possible introduction of these organisms in a stream of vapour passed through the apparatus. It seems more probable, as I have proved, that the bacteria flourished in spite of the treatment with mercuric chloride.

Further, Stoklasa does not state whether he re-calcined the charcoal. During re-calcination, various gases are given off, and microscopic examination shows that the cell-wall in ordinary charcoal is often incompletely charred. Thus the combined carbon in ordinary charcoal would be readily attacked by micro-organisms, and on this account the evolution of CO_2 is much greater from uncalcined charcoal than after reheating to about 1200° C.

If the oxidation of carbon takes place through the action of an enzyme, this naturally assumes the presence of a living cell, or, in other words, the oxidation is due, primarily, to bacteria.

General Conclusions.

The methods of experiment which have been dealt with attack the problem from totally different standpoints, and the accumulated evidence affords convincing proof that amorphous carbon slowly undergoes oxidation through the agency of bacteria.

The dependence on antiseptics is shown to be very treacherous, but it is clear that when complete sterilisation is secured by discontinuous boiling, there is no production of CO_2. The results obtained by the experiments with the varying degrees of temperature and under the dry conditions are also of critical importance and establish beyond question my contention that the amounts of CO_2 given off are really due to bacterial activity, and not to any chemical action in the coal or charcoal. If the evolution of CO_2 had proceeded steadily beyond the supra-vital temperature, it would have pointed to a non-vital change, but the fall of CO_2 at the death point clearly indicated the cessation of a vital process.

The determination, by measurement with the thermopile, of the rise of temperature due to oxidation becomes a very valuable confirmatory experiment in conjunction with the method of testing the evolution of CO_2 by titration.

It has been conclusively established by means of the thermopile that a definite rise of temperature occurs when carbonaceous substances such as charcoal, coal, peat, etc., are subject to the action of certain bacteria. And in this connection it must be remembered that the rise of temperature is maintained for some considerable time, and that the double-walled vacuum-flask, with at least a portion of the wires composing the thermopile, are steadily preserved at this temperature above the surrounding medium, in spite of any loss from radiation. There must thus be a continuous dissipation of heat and the amount generated is therefore more than actually appears; it must also be taken into consideration that this rate only applies to conditions *in vitro* and in a laboratory. Probably, in the soil, the carbon would be attacked by micro-organisms under circumstances more favourable to their activity and the oxidation would proceed much more rapidly. It is of importance to recognise that every process of oxidation raises the temperature in an appreciable degree, and this is a factor which should be taken into account in all problems relating to the soil.

Indeed, the action of bacteria in promoting exothermal changes is a subject too generally neglected. It must now be recognised as possessing a practical bearing upon investigations connected with oxidation of coal. The suggestion may also be made that in some cases of spontaneous combustion of coal, the heat generated by microbial activity is an influence to be taken into consideration, and may be a dangerous motive force acting upon explosive gases.

That carbon should be proved to undergo oxidation by bacteria is not surprising when we consider the fact that nitrogen undergoes the same process, while the oxidation of sulphur by bacteria has been established by Beijerinck (1) and quite recently that of hydrogen by Kaserer (4). The author has previously shown that oxalic acid undergoes decomposition into CO_2 and H_2O by the agency of a soil bacterium (6), a result which has since been confirmed independently by Hall (3).

Incidentally the present investigation throws some light upon the formation and decomposition of coal. In the ordinary course of events vegetable matter gradually undergoes a process of decay, countless bacteria and fungi deriving their sustenance from it and gradually effecting the dissolution of the cellulose and other compounds, until, ultimately, the end-products are reached. This process demands, among other things, a constant supply of oxygen, and in the absence of this element only a partial reduction

can be attained. This is well exemplified in the case of peat, where the vegetable *débris* in a wet and sodden condition is excluded from a sufficient supply of oxygen, and therefore the oxidising organisms are unable to carry on their work, any further changes must be due to anaerobic forms, and the decay is necessarily incomplete. In a similar manner it may be supposed that the large deposits which form the basis of coal are due to plant remains which, in the first instance, were precluded from complete oxidation owing to their submerged situation, where vegetable matter could only be acted upon by anaerobic bacteria.

In this connection, Renault's (10) researches upon Fossil Bacteria are of great interest. He brings forward evidence to show that bacteria have existed since Devonian times, and have played a considerable part in the destruction and decomposition of vegetable and animal tissues from this remote period. Microscopic sections have furnished remarkable proof of their presence in the upper Jurassic beds, in the Permian Strata, the Upper, Middle, and Lower Coal Measures, in the Carboniferous Limestone, and in the Devonian, and these illustrate in a remarkable manner the destructive action of numerous micro-organisms upon organic remains imbedded in these formations. Renault states that in many cases the minutest details have been preserved in such perfection that it has been possible to detect the bacteria often more easily in the fossilised than in the living state. All stages in the disintegration of the cell-tissues are clearly exhibited, and it is proved that the *rôle* of these micro-organisms has been identical with that which they perform in the present day.

According to Renault, "If in the formation of coal there are two distinct phases, one, purely chemical, which has brought the remains of plants to a certain composition answering roughly, in the case of 'houille de bois pur,' to the formula $C_{10}H_8O$, the second, simply mechanical, due to a slow compression in a permeable medium, the first of these phases can be attributed to a bacterial fermentation developed in the marshes, ponds, deltas, and arrested by periodic floods, carrying away a portion of the macerated plants and transporting them into lakes and seas, where maceration became impossible." In later times heat and pressure would convert this partially decayed vegetable *débris* into coal. The decay has, however, only been arrested, and, like peat, when a sufficiency of oxygen is available and the necessary conditions for the life of aerobic organisms are presented, the decomposition proceeds, the elements are reduced to their simplest compounds, and the carbon is once more liberated in the form of CQ_2 to play its *rôle* in the life cycle.

Coal and peat are shown to be subject to the same laws as other organic

matter, and such substances remain unsusceptible to change only so long as bacterial action is excluded.

It is quite evident that where there is surface exposure bacteria must play a large part in the disintegration of coal, which is one of the most insoluble substances known, and that these organisms form an invaluable agency in assisting the circulation of carbon and again converting it to the uses of Nature.

Summary.

Under conditions of exposure to the air, a slow oxidation of amorphous carbon takes place through the agency of bacteria. This has been conclusively established by experiments upon such carbonaceous substances as charcoal, lamp-black, coal, and peat.

When these substances are subjected to bacterial action carbonic acid is given off, as estimated volumetrically by absorption in baryta solution and titration with standard oxalic and hydrochloric acids.

The amount of CO_2 given off increases in proportion to the rise of temperature and ceases to be evolved at a supra-vital temperature. There is no evolution of CO_2 under perfectly dry conditions such as preclude the possibility of bacterial life.

A distinct rise of temperature occurs through the action of bacteria. The heat generated was determined by measurement, with a galvanometer, of the electromotive force produced by the difference of temperature between two thermo-elements, one placed in a sterile and the other in an inoculated flask.

The evolution of CO_2 and the accompanying rise of temperature does not take place when carbonaceous substances are preserved from the intrusion of micro-organisms.

The heat generated by microbial activity is an influence to be taken into account in connection with the oxidation and spontaneous combustion of coal; it may be a dangerous motive force acting upon explosive gases.

The oxidising action of bacteria must be largely responsible for the disintegration of coal and the high percentage of depreciation which it undergoes in store.

Coal and peat, like other organic matter, are liable to decomposition as soon as conditions are presented suitable for the life of aerobic organisms. The carbon is then once more liberated in the form of CO_2 to play its *rôle* in the life cycle. It is thus conceivable that the vast supplies of carbon locked up in the world's coal-fields may become available for plant nutrition without the intervention of direct combustion.

I have to express my indebtedness to my colleague, Dr. Morris-Airey, for much help which he has given me in the preparation and testing of the electrical apparatus employed in this research, and I take this opportunity of thanking him for the kind way in which he has always been ready to give me the benefit of his assistance in any technical difficulty.

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LITERATURE.

1. Beijerinck, 'Ueber die Bakterien, welche sich im Dunkeln mit Kohlensäure als Kohlenstoffquelle ernähren können," 'Cent. f. Bakteriologie,' abt. II, vol. 11, 1903—04.
2. Brown, A. J., "On the Existence of a Semi-permeable Membrane enclosing the Seeds of some of the Gramineæ," 'Annals of Botany,' vol. 21, 1907.
3. Hall, "The Effect of Plant Growth and of Manures upon the Retention of Bases by the Soil," 'Roy. Soc. Proc.,' B, vol. 77, 1905.
4. Kaserer, "Die Oxydation des Wasserstoffes durch Mikro-organismen," 'Cent. f. Bakteriologie,' abt. II, vol. 16, 1906.
5. Nägeli, "Ueber oligodynamische Erscheinungen in lebenden Zellen, mit einem Vorwort von S. Schwendener und einem Nachtrag von C. Cramer," 'Denkschriften d. Schweizerischen Naturforschenden Gesellschaft,' vol. 33, 1893.
6. Potter, "On the Decomposition of Oxalic Acid by Bacteria," 'Univ. of Durham Phil. Soc. Proc.,' vol. 2, 1903.
7. ——— "Bacteria as Agents in the Oxidation of Amorphous Carbon," Brit. Assoc., Cape Town, 1905.
8. ——— "On a Bacterial Disease of the Turnip (*Brassica napus*)," 'Roy. Soc. Proc.,' vol. 67, 1900.
9. ——— "On the Occurrence of Cellulose in the Xylem of Woody Stems," 'Annals of Botany,' vol. 18, 1904.
10. Renault, "Recherches sur les Bactériacées Fossiles," 'Annal. d. Sci. Nat.,' Bot., series 8, vol. 2, 1895.
11. Stoklasa, "Ueber die anaërobe Atmung der Samenpflanzen und über die Isolierung der Atmungsenzyme," 'Ber. d. Deut. Bot. Gesell.,' vol. 25, 1907.
12. ——— "Der anaërobe Stoffwechsel der höheren Pflanzen und seine Beziehung zur alkoholischen Gärung," 'Beit. z. Chem. Physiol. u. Path. v. Fr. Hofmeister,' vol. 3, 1903.
13. True and Oglevee, "The Effect of the Presence of Insoluble Substances on the Toxic Action of Poisons," 'Botanical Gazette,' vol. 39, 1905.