LII.—*On Nitrification.* Part III.

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CONTENTS.

	Page		Page
Introduction.....	637	1. Alkalinity of the solution	653
Some additional proofs that nitrification is due to an organism..	640	2. Concentration of the solution.....	662
Present position of this theory of nitrification	641	3. Character and amount of nitrifying organism	664
Mode of work in recent Rothamsted experiments	642	4. Depth of solution	666
The distribution of the nitrifying organism in the soil.....	645	5. Proportion of organic carbon	667
Nitrogenous substances susceptible of nitrification	650	Minimum temperature at which nitrification occurs	668
Influence of various conditions on the process of nitrification :—		Reduction of nitrates by soil	669
		The nitric acid contained in human urine.....	669

FIVE years have elapsed since I last reported to the Society (Trans., 1879, 429) the results of my investigations on the process of nitrification, conducted in the Rothamsted Laboratory. During these five years the investigations have been continued, but have been chiefly confined to an attempt to elucidate the causes which determine the nitric or nitrous character of the final product. This special branch of the inquiry, in spite of much labour, is not yet ripe for publication.

Various results, however, have been obtained from time to time, and these I now propose to lay before the Society.

Before describing the new results obtained at Rothamsted, it will be well to notice the recent work of other investigators in the same field, and also to say a few words on the present position of the theory which ascribes nitrification to the action of a living organism.

I must in the first place refer to the excellent paper by J. Soyka (*Zeitsch. f. Biol.*, 1878, 449), with which, though published previously to my last report, I have only lately made acquaintance. It treats chiefly of the nitrification of urine under various conditions. Soyka's experiments directly support the conclusion of Schloesing and Müntz, that nitrification is due to the action of a living organism.

Two papers have been published by Schloesing and Müntz (*Compt. rend.*, 89, 891, 1074*) since the date of my last communication. In these papers, the authors describe the character of the organism producing nitrification, which they separated by means of systematic cultivations in a fluid medium: the organism is apparently a micro-coccus. They also state the range of temperature to which the action of the organism is limited. The influence of various other conditions is described.

There are a few points of difference between the conclusions arrived at by these French chemists and those which I have already published. The first is apparent only. It was stated in my last paper that "bacteria are incapable of producing nitrification." This statement was far too general. It is, in fact, now shown that the nitrifying organism is a member of the great family of bacteria. In the sense, however, in which the phrase was employed it was correct. It was, in fact, used in reference to results showing that nitrification did not commence in solutions largely infected with ordinary atmospheric bacteria, a result with which the later statements of Schloesing and Müntz entirely agree. The second point of difference concerns the temperature at which nitrification ceases. Schloesing and Müntz found 37° to be the most favourable temperature. Nitrification occurred, though at a greatly diminished rate, at 45°, and to a very slight extent at 50°. In my own experiments, already published, I was unable to obtain nitrification at 40°. It is probable that the different result is owing to the different conditions which obtained in the two series of experiments. Unfortunately the methods of work adopted by the French chemists have never been published. The third point of difference is regarding the conditions which determine the formation of nitrites rather than of nitrates in a nitrifying solution. Schloesing and Müntz regard the difference in the product as due to differences

* Some fuller details are given in the *Leçons de Chimie Agricole professées par Th. Schloesing*, 1883.

in the conditions of temperature and aëration. Without denying the influence of certain external conditions, my own results clearly point to a difference in the character of the organism as being in many cases the chief determining factor. It is indeed possible to have two similar solutions, under identical external conditions, in one of which only nitrites, and in the other only nitrates are being produced; the difference being determined by the character of the organisms with which the respective solutions have been seeded.

The late Dr. Angus Smith, in his Report made in 1882 to the Local Government Board, as Inspector under the Rivers' Pollution Prevention Act, has described many experiments on nitrification. While speaking most courteously of the results published by other investigators, leading to the conclusion that nitrification is the work of a living organism, he maintains that nitrification may also take place by a process of simple oxidation by atmospheric oxygen. He says, for instance, on p. 26, "Bodies containing protein compounds, when in abundance of water and in common air, may oxidise and form nitric acid." As this view seems still to be held by others, it will be well to consider briefly the evidence which is adduced.

Dr. Angus Smith's experiments show (as was already known) that ammonia is readily converted into nitrate both by hydrogen peroxide, and by potassium permanganate in the presence of free alkali. They also show that nitrates are abundantly formed when a weak solution of blood is left in contact with permanganate. These results clearly furnish no argument in favour of the view that nitrates may be formed by simple contact with atmospheric oxygen. Dr. Angus Smith further describes experiments in which nitrates were formed in a dilute solution of blood without the addition of other matter. Here we should certainly have evidence of nitrification by simple oxidation, if only precautions had been taken to eliminate the influence of living organisms by a preliminary boiling of the solutions, and their subsequent protection from access of life. Of such precautions, however, there is no mention, and it becomes impossible therefore to attach any weight to the evidence. In my own earlier experiments, unsterilised solutions of ammonium salts, containing the usual nutritive ingredients, were found to nitrify (*Trans.*, 1879, 440); but no nitrification has been observed in solutions sterilised by heat, and afterwards protected from the entrance of organisms. Instances of this will be presently given.

Pichard has recently published experiments (*Ann. Agronomiques*, 1884, 302) on the nitrification of ammonium sulphate, and of earth-nut-cake, mixed with a relatively small proportion of sand, various salts being added, each mass in most cases intentionally receiving a certain quantity of ordinary soil. He concludes that ammonium salts undergo

a partial nitrification by simple exposure to air. Nothing is said, however, about the sterilisation of the sand and calcium carbonate with which the ammonium salt was mixed; the moist sand was indeed placed on a layer of earth, and the air having access was, apparently, unfiltered!

In experiments of this nature, it must always be borne in mind that moist substances, or liquids, exposed to air for a long time, may acquire distinct traces of nitrous acid by mere absorption of the nitrites present in the atmosphere, no nitrification of the substance under investigation having really taken place (Trans., 1881, 229).

During the present year, C. F. A. Tuxen has published (*Tidsskrift for Landökonomie*, 1884) the results of experiments on nitrification, in which different nitrogenous manures were mixed in relatively large quantities with a clay and with a sandy soil, and the course of change studied for more than a year. In a later section, reference will be made to some of his results.

Before discussing the present position of the theory of nitrification, it may be well to describe a few unpublished results which supply evidence on the question.

Some additional Proofs that Nitrification is determined by an Organism.

One important proof that nitrification is determined by the action of an organism is furnished by the fact that the process can be started at pleasure in any suitable solution by adding to the liquid a small quantity of a solution lately nitrified. For this proof to be effective, it must, of course, be shown that without such addition no nitrification will occur. As in the former report, the investigation was still too young for it to be possible to speak of solutions long preserved without nitrification, some examples of this fact will now be given.

On August 11, 1878, two bottles, containing an ammoniacal mixture sterilised by heat, were left unseeded, one having the neck plugged with cotton-wool, the other with the mouth simply covered with muslin. The solutions are particularly described in the Sixth Series of Experiments in the last Report. In duplicate solutions, which had been seeded, nitrification was practically completed by November 4. The unseeded bottle closed with cotton-wool was examined in April, 1879, July, 1880, and August, 1881; at the last date nitrification had not taken place, although three years had elapsed since the solution was prepared. The solution simply protected by muslin was found unnitrified in April, 1879, but nitrification afterwards occurred.

In the present paper, experiments are described (p. 650) in which solutions containing various nitrogenous substances were preserved

for more than a year without nitrification occurring, the seeded duplicates being all nitrified.

It may occasionally happen, from some condition of the experiment, that a solution with which the process of seeding has been gone through is not really seeded, no living nitrifying organism having been introduced; the solution in such a case remains unnitrified, and affords testimony similar to that given by solutions intentionally unseeded. Thus two solutions were seeded in June, 1881, with a nitrified solution which had in one case been previously long exposed to bright sunlight, and in the other had been kept for two hours at 50° ; in neither case did nitrification occur, although the subsequent examination of the solutions extended over 19 months. Of 15 solutions seeded with only $\frac{1}{100}$ of a drop of a weak nitrified solution, 9 have remained unchanged. Of 20 solutions seeded with clay subsoil, 17 have not nitrified (see p. 646). This list of illustrations might easily be extended, but the above examples seem sufficient.

We pass next to a new line of proof. If nitrification is due to the vital activity of an organism, it is clear that it can only occur when all the substances necessary for the nutrition of that organism are present. One substance indispensable to all life is phosphoric acid. The following experiments were made as to the effect of leaving out phosphoric acid from the usual nitrifying media. On October 8, 1883, three cultivations were started in the usual ammoniacal mixture; the potassium phosphate being omitted in two cases. All three solutions were seeded with a few drops from a lately nitrified solution. On October 22, nitrification had commenced in the solution containing phosphate, and by November 10 the nitrification of the ammonia was completed. The bottles not supplied with phosphate were examined up to the following June, but in neither case did nitrification take place. It would appear therefore that the presence of phosphates is an essential condition for nitrification, and in this fact we have a further corroboration of the theory which ascribes nitrification to the action of a living organism.

Present Position of the Theory that Nitrification is due to a living Organism.

The evidence in favour of this theory of nitrification appears now to be very complete. Nitrification in soils and waters is found to be strictly limited to the range of temperature to which the vital activity of low organisms is confined. Nitrification is also dependent on the presence of plant-food suitable to organisms of low character. Further proof is afforded by the fact that antiseptics are fatal to nitrification. The action of heat is equally confirmatory. By raising sewage or soil to the temperature of boiling water, its

nitrifying power is entirely destroyed. Finally, nitrification can be started in boiled sewage, or in other sterilised liquids of suitable composition, by the addition of a few particles of surface soil, or a few drops of a solution which has lately nitrified; while if no such addition is made, these liquids may be freely exposed to filtered air without nitrification taking place.

It is difficult to conceive how the evidence in favour of the ferment theory of nitrification could be further strengthened. Although, however, the greater part of this evidence has been before the scientific public for more than seven years, the theory that nitrification is due to an organism can hardly be said to have obtained any general acceptance; it has not, indeed, been seriously controverted, but neither has it been embraced. It is surely incumbent on those who discuss sanitary and agricultural questions either to accept the theory, and henceforth regard the oxidation of nitrogenous organic matter as effected solely by living organisms, and confined to the conditions suitable for their activity, or to adduce some evidence that nitrification may take place in soils and waters by simple contact with oxygen, when all organisms have been absolutely excluded. Schloesing has apparently given a final blow to the theory that air exists in a condensed state in the pores of a soil, and may on that account exert special oxidising powers, by his recent experiments (*Ann. de la Science Agronomique*, 1884, 1), showing that the gases in a vegetable soil simply occupy the normal volume due to temperature and atmospheric pressure.

Mode of Work in Recent Rothamsted Experiments.

It will be convenient to describe briefly the general mode of work adopted in my own experiments before mentioning the results obtained.

The results to be described have all been obtained by experiments in a fluid medium. The solution most generally employed contained in 1 litre—

Ammonium chloride	80 milligrams.
Sodium potassium tartrate	80 ,,
Potassium phosphate	40 ,,
Magnesium sulphate	20 ,,

To this solution, calcium carbonate was generally added to supply the necessary base. When calcium carbonate was not used, 50 mgrms. of gypsum per litre, or in some cases a little tricalcium phosphate, was added. It is necessary to dissolve the magnesium salt separately, and add it to the other ingredients after they have been largely diluted, or precipitation may occur.

Considerable use has also been made of diluted urine, usually of the

strength of 4 c.c. per litre; such a solution has the advantage of simplicity of preparation, but as its strength varies at different times, it is not suitable for standard or comparative experiments.

In special experiments, solutions of various compositions have been employed.

The greater number of experiments have been conducted in short wide-mouthed bottles. The volume of the solution at starting has been 100 c.c., or rather more; the depth of liquid $1\frac{1}{2}$ to 2 inches. The depth has been the same in all comparative experiments.

The bottle containing the solution has its neck closed by a plug of cotton-wool, which is protected from dust by a cap of filter-paper tied over it. Air has thus free access to the solution. The bottle with the solution thus protected is placed in a water-oven, and kept near the temperature of boiling water for six or eight hours to destroy any organisms present. When cool the solution is ready for use.

The calcium carbonate usually employed to furnish the necessary base is prepared by precipitation, and generally added in a moist state. In the case of urine solutions, the calcium carbonate is added before the sterilisation of the liquid; in the case of solutions of ammonium salt, it is added afterwards, the wet calcium carbonate being in this case freshly boiled before use.

The seed employed to start nitrification in the solutions consists either of a small quantity of fresh soil, usually about 0.1 gram, or more generally of 1 c.c. of a solution already nitrified. The nitrified solution was always shaken before the removal of a portion to serve as seed.

In all the earlier experiments, the cotton-wool plug was lifted out, both during seeding, and during each subsequent removal of the solution for examination. These operations were, however, always conducted in a separate room, kept locked until the arrival of the operator, and great pains were taken to avoid raising any dust. With these precautions it was found, as a rule, that bottles might be safely opened without the introduction of any nitrifying organisms. During the last year and a half, however, the removal of the plug has been discontinued; the plug is now simply raised, and the end of a pipette, drawn out to capillary dimensions, is introduced between the plug and the side of the neck; in this way liquids may be introduced or removed at pleasure. When a portion of the liquid is being removed by suction, some cotton-wool is pressed round the tube of the pipette, so that only filtered air may gain admission to the bottle. These precautions are adopted from Klein's method. In both the earlier and later experiments, the pipette was kept immersed in boiling water till the instant it was required for use; after use it was washed, and again replaced in the boiling water till next required. For many

experiments, precautions against the entrance of nitrifying organisms were quite unnecessary, but the method described was still generally employed with the object of keeping the solutions free from moulds and other foreign growths.

The bottles, after seeding, were usually placed in a dark cupboard,* and exposed to the ordinary temperature of the laboratory, which was warmed by a stove in winter time. When the cultivation was to proceed at a higher temperature, the bottles were placed in a covered metal box, having a water-jacket, the temperature of which was maintained at the desired point by means of Page's gas-regulator.

The commencement of nitrification in a solution was determined by testing with diphenylamine. 1 c.c. of the solution was placed in a very small beaker, a drop of solution of diphenylamine sulphate in sulphuric acid added, and then 2 c.c. of concentrated sulphuric acid, and mixture effected by agitation; the development of a violet-blue colour shows the presence of nitric or nitrous acid. This test will detect 1 part of nitric nitrogen in 20,000,000 of water.

The subsequent course of the nitrification was ascertained by repeated examinations for ammonia by Nessler's reagent, and for nitrous acid with metaphenylenediamine; each experiment was made with 5 c.c. of the solution, and the test-tubes employed were always of the same size. The reactions obtained were recorded as "trace," "small," "moderate," "considerable," "large," or "abundant." If the change were to nitrites only, the ammonia would fall from "large" to "trace," while the nitrous acid increased from "trace" to "large;" if the change were to nitrates only, the ammonia would diminish without any corresponding production of nitrous acid. It must be recollected, however, that in solutions kept at a high temperature, and in the case of solutions at ordinary temperatures if the ammonia was present as carbonate, the ammonia may gradually disappear without any corresponding production of nitrates or nitrites, if the bottle is not stoppered but merely closed with cotton-wool. Working in the manner now described, with solutions always containing the same proportion of ammonium chloride at starting, a good idea was generally obtained of the course of the nitrification. The disappearance of all ammonia marked the completion of the process. This method of examination was adopted with the special object of studying the effect of various conditions on the nitric or nitrous character of the change, a subject with which we are not at present concerned; it

* The paper by Messrs. Downs and Blunt, showing the prejudicial influence of light on the development of bacteria, was read at the Royal Society on the same evening that my own communication, mentioning the same fact with regard to the nitrifying organism, was made to the Chemical Society. In Soyka's experiments the prejudicial effect of light on nitrification is plainly shown.

equally served, however, to fix in every case the commencement and termination of nitrification. In some few cases, quantitative determinations of nitrous and nitric acid were made.

The Distribution of the Nitrifying Organism in the Soil.

That the special organism which nitrifies ammonia exists in abundance in the surface soil of all fertile land may be considered as now established by a variety of evidence; it appeared of considerable interest to ascertain to what depth in the soil its presence extended.

In the autumn of 1883, samples of soil were taken for analysis from various depths in the experimental Rotation Field (Agdell) at Rothamsted; the sampling of the soil extended to the depth of 9 feet. The pits formed for this purpose were turned to account for the present inquiry. The surface soil in this field (that which is annually turned by the plough) is about 6 or 7 inches deep, and of a grey colour when moist. Beneath this, with often a sharply defined boundary, comes a reddish-yellow clay containing many flints. Into this clay the deeper root and worm-holes pass, the former often extending 4 feet or more. The former course of a root is indicated by a dark streak in a section of the clay, and by the bleaching of the clay in the immediate vicinity. Below the clay subsoil comes chalk, but this was not reached in the samples of soil with which we are at present concerned.

On November 17, 1883, a series of samples of soil and subsoil were taken from the pit on Plot 1;* the samples being taken from a depth of 2 inches, 9 inches, 18 inches, 3 feet, 6 feet, and 8 feet from the surface; the samples from the lowest three depths were taken in duplicate. The samples of subsoil were taken from the solid clay, all channels being avoided. A fresh surface was first cut on the side of the pit at the spot selected for sampling; this surface was scraped with a freshly ignited platinum spatula; then, the spatula having been washed, re-ignited, and cooled, a portion of soil was detached by the spatula and at once transferred into one of the usual bottles containing a sterilised urine solution of the strength of 4 c.c. per litre; each bottle contained some precipitated calcium carbonate. The quantity of soil taken varied of course; it was about 0.1 gram. It is obvious that as the cotton-wool stopper had to be lifted to introduce the soil, opportunity was given for the entrance of any organisms floating in the air. Experience, however, has shown that air free from soil dust very seldom contains nitrifying organisms, and this

* The rotation on this Plot is—turnips, barley, bare fallow, wheat. The turnips receive a liberal nitrogenous and cinereal manuring, and are fed on the land. The soil samples were taken after the wheat crop.

view is confirmed by the results of the present experiments. The seeded bottles were placed in a dark cupboard of moderate temperature.

On December 15, 1883, an exactly similar series of samples was taken from a pit dug in Plot 8 in the same field,* the whole operation being conducted precisely as before.

The two series of urine solutions thus seeded with soil and subsoil were examined from time to time to ascertain when nitrification commenced, and when this had happened the approximate date of its completion. The results of these examinations are given in the following tables. The completion of nitrification is indicated by a small vertical line.

Nitric or Nitrous Acid produced in Dilute Urine, seeded with Clay Soil (Agdell Field, Plot 1) from various depths, on November 17, 1883.

Depth from which Soil taken.	Jan. 30 (74 days).	March 8 (111 days).	May 6 (170 days).	June 14 (209 days).	July 22 (247 days).	Sept. 17 (304 days).	Oct. 18 (335 days).
2 inches....	large	—	—	—	—	—	—
9 "	none	none	trace	large	—	—	—
18 "	"	considerable	large	—	—	—	—
3 feet (A) ..	"	none	none	none	none	none	none
" (B) ..	"	"	"	"	"	"	"
6 " (A) ..	"	"	"	"	"	"	"
" (B) ..	"	"	"	"	"	"	"
8 " (A) ..	"	"	"	"	"	"	"
" (B) ..	"	"	"	"	"	"	"

Nitric or Nitrous Acid produced in Dilute Urine, seeded with Clay Soil (Agdell Field, Plot 8) from various depths, on December 15, 1883.

Depth from which Soil taken.	Feb. 18 (65 days).	March 22 (97 days).	May 6 (142 days).	June 14 (181 days).	July 22 (219 days).	Sept. 17 (276 days).	Oct. 18 (307 days).
2 inches....	moderate	large	—	—	—	—	—
9 "	none	none	none	trace	large	—	—
18 "	small	large	—	—	—	—	—
3 feet (A) ..	none	none	none	none	none	moderate	considerable
" (B) ..	"	"	"	"	"	none	none
6 " (A) ..	"	"	"	"	"	"	"
" (B) ..	"	"	"	"	"	"	"
8 " (A) ..	"	"	"	"	"	"	"
" (B) ..	"	"	"	"	"	"	"

* The rotation on this Plot is—turnips, barley, beans or clover, and wheat. The turnips receive a liberal nitrogenous and cinereal manuring, and are carted off the land. The soil samples were taken after the wheat crop, which followed clover.

When examined on January 30, 1884, the solution seeded with soil from Plot 1, 2 inches from the surface, was found to be completely nitrified. The other solutions seeded with soil 2 inches, 9 inches, and 18 inches from the surface nitrified after a longer period, and in the case of the soil taken at 9 inches from the surface the commencement of nitrification was greatly delayed. It may be assumed that long delay in the commencement of nitrification (with favourable external conditions) implies the presence of a very minute quantity of organism in the solution, or that this organism is of exceptionally feeble character.

It will be seen that the subsoils from the depth of 3 feet, 6 feet, and 8 feet did not produce nitrification, except in the case of one bottle out of four receiving subsoil from a depth of 3 feet; in this case, nitrification commenced so extremely late that I am disposed to think it more probable that some nitrifying organism gained access to the solution during its examination on July 22, than that the organism was originally present in the subsoil. The question, however, must remain open to doubt.*

In the above experiments, the nitrifying organism was not certainly found below 18 inches from the surface. A third series of experiments was started on April 5, 1884, with the view of examining in more detail the soil lying within a moderate distance below the surface. In this case, the soil and subsoil were taken from a pit dug at the north end of Agdell Field.† Samples were taken from 3 inches, 6 inches, 9 inches, 15 inches, 2 feet and 3 feet from the surface; the experiments with soil from the last four depths were made in duplicate. All the operations were conducted precisely as before. The results yielded by the subsequent examinations of the solutions were as follows:—

* If the nitrifying organism was actually furnished by the soil, its period of incubation was more than 219 days; this would be far longer than has occurred in any other experiment. In dilute cold solutions the period of incubation has extended to 50 or 70 days, when the organism supplied has been small in quantity and feeble in character. The longest periods yet noticed are those mentioned in the above experiments with clay subsoil.

† This portion of the field is unmanured; it is cropped with turnips (carted), barley, beans or clover, and wheat. The preceding crop was wheat.

Nitric or Nitrous Acid produced in Dilute Urine, seeded with Clay Soil (Agdell Field, North Enl) from various depths, on April 5, 1884.

Depth from which Soil taken.	May 26 (51 days).	June 16 (72 days).	July 22 (108 days).	Sept. 1 (149 days).	Oct. 27 (205 days).
3 inches	trace	small	large	—	—
6 "	none	trace	considerable	large	large
9 " (A)	"	none	trace	considerable	large
" (B)	"	trace	moderate	considerable	considerable
15 " (A)	"	none	none	none	none
" (B)	"	"	"	"	"
2 feet (A)	"	"	"	"	"
" (B)	"	"	"	"	"
3 " (A)	"	"	"	"	"
" (B)	"	"	"	"	"

The urine solution employed in this series of experiments was in reality, though not intentionally, considerably stronger than that used in the two earlier series, the conclusion of nitrification was thus in all cases delayed. The sample of soil taken nearest to the surface was the first to start nitrification in the urine; the samples from 6 inches and 9 inches depth also proved effective. The subsoil from 15 inches downwards failed to produce nitrification. The time allowed for trial was indeed shorter than in the two preceding series, but it included the whole of an exceptionally warm summer, when the temperature was especially favourable for nitrification. The results of this latter series of experiments thus show a more limited distribution of the nitrifying organism in the subsoil than that indicated by the earlier series; there soil taken from a depth of 18 inches proved effective, here soil from a depth of 15 inches fails.

The three series of results just described may be thus summarised: In four experiments made with soil 2, 3, and 6 inches from the surface, and four experiments with soil 9 inches from the surface, there was not one case of failure in the production of nitrification. With soil below 9 inches the result was irregular. Out of twenty experiments, nitrification has occurred in three instances only, namely, in two instances with soil from 18 inches, and in one instance with soil 3 feet from the surface; these results appear exceptional, as in two experiments with soil 15 inches from the surface no nitrification was produced, neither did any occur in two experiments with soil 2 feet, and in five experiments with soil 3 feet from the surface. There is also some probability that the nitrification occurring with soil 3 feet from the surface was really due to some accidental contamination, as

the solution remained for 219 days without any appearance of nitrification, which occurred only after a still longer period.

I am disposed to conclude that in our clay soils the nitrifying organism is not uniformly distributed much below 9 inches from the surface. On much slighter grounds it may perhaps be assumed that the organism is sparsely distributed down to 18 inches, or possibly somewhat further. At depths from 2 feet to 8 feet there is no trustworthy evidence to show that the clay contains the nitrifying organism. It is however probable that the organism may occur in the natural channels which penetrate the subsoil at a greater depth than in the solid clay. In the case of sandy soils, we may probably assume that the organism will be found at a lower depth than in clay.

The only published researches I am aware of on the presence of the nitrifying organism in the subsoil, are those made by Müntz and Aubin with portions of rock taken near the summit of the Pic du Midi, 2877 metres above the sea level (*Annales de l'Institut National Agronomique*. Paris, 1883). A disintegrating schistous rock was blasted with gunpowder; a sample of rock taken more than 50 centims. from the surface produced a feeble nitrification when introduced into a sterilised medium.

The distribution of bacteria in soil has been made the subject of microscopical investigation by Koch (*Jahresb. d. Agricultur Chemie*, 1881, 43). He states that the micro-organisms in the soils he has examined diminish rapidly in number with an increasing depth, and that at the depth of scarcely a metre the soil is nearly free from bacteria. Pumpelly and Smyth (*Annual Report of the National Board of Health*, 1883) have made some experiments with soil carefully removed from the side of a well newly dug in a hard blue clay. In four experiments, clay 1 foot, 2 feet, and 6 feet from the surface, produced infection in a sterilised solution, while clay 9 feet from the surface was without effect. It is of course probable that those organisms which do not require a considerable supply of oxygen to exercise their functions will be able to exist at lower depths in the soil than the nitrifying bacterium.

Some important practical conclusions seem to follow from the limitation of the nitrifying organism to the upper layer of the soil. Thus it is evident that the oxidation of nitrogenous matter in soil will be confined to matter near the surface. The nitrates found in the subsoil, or in the subsoil drainage waters, have really been produced in the surface soil, and have passed down by diffusion, or in a descending column of water. Again, in arranging a filter-bed for the oxidation of sewage, it is obvious that when dealing with a heavy soil lying in its natural state of consolidation very little will be gained by

making the filter-bed of considerable depth. If, on the other hand, an artificial bed is to be constructed, it is clearly the top soil, rich in oxidising organisms, which should be exclusively employed.

Nitrogenous Substances susceptible of Nitrification.

The laboratory experiments described in the previous reports were made either with soil, or with solutions containing ammonia. Since then many experiments have been made with diluted urine as the nitrifying medium. Successful trials have also been made on the nitrification of solutions of asparagine, milk, and rape-cake. Thus, besides ammonia, two amides, and albuminoids in two forms, have proved susceptible of nitrification. The details of the last-named experiments were as follows. It will be observed that each series included experiments on the behaviour of the solution when left unseeded by the nitrifying organism.

Urine and Milk Solutions.—A solution of urine (3 c.c. per litre) was placed in three bottles. At the same time a solution of new milk (4 c.c. per litre) was also placed in three bottles. In every case, the liquid was 2 inches in depth. The bottles were closed with cotton wool, and heated in boiling water for one hour. To two bottles in each series calcium carbonate was added, and one of the solutions so treated was seeded on July 8, 1882, from a freshly nitrified solution. The seeded urine solution was examined on September 4; nitrification was then completed. The seeded milk solution was examined on September 25; a large amount of nitric acid, but no ammonia, was then present. A subsequent quantitative determination by Schloesing's method showed the presence of 18.1 parts of nitric nitrogen per million in the milk solution. The four unseeded solutions of urine and milk were examined for nitric and nitrous acid on August 20, 1883, nearly 14 months after the solutions were prepared; none was found in the milk, a trace only in the urine.* Since these experiments were made, diluted milk has frequently been nitrified in the laboratory; ammonia is always formed as a preliminary stage of the process.

Asparagine Solutions.—The asparagine solution was prepared on September 23, 1882; it contained, per litre, 107 mgrms. asparagine (dried over sulphuric acid), 80 mgrms. sodium potassium tartrate, 40 mgrms. potassium phosphate, and 20 mgrms. magnesium sulphate. The solution was placed in four bottles, to each of which calcium carbonate was added. The depth of liquid and mode of sterilising were the same as in the preceding experiments. Three of the

* Information as to the presence of nitric acid in fresh urine will be found on page 669.

solutions were seeded from freshly nitrified liquids. On October 2, one of the seeded solutions was tested, and a considerable amount of ammonia found; the sterilised unseeded liquid had shown only a trace of ammonia with Nessler's reagent. On October 23, nitrification was apparently completed in all the seeded solutions. The nitrification of the asparagine solution was exceptionally speedy; the formation of ammonia apparently preceded that of nitric acid. In October of the following year, a quantitative determination of nitric acid, made in a mixture of the seeded solutions, showed 26.1 parts of nitric nitrogen per million; the solutions had evidently concentrated by keeping. The unseeded solution when opened 12 months after its preparation contained only a trace of ammonia; no nitrification had occurred.

Rape-cake Solutions.—An extract of the cake in cold water was filtered, made slightly alkaline with sodium carbonate, boiled, and again filtered. As the amount of nitrogenous matter in the solution was unknown, it was divided into two portions, one, half as concentrated as the other. The bottles containing the solutions received calcium carbonate, and in other respects were treated as above described. One solution of each strength was seeded on October 14, 1882; one solution of the weaker strength remaining unseeded. The solutions then contained only a trace of ammonia. On November 20, ammonia was present in some quantity in both the seeded solutions, but no nitric or nitrous acid. On December 18, the ammonia had still further increased. On January 30, 1883, a trace of nitrous or nitric acid was found by diphenylamine in the weaker solution, none in the stronger. On February 19 nitrification had made scarcely any progress. More calcium carbonate was now added, and both bottles placed in an air-bath kept at 20°. On March 13, as the stronger rape-cake solution had not begun to nitrify, and but little change had taken place in the weaker solution, both were re-seeded with 1 c.c. of a recently nitrified solution. From this time nitrification proceeded rapidly. On April 14, nitrification was completed in the weak rape-cake solution, and almost completed in the stronger. In the following October, a quantitative determination of nitric acid was made in the weaker solution by Schloesing's method; it was found to contain 33.2 parts of nitric nitrogen per million. The unseeded rape-cake extract, examined at the same time, was unnitrified. The seeded rape-cake extracts were clearly nitrified with difficulty, some hindrance apparently occurring in the earlier stages of the process.

Evidence of the nitrifiability of rape-cake when applied to land as manure is afforded by the analysis of the drainage waters from the experimental wheat field at Rothamsted. Each plot in this field has a drain-pipe, 2 to 3 feet from the surface, running down the middle of

it. On one plot coarsely powdered rape-cake (1,700 lbs. per acre, 1878 to 1881, 1889 lbs. since 1881) is ploughed in towards the end of October. On 15 occasions during the last six years, the winter drainage waters from the rape-cake plot, and from two unmanured plots, have been simultaneously collected and analysed. The mean amounts of nitric nitrogen in the drainage from the two unmanured plots were respectively 5.3 and 5.1 per million, while in that from the rape-cake plot it amounted to 13.7 per million.

Boussingault nitrified 50 per cent. of the nitrogen of rape-cake when the latter was mixed with soil (*Compt. rend.*, 82, 477). Pichard, in the experiments already referred to, nitrified 46 per cent. of the nitrogen of earthen-cake, when the latter was mixed with sand, and seeded with fertile soil. Hatton (*Trans.*, 1881, 264) obtained but little nitric acid by filtering a solution of rape-cake during nine days through a column of sand, but he observed a considerable production of ammonia; the action in this experiment was clearly uncompleted.

The question of the production of nitric acid from rape-cake has been gone into in some detail, as it has been denied by some that vegetable substances are susceptible of nitrification. There is surely, however, no scientific ground for supposing that the amides and albuminoids of plants differ as to their capacity for nitrification from the amides and albuminoids of the animal body, nor are there any experiments indicating that such a difference exists. It is true that some nitrogenous vegetable substances are not easily nitrifiable, but the same may be said of such animal substances as hair. It is, however, freely admitted that, in a given time, ordinary vegetable tissue will yield far less nitric acid than animal tissue; partly for the obvious reason that it contains less nitrogen, but partly also because the excess of carbonaceous matter in the vegetable tissue has also to be oxidised. The available oxygen is thus, in the case of vegetable tissue, largely consumed in the production of carbonic acid, whilst in the case of animal tissue a much larger proportion is devoted to the oxidation of nitrogen.

Few comparative results have been obtained as to the relative rapidity of nitrification in solutions containing the same amount of nitrogen in various forms. At present, speaking only of very weak solutions provided with the proper nutritive ingredients, I should be disposed to say that nitrification takes place most speedily in solutions of ammonium salts and asparagine, less speedily in urine, and with distinctly more difficulty in solutions of milk and rape-cake.

In all cases in which non-ammoniacal substances were employed, the first stage of the process was the production of ammonia. C. F. A. Tuxen, in a paper already referred to, has published

experiments on the formation of ammonia and nitric acid in soils to which bone-meal, fish-guano, or stable manure had been applied; in all cases he found that the formation of ammonia preceded that of nitric acid. As far therefore as information goes at present, it would seem probable that ammonia is, strictly speaking, the only nitrifiable substance; practically, ammonium carbonate is the material from which nitric acid is in all cases produced. With this idea before us, we may safely assert that all nitrogenous bodies are nitrifiable which yield ammonia when attacked by the organisms present in fertile soils.

INFLUENCE OF VARIOUS CONDITIONS ON THE PROCESS OF NITRIFICATION.

1.—*Alkalinity of the Solution.*

It has been acknowledged by every investigator that the presence of some salifiable base is one of the conditions essential for nitrification. In the last Report (third series) some experiments illustrating this fact were described, and two more recent ones may here be mentioned. On August 25, 1883, six solutions of the composition given at p. 642, save that the sodium potassium tartrate was only 40 mgrms. per litre, were seeded from a freshly nitrified liquid. Two of the solutions received no added base, two received precipitated calcium carbonate, and two a small quantity of sodium carbonate. The six bottles were all placed in the same cupboard. In two of the solutions receiving base, nitrification was completed in 51 days; in the other bottles receiving base, nitrification terminated in 65 days. In the two solutions receiving no base, a small quantity of nitric acid was found at the end of a year, but the ammonia unnitrified was still abundant.

It appeared of some importance, from a practical point of view, to ascertain to what extent nitrification would proceed in the case of diluted urine, without the assistance of an added base. The experiment was made in wide-mouthed pint bottles provided with cotton-wool stoppers; 320 c.c. of diluted urine (4 c.c. per litre) were placed in each bottle, forming a column $3\frac{5}{8}$ inches deep. The series was as follows:—

U, containing diluted urine alone.

UCa, containing diluted urine with CaCO_3 .

UCaNa, containing diluted urine with CaCO_3 and Na_2CO_3 .

UG, containing diluted urine with glucose.

UGCa, containing diluted urine with glucose and CaCO_3 .

UGCaNa, containing diluted urine with glucose and CaCO_3 and Na_2CO_3 .

The proportion of glucose was 100 mgrms. per litre, of sodium carbonate 250 mgrms. All the bottles were seeded on May 1, 1882, with 1 c.c. of a solution recently nitrified by soil.

On June 19, no nitrous acid was present in U, and in UG a trace only; in the other solutions containing an added base, a distinct, and in some cases considerable amount was found.

On July 8, only a very small quantity of ammonia remained in UCa, UCaNa, and UGCaNa; the quantity was considerable in UGCa, and very considerable in U and UG.

On August 7, the ammonia had disappeared from UGCa, it was still considerable in the case of U and UG.

On September 4, nitrous acid having disappeared from the solutions, some quantitative determinations of nitric nitrogen were made by means of indigo; the results were as follows:—

Solution U,	9·7	of nitric nitrogen per million.
„ UCa,	25·6	„ „ „
„ UCaNa,	23·1	„ „ „

The solutions were left undisturbed till October 3 in the following year. The quantity of ammonia was then still considerable in the case of U and UG. Quantitative determinations were again made, this time by the more exact method of Schloesing. The results were as follows:—

Solution U,	12·8	of nitric nitrogen per million.
„ UG,	12·7	„ „ „
„ UCa,	25·9	„ „ „

Thus 17 months after the seeding of the urine solutions, and 14 months after nitrification had ceased in all the solutions supplied with a base, the nitrogen of the urine solutions not so assisted was only one-half nitrified. The reason of this result is not far to seek. The first stage in the nitrification of urine is undoubtedly the conversion of the urea into ammonium carbonate; this, of course, plays the part of a base, and nitrification may proceed vigorously till the whole of the ammonium carbonate is converted into ammonium nitrate (or nitrite); at this point the action must stop, unless some other base is available. We should infer therefore, on theoretical grounds, that in the absence of any additional base, only one-half of the nitrogen contained in a solution of ammonium carbonate would be nitrifiable, and this is precisely the limit of nitrification which has appeared in the diluted urine experimented with.

The practical bearing of the fact thus established is obvious. Neither urine, nor sewage containing it, necessarily includes within itself the constituents requisite for complete nitrification. Nor will

intermittent filtration through soil necessarily affect a complete oxidation of ammonia. For nitrification to be complete, there must always be present in the sewage, or in the soil through which it is filtered, a sufficient quantity of easily salifiable base to combine with the nitric acid produced. If the supply of base is insufficient, nitrification will be incomplete. If no additional base be present, nitrification can at most extend to only one-half of the ammonia, and will often fall short of this, as only in certain cases will the whole of the ammonia exist as carbonate.

In the course of the general investigation, cultivations have been made under various conditions, both as to the amount and character of the base present. In most of the experiments, precipitated calcium carbonate in excess was added to the solution, and has furnished practically the whole of the base required for nitrification. When calcium carbonate is added to a cold solution of ammonium chloride, the alkalinity of the liquid is of course very feeble, the conditions only admitting the formation of a very small proportion of ammonium carbonate. In other experiments, varying proportions of disodium carbonate, monosodium carbonate, or of the carbonates of ammonium, have been employed; in all these cases, the alkalinity of the liquid has been more or less increased.

It appears from the results at command, that the addition of a small proportion of disodium carbonate, Na_2CO_3 , to an ammonium chloride solution containing calcium carbonate, increases the speed of nitrification; whilst a larger proportion retards the process, or may prevent nitrification altogether.

In seven pairs of experiments made at various times, the period required for nitrification in a solution supplied only with calcium carbonate was compared with that required in a similar solution containing in addition 160—200 mgrms. of disodium carbonate per litre.* In four instances, nitrification was considerably more speedy where the disodium carbonate was present; in two instances, the speed was nearly alike in the comparative experiments; in one instance (a shallow solution), nitrification took place more speedily without sodium carbonate. The presence of the disodium carbonate was thus, on the whole, an advantage.

In sixteen instances, in which the effect of calcium carbonate, used alone, was compared with that of 320 mgrms. of disodium carbonate

* Nearly all the experiments on the comparative influence of calcium carbonate, disodium, and monosodium carbonate, were made with the ordinary solution containing 80 mgrms. of ammonium chloride per litre. In such a solution, 160 mgrms. of disodium carbonate will almost exactly suffice to combine both with the chlorine and with the nitric acid produced. The solution at the end of the operation would thus be nearly neutral.

per litre, also used alone, nitrification was in 13 instances distinctly less speedy with the sodium than with the calcium carbonate, whilst in 3 instances the progress was nearly alike under both conditions.

In five comparisons between the effect of calcium carbonate, and 480 mgrms. of disodium carbonate per litre, used alone, all the solutions receiving calcium carbonate nitrified long before those receiving sodium carbonate; in fact, nitrification did not take place in three of the latter.

From these comparisons, it would appear that in an ammonium chloride solution, with the conditions described, an alkalinity equal to 160—200 mgrms. of disodium carbonate per litre is on the whole favourable to nitrification, whilst an alkalinity of 320 mgrms., or more, is unfavourable.

To ascertain more completely the influence of different degrees of alkalinity, and also the comparative effect of different alkaline substances, the following series of experiments was made.

The chloride of ammonium solution employed was of the usual composition, save that it contained only 40 mgrms. of sodium potassium tartrate, and 50 mgrms. of gypsum per litre. 100 c.c. of this solution were placed in each of 15 bottles. To five of these, disodium carbonate was added, in the proportions 1, 2, 4, 6, 8, the unit taken being 160 mgrms. per litre. To five, monosodium carbonate (NaH_2CO_3) was added in such quantity as to equal the disodium carbonate in alkalinity. To the remaining five bottles, ammonium carbonate (white outside crust of lumps) was added in similar quantity and proportion. The ammonium carbonate solutions were placed in stoppered bottles, the others in bottles having cotton-wool stoppers. All the solutions received a liberal seeding on February 25, 1884. The results of the subsequent examinations for nitrous and nitric acids are given in the following table (p. 657). The entire disappearance of the ammonia is indicated, as before, by a vertical line.

According to the table, the solutions containing 1 and 2 units of disodium carbonate (160 and 320 mgrms. per litre) nitrified at the same rate; in fact, however, the less alkaline solution was always somewhat ahead, though not to such an extent as to require the use of different terms in the table. With 4 units of disodium carbonate (640 mgrms. per litre), the commencement of nitrification was delayed about 26 days. With 6 and 8 units of disodium carbonate (960 and 1280 mgrms. per litre), nitrification was certainly delayed from 90 to 100 days, and it is even doubtful whether nitrification took place at all in the case of the stronger solution. Several practical difficulties occur in experiments conducted in the above manner. If the trial is prolonged, and the temperature that of summer, the ammonia is volatilised through the cotton-wool stopper; the alkali-

Nitric or Nitrous Acid produced in Ammoniacal Solutions under different conditions of Alkalinity, seeded February 25th, 1884.

Proportion of Alkaline Salt present.	Mar. 22 (26 days).	April 17 (52 days).	May 7 (72 days).	May 24 (89 days).	July 7 (133 days).	Sept. 6 (194 days).
<i>Disodium Carbonate Series.</i>						
1.	trace	moderate	large	—	—	—
2.	trace	moderate	large	—	—	—
4.	none	trace	moderate	considerable	—	—
6.	"	none	none	none*	small	small
8.	"	"	"	" *	trace	"
<i>Ammonium Carbonate Series.</i>						
1.	moderate	abundant	—	—	—	—
2.	small	abundant	—	—	—	—
4.	none	none	trace	abundant	—	—
6.	"	"	small	abundant	—	—
8.*	"	"	none	none*	none	none
<i>Monosodium Carbonate Series.</i>						
1.	small	large	—	—	—	—
2.	"	"	—	—	—	—
4.	"	"	—	—	—	—
6.	"	"	—	—	—	—
8.	trace	considerable	large	—	—	—

nity of the solution is thus reduced below that fixed at starting; and if nitrification occurs at a late period it is prematurely concluded, there being little ammonia left to be nitrified.

In the ammonium carbonate series, we find the order of the commencement of nitrification similar to that observed in the case of the solutions containing disodium carbonate; the weakest solution is here again the first to exhibit nitrification. In this series, an alkalinity of 6 of the adopted units (probably about 260 mgrms. nitrogen per litre at the time of nitrification) is evidently nitrifiable, though slowly; but with an alkalinity of 8 units no certain evidence of nitrification was obtained.

In the monosodium carbonate series, we meet with wholly different results. On the first day of examination, nitrification was found to have commenced in all the solutions; the amount of nitrification was here again inversely in proportion to the alkalinity, but the differences, except in the case of the strongest solution, were too small to affect the terms adopted in the table. Nitrification was soon completed in all the solutions.

* At this date these solutions were again seeded from a nitrified solution.

It appeared from these experiments, that any increase in the alkalinity of the solution was attended with further delay in the commencement of nitrification; that with disodium carbonate a solution containing 960 mgrms. per litre could scarcely be nitrified; that ammonium carbonate presented rather less obstacle, the 6-unit solution (about equal in alkalinity to 960 mgrms. of disodium carbonate) nitrifying with some ease, but no nitrification occurring in a solution of one-third greater strength. Monosodium carbonate, on the other hand, had distinctly less prejudicial effect; and in this case, apparently, the limit of alkalinity at which nitrification ceases was not reached, though about 2050 mgrms. of monosodium carbonate per litre were employed.

As the maximum proportion of monosodium carbonate which may be present in a solution without preventing nitrification had not been ascertained, a further series of experiments was commenced on May 8, 1884. The solution employed was of the same composition as before. To one series of solutions 8, 12, 16, and 20 units of monosodium carbonate were added; and to another series 4, 6, 8, and 10 units of disodium carbonate, prepared by gently igniting the equivalent quantity of monosodium carbonate. The exact comparison of the two carbonates was thus insured.

The experiment was not as successful as could be wished, for during the warm summer months the ammonia almost entirely disappeared from the solutions not yet nitrified. In the solution containing 4 units (640 mgrms. per litre) of disodium carbonate, nitrification commenced in 53 days, but from the cause already mentioned no certain evidence of nitrification was obtained in the more alkaline solutions. In the monocarbonate series, the solution containing 8 units (2100 mgrms. per litre) showed nitrification in 32 days, but no distinct evidence of nitrification was obtained when 12 units were present. The results thus confirmed in great measure those already obtained, but without adding any new facts.

In a third series of experiments, carbonates of ammonium were compared containing different proportions of carbonic acid, this condition evidently having considerable influence on the result in the experiments with sodium carbonates. A solution was employed of the same composition as before, and to it was added ammonium carbonate taken in some cases from the white opaque crust of lumps of commercial carbonate, and in other cases obtained from the clear central mass of such lumps. As the composition of these carbonates was not strictly known, we may designate them for the present purpose as ammoniac and ammonious carbonates. The ammoniac carbonate was employed in the proportion of 4, 6, 8, and 10 units, the ammonious carbonate in the proportion of 2, 4, 6, and 8 units;

the unit was in all cases based on alkalinity. The experiments were made in rather loosely stoppered bottles. The quantity of ammonia was far too great to disappear entirely by volatilisation in the course of the experiment. The solutions were seeded on May 28, 1884.

The solution with 2 units of ammonious carbonate showed a commencement of nitrification in 36 days, that with 4 units in 71 days; no other solutions in the series nitrified. With ammonium carbonate, the solution with 4 units exhibited nitrification in 36 days, and that with 6 units in 57 days; no others nitrified. At the time of its nitrification, the 4-unit solution of ammonious carbonate exhibited an alkalinity equal to about 140 parts of nitrogen per million; the 6-unit solution of ammonic carbonate an alkalinity equal to about 220 parts of nitrogen per million. The maximum proportion of ammonium carbonate capable of nitrification is here shown to be the same as in the first series of experiments. As in the case of the sodium carbonates, it is further clearly shown that the larger the proportion of carbonic acid united with the base, the less is the prejudicial influence of the salt on nitrification.

Other experiments having shown that, under conditions unfavourable to nitrification, important aid is afforded by increasing the amount of the nitrifying organism present, a fourth series of experiments with ammonium carbonate was commenced, in which 1 gram of fresh arable soil was added to each solution. The solution employed was more concentrated than before; it contained per litre—sodium potassium tartrate, 1.0 gram; potassium phosphate, 0.3 gram; magnesium sulphate, 0.1 gram. The ammonium carbonate (tolerably clear pieces) was used in the proportion of 6, 8, 10, 12, 14, and 16 units. The experiment commenced on July 14, 1884.

In the solution containing 6 units of ammonium carbonate, nitrification commenced after 78 days; its alkalinity was then determined by experiment, and was equivalent to 245 parts of nitrogen per million. The solution containing 8 units of ammonium carbonate commenced to nitrify after 96 days, its alkalinity being then equal to 368 parts of nitrogen per million.* No other solution has at present nitrified, though 129 days have now elapsed since seeding. It will be noticed that under these more favourable conditions a stronger solution of ammonium carbonate was nitrified than in the preceding series.

On comparing the results with ammonium carbonate with those obtained in solutions containing sodium carbonate, it appears that nitrification is possible with an alkalinity equal to 368 parts of nitrogen (as ammonia) per million in the case of ammonium carbonate,

* This alkalinity is somewhat above that of the ammonium carbonate added ($N = 344$ per million): additional alkali had probably been derived from the soil, or by the decomposition of the sodium potassium tartrate.

and with an alkalinity equal to about 350 parts of nitrogen per million in the case of monosodium carbonate. As far therefore as these few experiments go, the maximum degree of alkalinity which nitrification will tolerate is much the same whether ammonium or monosodium carbonate is the alkaline substance present. On the other hand, when disodium carbonate is the alkaline substance, the limit of alkalinity, though imperfectly ascertained, is distinctly lower. Nitrification has twice certainly occurred with an alkalinity equal to 174 parts of nitrogen per million, and nitrification has more doubtfully occurred in one out of two trials with an alkalinity equal to 261 parts of nitrogen per million.

The prejudicial influence which soluble alkalis exercise on nitrification, when they exceed a certain amount, has been noticed by Schloesing and Müntz (*Compt. rend.*, **89**, 1075). They say "Une faible alcalinité des milieux est nécessaire à la production du nitre. Dans la nature, c'est généralement le carbonate ou plutôt de bicarbonate de chaux qui joue le rôle d'alcali. Les carbonates alcalins très étendus produisent le même résultat; mais, lorsque leur degré de concentration dépasse deux ou trois millièmes, ils deviennent défavorables ou même arrêtent complètement l'action du ferment nitrique. Il en est de même du carbonate d'ammoniaque et de la chaux." No details of their experiments have been published. The proportion of soluble alkalis which I have found to prevent nitrification is smaller than that mentioned by the French chemists, save in the case of monosodium carbonate; of this salt, two-thousandths did not prevent nitrification, but three-thousandths did.

The prejudicial influence of caustic lime on nitrification was noticed long since by Boussingault. The alkalinity of lime-water is indeed about twice as great as that shown in my own experiments as the maximum beyond which nitrification will not occur. A heavy dose of lime applied to land will thus probably retard, or even suspend for a time, the process of nitrification in the surface soil, but this action will soon cease as the lime unites with carbonic acid, and the final result will be favourable to nitrification if the land was originally deficient in lime.

The fact that nitrification will not occur in a solution exceeding a certain degree of alkalinity entirely prevents the nitrification of urine, except when largely diluted with water. The first action of soil organisms upon urine is the conversion of urea into ammonium carbonate; the second action—the nitrification of this ammonia—can only take place if the alkalinity of the solution, and consequently the proportion of ammonium carbonate, is below a certain very moderate amount. The limit imposed by concentration on the nitrification of urine will be seen by the results given in the following table. Pre-

vious experiments had shown that a 10 per cent. solution of urine was nitrifiable, though with some difficulty; further experiments were therefore made to ascertain the extreme limit of concentration at which nitrification would occur. In each experiment, 100 c.c. of diluted urine were placed in a loosely stoppered bottle, and 1 gram of arable soil added. The experiments commenced on May 6, 1884.

Nitric or Nitrous Acid produced in Diluted Urine of various strengths, Seeded with Surface soil May 6, 1884.

Urine in 100 of water.	May 17 (11 days).	May 26 (20 days).	June 23 (48 days).	July 7 (62 days).	Aug. 4 (90 days).	Sept. 30 (147 days).	Oct. 25 (172 days).
1.	trace	small	abundant	—	—	—	—
5.	none	trace	moderate	abundant	abundant	abundant	—
10.	„	none	none	trace	large	abundant	abundant
12.	„	„	„	none	trace	considerable	large
14.	„	„	„	„	none	none	none
16.	„	„	„	„	„	„	„

It is seen that nitrification commenced in the 1 per cent. solution of urine in 11 days; in the 5 per cent. solution in 20 days; in the 10 per cent. in 62 days; and in the 12 per cent. in 90 days. In the solutions of greater concentration, no nitrification has appeared.

The alkalinity of the 10 per cent. urine solution was determined by experiment at the commencement of nitrification, it was equivalent to 351 parts of nitrogen per million. The alkalinity of the 12 per cent. solution was found to be equal to 368 parts of nitrogen per million when nitrification started. The alkalinity of the 14 and 16 per cent. urine solutions on September 6, was equal respectively to 412 and 479 parts of nitrogen per million; in these solutions, no nitrification has up to the present time (November 20) appeared. The greatest alkalinity of a urine solution in which nitrification is possible, is thus equal to about 368 parts of nitrogen per million, a figure identical with that obtained in the previously described experiments made with ammonium carbonate. The idea with which the experiments were started is thus confirmed. The limit of concentration of a urine solution which shall be capable of nitrification is determined by the degree of alkalinity of the solution after the urea is converted into ammonium carbonate. It is of course possible that urine solutions stronger than those here described may finally undergo nitrification if placed in circumstances where a gradual volatilisation of ammonium carbonate will take place. Stronger urine solutions would also probably nitrify

if poured on a considerable mass of soil; partly because the urine would be further diluted by the moisture of the soil, and partly because a part of the ammonia would be removed from solution by the absorptive power of the soil. Strong solutions of urine could probably also be nitrified if mixed with gypsum, by which the injurious excess of ammonium carbonate would be removed, ammonium sulphate being substituted for it.

Some practical bearings of these results are obvious. Alkaline waste liquids may prove a very undesirable addition to streams in which the oxidation effected by living organisms is the natural means of purification. Farmers, too, will now better understand why liquid manures should always be applied to land in a state of great dilution. Where sheep or cattle are fed on land, there is undoubtedly in dry weather a considerable loss of ammonia, the fermented urine remaining on the surface in a condition too concentrated either for nitrification or the retention of the ammonia by the soil. The effect of liming soil will now also be better understood.

2.—*Concentration of the Solution.*

The influence of increased concentration of a solution in retarding, or altogether preventing the process of nitrification, has been already illustrated so far as those solutions are concerned in which, as in the case of urine, an increase of concentration implies an increase in the proportion of soluble alkali present; we have yet to say a few words as to the behaviour of solutions in which difference of concentration is not accompanied by a difference in alkalinity. Such conditions occur when solutions of ammonium chloride of various degrees of concentration are the subject of experiment, calcium carbonate being the only base supplied. In such solutions, it is found that an increase of concentration always retards the commencement of nitrification, this retardation being manifest whenever a strong and weak solution are seeded with equal amounts of organism of similar character, and then placed under identical external conditions. The extent of the retardation depends greatly on the conditions of the experiment. If a small amount of feeble organism is supplied to a weak, and to a more concentrated solution of ammonium chloride—say, for instance, to the solution described on p. 642, and to one of 8 or 16 times the strength—nitrification may commence much earlier in the weaker solution if the temperature is low, but only a few days earlier if the temperature is that most favourable to the growth of the organism. Thus in some experiments described in the previous Report, solutions of the comparative strengths of 1 : 8 were seeded on the same day; at a temperature of 7—10°, nitrification commenced in

the weaker solutions in 31—38 days, and in the stronger in 78 days; whilst at a temperature of 30° nitrification began in the weaker in 11—12 days, and in the stronger in 17—19 days. It has since been ascertained that an increased quantity, or special vigour of the ferment added, also greatly diminishes the hindrance to nitrification presented by a strong solution. It would appear that the nitrifying organism finds difficulty in multiplying in a strong solution; and that this is particularly the case when the organism is of feeble constitution, or has been previously cultivated in a weak solution. An organism of such character may produce no effect when added in small quantity to a strong solution. In one instance of this kind, nitrification had not commenced in 144 days. If, however, the temperature is considerably raised the multiplication of the organism is stimulated, and the results of its work soon appear. By adding a large quantity of the organism, or an organism already cultivated in a strong solution, the difficulty may equally be met without raising the temperature. Thus in an experiment with an ammonium chloride solution of eight times the usual strength, but seeded with 1 gram of fresh soil, nitrification appeared in 16 days at the ordinary temperature. Illustrations of the influence of different kinds and amount of seed will come more fitly in the following section; in every series, however, in which a uniform seeding is adopted nitrification will appear first in the weaker solution.

In the previous Report, the strongest ammonium chloride solution nitrified contained 0.640 gram of the salt per litre. While the system of small seeding was employed, it was found extremely difficult to nitrify stronger solutions. By the use of a more vigorous organism and larger seeding, the limit of concentration has been greatly extended. Solutions containing 1.280 gram of ammonium chloride per litre have been completely nitrified, and nitrification is now in active progress in solutions containing 2 grams, 4 grams, and 8 grams of ammonium chloride per litre. The first of these three solutions contained, besides the ammonium chloride, sodium potassium tartrate, 1 gram; potassium phosphate, 0.3 gram; magnesium sulphate, 0.1 gram per litre. The other two solutions contained twice the quantities just named. To 100 c.c. of each solution was added 1 gram of fresh soil. The experiment commenced on May 19, 1884; no artificial heat was used. Nitrification commenced in the weakest solution in 35 days, in the second solution in 42 days, and in the strongest solution in 56 days. The last-named solution would contain 2095 parts of nitrogen per million. A far stronger solution of ammonium chloride can thus be nitrified than of ammonium carbonate.

3.—*Character and Amount of the Nitrifying Organism.*

The general influence of the character and quantity of the organism has been necessarily mentioned in the preceding section. The subject is of considerable importance: in fact, the rapidity of nitrification very greatly depends on these conditions, and comparative experiments are of little value in which the conditions named have not been maintained as constant as possible. We will first describe some results obtained with different quantities of organism.

Three bottles, each containing 100 c.c. of a $\frac{1}{2}$ per cent. urine solution, were seeded respectively on October 8, 1883, with 1 drop, 5 drops, and 10 drops of a nitrified liquid. On December 13 (66 days after seeding) nitrification had just commenced in the solution which had received 1 drop, it had made considerable progress where 5 drops had been added, and still greater progress where 10 drops had been employed.

Three series of experiments were made in a dilute urine solution (4 c.c. per litre), the seed being limited to one-hundredth of a drop of nitrified solution; this was accomplished by adding 1 c.c. of the nitrified liquid (previously shaken) to 100 c.c. of boiled water, mixing well, and then seeding with 1 drop of the solution. In each series of experiments, a different nitrified liquid was employed as seed. In the first series, only one solution nitrified out of five. In the second series, all the five solutions nitrified, but only after a long time. In the third series, also of five, none of the solutions nitrified.

Three experiments were made with the strong solution containing 2 grams of ammonium chloride per litre, described in the preceding section; 0.01, 0.10, and 1.00 gram of fresh soil were employed as seed. With 1 gram of soil, nitrification started in 35 days, with 0.10 gram in 42 days, and with 0.01 gram in 56 days.

When the seed is of vigorous constitution, a diminution in its quantity is far less severely felt than when seed of feeble character is employed.

The different vigour of the nitrifying organism obtained in different cultivations is a fact that soon strikes an experimenter on nitrification. If two dilute solutions of the same composition have been completely nitrified by the addition of small amounts of organism, it seems fair to conclude that a similar amount of organism will be found in each solution at the close of the experiment, equal amounts of food having been present, and equal amounts of work done; and this probability is increased when second and third cultivations are made from each of these solutions. Notwithstanding, however, this similarity of condition, equal volumes of these two nitrified solutions may possess a

very different capacity for producing nitrification; one may be a strong ferment, starting nitrification in a very short time when added to a new fluid, and the other a weak ferment, extremely slow in action, and these characteristics will remain throughout many successive generations.

The character of the organism is in these cases determined by its previous history. A vigorous organism is obtained by cultivation in a fairly strong solution, well supplied with nutritive ingredients; such an organism will more or less maintain its character even during successive cultivations in a weak solution.

A weak organism is obtained by long repeated re-cultivation in very dilute solutions; by proceeding thus, the organism may at last become scarcely able to produce nitrification at all, even at the most favourable temperature. When a weak organism is placed in a strong nutritive solution, it may produce no effect; but if nitrification sets in it may become vigorous, the new growth of organism gathering strength from the favourable conditions surrounding it.

With the experience thus gained as to the circumstances which determine the vigour of the nitrifying organism, it has been possible, as already stated, to nitrify solutions which before had proved refractory. Thus four solutions, two of 8, and two of 16 times the usual strength (0.64 gram and 1.280 gram of ammonium chloride per litre), were seeded on May 22, 1883, by the addition of 1 per cent. of a previously nitrified solution. On August 4, nitrification had made some progress in the weaker pair of solutions, but had not commenced in the stronger. On October 13 (144 days after seeding), no nitrification had yet appeared in the two stronger solutions. These were now re-seeded, not from the weak solution previously employed, but from the solution of eight times the usual strength, the nitrification of which was now nearly completed. The use of organism grown in this more concentrated solution was quite successful, the solutions of 16 times the usual strength were both nitrified.

The plan that has proved most effective in producing rapid nitrification, is to make use of the sediment lying at the bottom of the vessel in which the nitrification of a fairly strong solution has been conducted. As calcium carbonate is employed in excess in the operation, the sediment largely consists of this substance; but with this, after standing, the greater part of the nitrifying organism is associated. If at the conclusion of nitrification the clear solution is decanted from the sediment, and the new solution which is to be nitrified poured on in its place, conditions extremely favourable for rapid nitrification are obtained. This method ensures the presence of a large quantity of the nitrifying organism, and if the previous cultivation has been made in a fairly strong solution, the organism is also of high quality.

When a pure nitrifying organism, the result of previous cultivation, is not available, the same conditions favourable to rapid nitrification may be obtained by adding sufficient moist arable soil (taken an inch or two below the surface), to slightly cover the bottom of the vessel employed.

Illustrations have already been given of the successful action of large applications of a vigorous organism in the case of solutions far stronger than those previously nitrified; a few examples of the increase of speed resulting from this mode of work may now be added.

100 c.c. of a 2 per cent. urine solution were seeded on October 8, 1883, with two drops of a previously nitrified solution; nitrification was nearly completed on February 5, 1884, 122 days after seeding. At this date, the solution was decanted from the sediment at the bottom of a $\frac{1}{2}$ per cent. urine solution just nitrified, and after washing the sediment once by decantation, 100 c.c. of a 2 per cent. urine solution were introduced. This solution was completely nitrified by March 31, 54 days after seeding, or in far less time than under the previous conditions.

100 c.c. of a 5 per cent. urine solution, seeded as before with two drops of a previously nitrified solution, showed a rather considerable amount of nitrification at the end of 86 days. A similar solution placed on a sediment showed a very large amount of nitrification in 31 days. A solution of similar strength treated with 1 gram of soil showed a commencement of nitrification in 20 days.

On May 8, 1884, 100 c.c. of the usual dilute ammonium chloride solution (see p. 642) were placed on the sediment left after the nitrification of a solution eight times as strong. In nine days, nitrification had made very distinct progress, and in 16 days the whole of the ammonia had disappeared. This is the speediest nitrification I have yet observed in experiments made in solutions; only once has nitrification been completed in 16 days at the favourable temperature of 30° , and generally 19 days are required at this temperature for solutions of similar strength, receiving only a small quantity of seed. The use of a large amount of vigorous organism thus effected nitrification at ordinary temperatures, at a speed equal to the most favourable result previously obtained at optimum temperatures.

There can be no doubt that the extremely rapid nitrification which certain nitrogenous matters undergo in arable soil is largely due to the immense mass of nitrifying organism with which this matter is brought in contact.

4. *Influence of Depth of Solution.*

In the earliest experiments, the solutions were placed in wide-mouthed pint bottles, the column of liquid at starting being 5 inches

deep. The greater number of experiments have, however, been made in smaller bottles, the column of liquid not exceeding a depth of 2 inches. On three occasions a comparison of the two methods was made, the volume of seed added in each experiment being proportional to the bulk of the solution. Out of nine experiments, nitrification was in seven instances considerably more rapid in the shallower liquids, whilst in two cases there was a slight advantage with the deeper solutions. In all cases there was free access of air through a cotton-wool stopper.

There can be no doubt that for vigorous nitrification a rapid renewal of the dissolved oxygen of the solution is required. For this reason nitrification in a stagnant solution can never proceed so rapidly as in a solution which moistens an aerated porous solid. Partly for this reason, though still more for the reason mentioned at the close of the last section, any suitable solution poured upon a mass of porous soil will be nitrified in a far shorter time than has ever been obtained in experiments made with stagnant liquids of considerable depth.

5. *Influence of Proportion of Organic Carbon.*

In the previous Report, three experiments were described in which the solutions contained different proportions of sodium potassium tartrate, the ammonium chloride remaining constant, the phosphates and remaining ingredients varied however with the tartrate. The trials were made at 20°, afterwards raised to 30°. Nitrification commenced first in the solution containing least tartrate (47 per cent. of the ammonium chloride), but concluded first in that containing most tartrate (187 per cent. of the ammonium salt). A few more experiments have been made on the subject.

In 15 pairs of experiments in which the solution was similar to that described on p. 642, excepting that one series received 40 mgrms., and the other 160 mgrms. of sodium potassium tartrate per litre (50 per cent. and 200 per cent. of the ammonium chloride), the advantage as to speed of nitrification was in 7 instances with the larger proportion of tartrate, in 3 instances with the smaller, and in 5 instances the rate of nitrification was quite similar. There was thus on the whole some advantage from the use of the larger proportion of tartrate; probably tartrate 100 per cent. of the ammonium salt is sufficient for every purpose.

Two series of experiments were also made in which glucose was substituted for the tartrate in the usual ammonium chloride solution; the glucose was used in the proportion of 20, 50, 100, and 200 mgrms. per litre. In each series, the solution containing least glucose was the first in which nitrification was completed.

In two pairs of experiments, diluted urine (4 c.c. per litre) was nitrified both with and without the addition of 100 mgrms. of glucose per litre; the rate of nitrification was not distinctly affected by the glucose.

There is thus no advantage to be derived from the presence of organic carbon above the very small proportion essential for the nourishment of the nitrifying organism; indeed the presence of an excess of the carbon compound must tend to diminish the rate of nitrification by appropriating a portion of the otherwise available oxygen. Urine apparently contains quite enough organic carbon for its own nitrification.

Minimum Temperature at which Nitrification occurs.

During the cold winter of 1879-80, some quantitative determinations of nitrous and nitric acids were made in a series of solutions standing in a cold cupboard. The solution was the ordinary one, containing ammonium chloride equal to nearly 21 parts of nitrogen per million. Weekly determinations in four solutions undergoing a nitrous, and in one solution undergoing a nitric fermentation, gave the following results:—

Production of Nitrous and Nitric Acid in Ammoniacal Solutions during a Period of Low Temperature, Nov. 20—Dec. 18, 1879.

	Nov. 20.	Nov. 27.	Dec. 4.	Dec. 11.	Dec. 18.
Mean temperature of preceding week	7°·8	6°·2	4°·0	3°·2	5°·6
Maximum temperature of preceding week	10°·9	7°·2	6°·5	5°·1	6°·4
Nitrogen as nitrous acid per million.					
Solution N	4·2	6·2	8·8	12·1	16·3
„ N3	5·4	7·9	10·4	13·5	17·2
„ N4	2·2	4·0	5·9	7·8	11·3
„ N5	1·7	2·7	4·6	5·4	7·6
Nitrogen as nitric acid per million.					
Solution N'4	5·3	5·4	5·8	—	6·8

From these results, it appears that nitrification may proceed slowly

in a solution even at a low temperature; the mean temperature of the coldest week is indeed as low as $3\cdot2^{\circ}$, the maximum temperature during the same period being about $5\cdot1^{\circ}$. It appears that the nitric fermentation was more affected by cold than the nitrous; of this fact, however, we have only a single instance.

The Reduction of Nitrates by Soil.

Of the general conditions under which reduction of nitrates occurs it is not my intention to speak; I wish simply to call attention to the curious fact that when fresh soil is added to diluted urine, or to other solutions suitable for nitrification, a destruction of the nitrates already present precedes the commencement of nitrification. This disappearance of the nitrates is completed in a few days. Dehérain and Maquenne have shown that the reduction of nitrates by soil is accomplished by bacteria. It would appear that the reducing bacterium multiplies itself far more rapidly than the nitrifying bacterium, and runs its course before the other becomes active. The reduction of nitrates now in question is always accompanied by turbidity of the fluid.

The Nitric Acid contained in Human Urine.

In the course of the experiments described in the present paper, I became aware that nitrates are a usual constituent of fresh human urine, the fact has however long been known. If half a cubic centimetre of fresh urine is diluted with its own volume of water, a drop of solution of diphenylamine sulphate added, and then 1 or 2 c.c. of concentrated sulphuric acid, the violet colour characteristic of the presence of nitric and nitrous acid will be produced. It is advisable to stand the beaker, or test-tube, containing the urine, in cold water during the addition of the sulphuric acid, to prevent the charring of the organic matter. As I have failed to find nitrous acid even with the extremely delicate naphthylamine test, it would appear that nitric and not nitrous acid is present. Quantitative determinations, made by Schloesing's method, have given me the following amounts of nitric nitrogen in fresh human urine.

Time of collection.	Nitric nitrogen per million.
October 1883, 11 A.M.	8·18
January 15, 1884, 11 A.M.	20·56
June 3, 1884, 8 A.M.	16·23
„ 25, 1884, 8 A.M.	7·08
October 21, 1884, 8 A.M.	4·76

In employing Schloesing's method, it is advisable not to take more

than 25 c.c. of urine, as the amount of carbonic acid produced by the splitting up of the urea is very large.

The amount of nitric acid occurring in urine appears from these determinations to be very variable, and in some cases rather considerable. The origin of this nitric acid is a subject of considerable interest. Bence Jones (*Phil. Trans.*, 1851, ii, 399) maintained that ammonia is oxidised to nitric acid in the human body, and the same conclusion has been recently arrived at by Th. Weyl. That the animal body is an organism possessed of wonderful oxidising powers is well known, and it would perhaps excite little surprise if it were shown that nitrates were produced in the process of oxidation. The evidence brought forward to support this conclusion is however at present very incomplete. That nitrates are present in many articles of food—notably in drinking water, and in most succulent vegetables—is an undoubted fact, and these nitrates, unless destroyed in the system, must appear in the urine; the evaporation from the body will also frequently determine the appearance of nitrates in the urine in a more concentrated form than in the food. To prove that nitrates are normally produced in the human body it would be necessary absolutely to exclude nitrates from the food. This has been partially done by Wulffius, who found that under these circumstances the nitrates in the urine were greatly diminished. The influence of diet probably explains the low contents of nitrate in the last two analyses just quoted; the urine in these cases was collected after a supper of bread and milk only. An entirely different mode of attacking the question is by administering ammonium salts, and observing whether the quantity of nitrates in the urine is increased under these conditions. This mode of inquiry has been adopted both by Bence Jones and by Th. Weyl. Both experimenters distil the urine with concentrated sulphuric acid, and examine the distillate for nitrous acid. Bence Jones seldom found nitrates by this method in normal urine; but after the administration of ammonia, ammonium carbonate, tartrate, or chloride, nitrous acid was found in the distillate by means of potassium iodide and starch; the same result followed the administration of urea. No quantitative experiments were made. Wulffius, on the other hand, found no increase of nitrates to follow the administration of ammonium chloride.

A recent research on the subject has been made by Th. Weyl. A summary of his communication to the Physiological Society of Berlin appears in *Nature* of April 3, 1884, p. 543. Apparently only a portion of the communication has been published, the paper by Th. Weyl, "Ueber die Nitrate des Thier und Pflanzenkörpers" (*Archiv für Pathologische Anatomie und Physiologie und für Klinische Medicin*, 1884, 462), dealing only with the historical part of the subject, and

with his results showing that nitrates are generally present in human urine, but not in that of dogs fed on flesh. According to the summary in *Nature*, Weyl found that in the case of men a very considerable increase in the nitrates of the urine followed the administration of ammonium citrate.

As it appeared of considerable interest to obtain further evidence as to this alleged oxidation of ammonia in the human body, I have made a few experiments with myself as subject. The method employed was to make a comparative experiment on two days of similar diet. At the end of the first day the urine passed between 10 P.M. and 8 A.M. was measured, and the nitric acid present determined. On the second day of similar diet, a known quantity of ammonium salt was taken in distilled water at 10 P.M., and the urine collected as before at 8 A.M., measured and analysed. In each determination, 25 c.c. of urine were evaporated to about 1 c.c., mixed with freshly boiled ferrous chloride and hydrochloric acid, and then distilled to dryness in Schloesing's tube retort, from which all air had previously been expelled. The nitric oxide in the mixed gases obtained was determined with considerable accuracy by the use of a Regnault-Frankland gas analysis apparatus. The results obtained in three comparative experiments, in which ammonium chloride, carbonate, and citrate were administered, were as follows:—

Date (1884).	Ammonium salt taken.	Urine collected 10 P.M. to 8 A.M.	Nitric nitrogen	
			Per mil- lion of urine.	Eliminated in urine during 10 hours.
June 3..	None	c.c. 587	16·23	mgrms. 9·5
„ 4..	Ammonium chloride, 1 gram	785	12·20	9·3
June 25..	None	595	7·08	4·2
„ 26..	Ammonium carbonate, 1 gram	670	5·98	4·0
Oct. 21..	None	650	4·76	3·1
„ 22..	Ammonium carbonate, 2 grams, neutralised with citric acid	910	4·28	3·9

The amounts of nitric nitrogen found per million of urine in each experiment are of little importance, as the degree of concentration of the urine varied a good deal; we must look rather at the absolute quantity of nitric nitrogen eliminated in equal times during each experiment. Thus viewed we find that the production of nitric acid

was practically unaffected by the administration of ammonium salts. In the case of ammonium chloride and carbonate, the nitric acid is very slightly diminished by their use, in the case of ammonium citrate it is slightly increased. The experiments with ammonium carbonate and citrate were made on a similar diet.

The few experiments here described appear to lend no support to the theory that ammonia is oxidised to nitric acid in the human body.