

LIX.—*The Formation and Destruction of Nitrates and Nitrites in Artificial Solutions and in River and Well Waters.*

By J. H. M. MUNRO, D.Sc., College of Agriculture, Downton, Salisbury.

CONTENTS.

	PAGE
Introduction	632
I. Nitrification of ethylamine by soil.....	633
II. " potassium thiocyanate by soil.....	637
III. " ammonium thiocyanate by soil.....	638
Experiment with thiocarbamide	639
IV. Nitrification of urea	639
V. " of gelatin by soil.....	641
VI. " of urine in accidentally fertilised solutions.....	642
VII. " of ammonium salts	643
Nitrification in strong and weak solutions of ammonium chloride	643
" in unfertilised solutions of ammonium chloride,	
sterilised by heat, and not sterilised	645
" of ammonium oxalate; decomposition of oxalates by	
soil ferments.....	645
" of ammonium chloride without purposely added	
organic carbon.....	646
" in boiled, filtered, transparent solutions	648
VIII. Is organic carbon essential to nitrification?	651
IX. Nitrification of ammonium salts in natural waters	654
Experiments on the nitrifying power of river and well waters....	658
X. Denitrification, and the influence of organic matter on nitrification..	667
Alternation of nitrification and denitrification	669
XI. Period of incubation of the nitrifying organism.....	679
Appendix	681

IN the spring of 1883, attracted by MM. Schloesing and Müntz's account of the nitrifying organism of the soil, and by Mr. R. Warington's extremely interesting researches on "Nitrification," which had been published in the *Journal of the Chemical Society* (Trans., 1878, 44, and Trans., 1879, 429), I commenced a few observations on the same subject. My first series of experiments was commenced before I became acquainted with Mr. Warington, and indeed, without the knowledge that he was pursuing his investigations on this subject. This series was designed to repeat and corroborate some of Mr. Warington's observations with ammonium salts, and to extend the results to three or four other nitrogenous compounds. Later on, I communicated with that gentleman, and received from him advice derived

from his own experience, and encouragement to pursue my observations with the view of eliciting new facts.

So slow, however, is the nitrifying process under some conditions, that this first series remained under observation for nearly two years, whilst subsequent nitrifying solutions, started in the summer and autumn of 1883, are only just done with. The great length of time occupied in these experiments is perhaps the reason why so few English chemists appear to have been attracted to the subject. Under these circumstances observations which corroborate the statements of others have some value, and in accordance with Mr. Warington's wish, I now condense into a brief form such of my results as appear of any interest, in view of the continuation of his researches (Trans., 1884, 637), together with some experiments on substances other than ammonium salts, on nitrification in natural waters, and on denitrification, which I believe to be original. The observations now described were made in 1883 and the spring of 1884, that is, between the publication of Warington's second (1879) and third (1884) reports; the only exceptions are certain experiments which I have made within the last two months, to elucidate points which occurred to me during the preparation of the paper for publication.

To attain as much clearness as possible, I have arranged the experiments in something like chronological order under separate headings, and will consider in succession—

Nitrification of Nitrogenous Compounds other than Ammonium Salts.

Nitrification of Ammonium Salts in Artificial Solutions.

Nitrification of Ammonium Salts in Well and River Waters.

Denitrification, and the Influence of Organic Matter on Nitrification.

I. *Nitrification of Ethylamine by Soil.*

We have direct and conclusive evidence that plants avail themselves of nitrogen supplied as nitrates, and that the nitrates are absorbed as such by the plants, and in some cases stored up without immediate alteration in their tissues. Taking into consideration with this the fact that all organic manures evolve ammonia during decomposition, and that ammonia in the soil is rapidly transformed into a nitrate, it may well be doubted whether plants ever assimilate nitrogen directly from any other compounds than nitrates. It was for this reason that it seemed to me interesting to ascertain whether ethylamine is nitrified by contact with soil, for G. Ville has long ago shown (*Recherches sur la Végétation*, 1857) that ethylamine and ammonia are equally efficacious as nitrogenous manures. Trimethylamine has also been successfully tried as a manure in France.

The sample of ethylamine hydrochloride employed was tested for purity by conversion into aurochloride. The crystals of ethylammonium aurochloride on ignition gave 50.78 per cent. metallic gold, whereas the formula $C_2H_5 \cdot NH_3Cl, AuCl_3$ requires 51.04 per cent.

I experimented in all with three solutions of ethylamine hydrochloride. Two of these were made up on March 9, 1883, and the description of the mode in which they were prepared and examined applies to all the solutions of my first series, to which the same date is affixed. The modifications adopted in later series are noticed in their place.

309 mgrms. (B1) and 618 mgrms. (B2) ethylamine hydrochloride were dissolved in ammonia-free distilled water in two stoppered bottles, to each of which I then added—

222 mgrms. potassium hydrogen tartrate,
 222 „ magnesium sulphate, cryst.,
 222 „ sodium phosphate, cryst.,

and water to 500 c.c. Half a teaspoonful of precipitated calcium carbonate to render the mixture permanently alkaline, and 2 grams moist arable soil, were put in afterwards. All the solutions of this date were kept in stoppered or corked bottles or flasks, about half full, which were placed in a cupboard in the laboratory, and exposed to the ordinary variations of temperature of the place. Chiefly on account of the low temperature prevailing, nitrification did not take place in any of the solutions of this series until the beginning of June, but when once commenced, it proceeded in several cases with great rapidity. The solutions were examined from time to time, for ammonia by the Nessler reagent, for nitrites by permanganate, and for nitrates or nitrites by the indigo test as used by Boussingault. It was only in later experiments that I adopted the more delicate reagents, metaphenylenediamine and diphenylamine, in place of permanganate and indigo for qualitative testings. The quantitative determinations were made as follows:—

Ammonia, by Nesslerising a diluted fraction of the liquid.

Nitrites, by weak standard permanganate (1 c.c. = 0.0001 gram available oxygen) in a pipetted fraction of the liquid acidified with sulphuric acid.

Nitrate in absence of nitrite, by the indigo process as modified by Warington.

Nitrate in presence of nitrite, by oxidising the nitrite in a pipetted fraction by cautious addition of permanganate, then determining the total nitrate with indigo, and deducting the nitrate formed from nitrite.

These methods, which are the same as those used by Warington,

enable one to follow very fairly the progress of a nitrifying solution, but they do not yield results of great accuracy. In some of my later experiments, for example, I wished to ascertain whether the nitrification was *solely* nitrous, or whether a small quantity of nitrate co-existed with the nitrite, and this it was extremely difficult to do, if not impossible. The estimations of ammoniacal nitrogen are merely approximate in all cases, since any error made in Nesslerising the very small fraction of liquid available for each testing is greatly multiplied when calculated on the whole 500 c.c.

The progress of the solutions B1 and B2 is recorded in the summary of observations made at the following dates :—

B1.—Made up March 9, 1883. 500 c.c. contained 309 mgrms. ethylamine hydrochloride = 53 mgrms. nitrogen. No ammonia, nitrites, or nitrates, were detected up to March 26. Between this and April 13, however, the solution became turbid, small patches of mycelium began to appear throughout the liquid, and ammonia was formed. By July 11 the ammonia present amounted to 16 mgrms. nitrogen, and nitrate was present. On July 26 the N as nitrite amounted to 28 mgrms., but between this date and August 16 it was completely converted into nitrate. Nitrification was practically complete on this date, and the N present as nitrate was estimated as $47\frac{1}{2}$ mgrms., or 90 per cent. of that originally taken.

B2.—Made up March 9, 1883, was double as strong as the preceding, and contained 106 mgrms. N in 500 c.c. For a time this followed a similar course to B1. On July 11 the ammonia present was equivalent to 33 mgrms. nitrogen, and the nitrous fermentation proceeded with such rapidity from this date, that by July 26 *all* the nitrogen was present as nitrite (105 mgrms. estimated). Instead of suffering conversion into nitrate by August 18, however, the nitrite was quite unaltered on that date, the quantity of permanganate consumed being exactly the same as before. Conversion into nitrate set in some time during the long vacation, for on October 18 *no* nitrite was present, and the N as nitrate was estimated as 104 mgrms., again over 90 per cent. of that originally present as ethylamine.

These two experiments show that ethylamine is easily nitrified, and that the process is divisible into three stages. At first ammonia is produced, and this may suffer total conversion into nitrite before any nitrate is formed (B2); the nitrite may be rapidly converted into nitrate, or may persist for a time without alteration (B2), ultimately, however, undergoing complete oxidation. The apparently capricious persistence of nitrite, ending sometimes in sudden and rapid conversion into nitrate, occurred in many of my experiments, as well as in those of Warington.

The third solution of ethylamine was made up on July 20, 1883,

and belongs to a series of mixtures nearly all of which failed to nitrify for 13 months, and then succeeded only after re-seeding with soil. At that date, I was anxious to obtain nearly clear solutions in which to observe nitrification, and I therefore tried to replace the carbonate of lime by soda or potash, and to substitute some other form of organic matter for the alkaline tartrate hitherto generally employed, and supposed to serve as aliment for the nitrifying organism. When an alkaline tartrate or any similar substance is employed, a bacterial fermentation is set up almost immediately, which destroys nitrates if they happen to exist already in the liquid experimented on, and at any rate renders the whole liquid turbid from the presence of myriads of organisms. These die down after a few days and form a deposit at the bottom of the liquid, but they never leave the liquid absolutely clear. At a later stage I was able to avoid these bacteria altogether, to do without tartrates or similar substances, and to obtain almost perfectly clear nitrifying solutions, but my earliest attempts almost all failed at the first seeding, apparently because 1 or 2 mgrms. soil is insufficient to induce nitrification in *unfavourable solutions*.

B3 contained no organic matter beyond the ethylamine itself, and that in the milligram or two of added soil. The solution was made up to contain in 100 c.c. of ammonia-free distilled water—

31	mgrms.	ethylamine hydrochloride (= 5.3 mgrms. N).
20	„	MgSO ₄ , cryst.
20	„	Na ₂ HPO ₄ , cryst.
17.7	„	KHO.

The alkalinity of this solution is equal to about 145 mgrms. KHO per litre (= 36.25 ammoniacal N per million), after allowing for complete saturation of the phosphoric acid. The solution about half filled a 6-oz. stoppered reagent bottle, and was seeded with 1 or 2 mgrms. *only* of dry soil from 3 or 4 inches depth. It remained quite clear (except for a few flocks of magnesium phosphate) until February 2, 1884, and contained no ammonia, nitrite, or nitrate on that date. On April 7, 1884, it was still clear, but a little ammonia was present, and on August 21, 1884, more than a year after seeding, although ammonia was still present, no nitrate or nitrite could be detected, even with diphenylamine. Some time before this, viz., on November 10, 1883, thinking that the failure to nitrify might be due to the exclusion of the alkaline tartrate, I had divided this solution (together with others which had been made without tartrate, and had refused to nitrify) into two portions, one of which I placed in an open wide-mouthed bottle, with a paper cap. To this part of the solution I added a small crystal of Rochelle salt, and the bottle was

kept in a cupboard in a warm room all the winter. Yet although a delicate cloudlike fungus gradually grew in the solution, no nitrite or nitrate had developed by April 7, 1884; the solution at this date having almost dried up, was thrown away. I may mention here that in no instance did the addition of Rochelle salt to the solutions which had refused to nitrify, produce the slightest formation of nitrite or nitrate, although in every case it was speedily followed by various growths of mould. Having thus come to the conclusion that absence of the alkaline tartrate was not the reason of non-nitrification in the portion of solution which had been kept in the stoppered bottle until August 21, 1884, I on that date re-seeded it with a few more milligrams soil, and on November 13, 1884, I had the satisfaction of finding it in full nitrification, both nitrite and ammonia being present in considerable quantity. This solution was unfavourable for nitrification, since it contained a caustic alkali, but the insufficient quantity of soil added at first was evidently *the* prohibitive circumstance. It remained free from mould throughout, and is at present nearly clear. The nitrite present on November 13, 1884, has since been converted into nitrate, but ammonia is still present (March, 1886). The original alkalinity of the solution, in fact (= 14.5 mgrms. KHO), is insufficient to allow of complete nitrification, since the hydrochloric and nitric acids produced from 31 mgrms. ethylamine hydrochloride require 42.4 mgrms. KHO for saturation.

A plain solution of ethylamine hydrochloride in distilled water, containing 3 grams per litre, has remained perfectly free from ammonia and nitrite from July 20, 1883, until now (March, 1886).

II. *Nitrification of Potassium Thiocyanate.*

The thiocyanates, it is well known, are poisonous to plants, and it occurred to me that possibly nitrogen in this form is not susceptible of fermentation into nitrate. I therefore included potassium thiocyanate in my first series of solutions, made on March 9, 1883. Solution (C1) contained 243 mgrms. KCNS; 222 mgrms. potassium bitartrate; 222 mgrms. MgSO₄ cryst.; and 222 mgrms. Na₂HPO₄ cryst., in 500 c.c. ammonia-free distilled water. Calcium carbonate and 2 grams moist soil were added. Total N = 35 mgrms. Until March 26 no change occurred. Ammonia then slowly developed, and a mould began to grow. On July 19, 33 mgrms. N existed as ammonia, and a trace of nitrite was present. On August 16 the ammonia had fallen to 12 mgrms. N, and the nitrite had increased; as the thiocyanate itself decolorises permanganate, no estimation was possible. A little nitrite was still present on October 18, although there was then no ammonia. On October 29, both nitrite

and ammonia were absent, and the N as nitrate was estimated at 27 mgrms., a nitrification of nearly 80 per cent. As showing the complete oxidation of the thiocyanate, I may mention that when made up, and even on March 26, 5 c.c. of the solution decolorised 14.4 c.c. of standard permanganate; on October 29, one drop of permanganate sufficed to colour the liquid. The reaction with ferric salts disappeared before June 9.

It appears from this experiment that thiocyanates are easily nitrified by soil, and that as in other cases, ammonia, nitrite, and nitrate appear in succession. A plain dilute solution of potassium thiocyanate in distilled water, made up March 9, 1883, is now, three years later, quite free from nitrite and nitrate. It has grown a few spots of mould, however, and contains a trace of ammonia.

III. *Nitrification of Ammonium Thiocyanate. Experiment with Thiocarbamide.*

Although ammonium thiocyanate is a plant poison, its isomeride, thiocarbamide, is stated by Dr. Emerson Reynolds to act as a nitrogenous manure. It is curious that in the following experiments I should have succeeded in nitrifying the former, whilst the latter has resisted nitrification for nearly three years. The ammonium thiocyanate resisted nitrification for 13 months, because of the sterility of the 1 or 2 mgrms. of soil with which the solution was seeded, but it commenced to nitrify very shortly after being re-seeded with soil; re-seeding, however, has *not* induced nitrification of the thiocarbamide.

Ammonium Thiocyanate.—Solution G1, made up July 20, 1883. The 100 c.c. ammonia-free distilled water contained 35 mgrms. NH_4CNS ; 20 mgrms. MgSO_4 cryst.; 20 mgrms. Na_2HPO_4 cryst., and 20 mgrms. oxalic acid crystals neutralised by addition of 17.7 mgrms. KHO . 1 or 2 mgrms. dry soil were added, and a little magnesium carbonate. This bottle, it will be seen, belongs to the first series, from which I excluded tartrates, on account of their liability to encourage reducing bacteria and moulds. Organic carbon was supplied in the form of an oxalate, and this made no difference to the eventual nitrification of the solution, although I for some time believed that it would. I now believe that the oxalate might also have been omitted, but prefer to discuss the influence of organic matter on nitrification in the light of subsequent experiments.

Progress of the solution:—Up to November 10, 1883, no change could be detected, the solution giving the reactions for oxalates, ammonia, and thiocyanates, remaining perfectly clear, and giving no evidence of nitrite or nitrate with metaphenylenediamine or

indigo. Half of the solution was then removed to a shallow bottle, a crystal of Rochelle salt added, and the bottle placed in a warm cupboard for the winter. On February 2, 1884, it began to grow a mould, which increased in amount until April 7, 1884, when the solution was thrown away, having completely failed to nitrify, notwithstanding the addition of tartrate. The half solution containing no tartrate was kept in its stoppered bottle until August 14, 1884, and as it showed no nitrification or other apparent change at this date, 13 months after being made up, it was re-seeded with a few milligrams of soil. By November 13, 1884, abundance of nitrite was formed, but ammonia was still present. Since then, both ammonia and nitrite have been converted into nitrate, and the reaction with ferric salts has disappeared. The addition of a sufficient quantity of soil was therefore the indispensable condition of nitrification with this solution.

Thiocarbamide.—I prepared this substance in the ordinary manner by heating ammonium thiocyanate at 170° C. for some time, but I could not free it entirely from traces of that salt. The solution for nitrification was of the same strength, and was made up on the same date (July 20, 1883) as the corresponding one of ammonium thiocyanate, and it was in all respects similarly treated. I need therefore only say that it entirely failed to nitrify, even after re-seeding with soil, and on March 13, 1886, it contained neither nitrite nor nitrate. The reaction with ferric salts, due to the small admixture of thiocyanate, gradually disappeared, but the thiocarbamide appears to have suffered no change.

Quite recently I have made a third attempt to nitrify this solution. A gram of fresh soil was added three months ago, but as I write (June 8, 1886) the solution is unaltered, and there is no sign of nitrification. After three seedings, and a period of trial extending over nearly three years, we may conclude with tolerable certainty that thiocarbamide is not nitrifiable; very probably it exercises an antiseptic action fatal to nitrification, for otherwise the traces of ammonium thiocyanate with which it was contaminated would certainly have furnished nitrate sufficient for detection.

Plain solutions of ammonium thiocyanate and of thiocarbamide in distilled water (1 mgrm. per c.c.) have remained free from nitrite or nitrate, and without apparent alteration, for nearly three years.

IV. *Nitrification of Urea.*

Diluted urine has been used as a medium for nitrification in many of Mr. Warington's experiments, and in the summer of 1883 I made up a few solutions from instructions which he was kind enough to

give me. I need only say that they nitrified quickly, passing through the usual stages of ammonia, nitrite, nitrate. The two experiments now to be described were made with pure crystallised urea, in order to see whether nitrification would take place without the presence of the organic matter which accompanies urea in urine. To the first bottle (F1) I therefore added organic carbon in the shape of an oxalate only; the solution eventually nitrified (and I believe would have done so without the oxalate). The second bottle (F2) was like the first, but soil was entirely omitted. The behaviour of these two solutions exhibits very well the vital relation of soil to the process of nitrification. F1 seeded at first with only a milligram or two of soil, refused to nitrify for 13 months, when re-seeding with a few milligrams of soil speedily set up nitrification. F2, although not sterilised by heat, has remained for 2 years and 8 months without showing a sign of nitrification. It developed ammonia, though more slowly than F1; but in the absence of soil the ammonia showed no tendency to pass into nitrite or nitrate, notwithstanding the supply of salifiable base at hand. All the experiments have tended to convince me that the ammoniacal ferment is far more generally diffused than the nitric ferment.

The solutions were made up July 20, 1883. Total N in each = 9.3 mgrms.

F1. 100 c.c. contained 20 mgrms. urea; 20 mgrms. $MgSO_4$ cryst.; 20 mgrms. Na_2HPO_4 cryst.; and 20 mgrms. crystallised oxalic acid neutralised with 17.7 mgams. KHO. A little magnesium carbonate and 1 or 2 mgrms. soil were added.

F2. Exactly like F1, but with no soil.

Aug. 1, 1883. F1 contains 0.2 mgrm. NH_3 . F2 contains no NH_3 .

Aug. 16, 1883. F1 contains 0.4 mgrm. NH_3 . F2 „ „

Aug. 21, 1883. F1 contains 0.7 mgrm. NH_3 . F2 „ „

Oct. 18, 1883. F2 contains trace NH_3 .

Nov. 10, 1883. F1 contains 1.0 mgrm. NH_3 . F2 contains 2.5 mgrms. NH_3 .

When once contaminated, therefore, F2 developed ammonia faster than F1. No nitrite or nitrate having appeared in either solution to this date, half of each solution was removed to another bottle, and a crystal of Rochelle salt added as explained in previous sections. These tartrated solutions remained without nitrification (although the one with soil eventually grew a mould) until April, 7, 1884, when they were thrown away.

Aug. 14, 1884. F1 and F2 (not tartrated) are clear, free from mould, contain ammonia but no nitrite or

- nitrate. F1 was now re-seeded with a few milligrams of soil.
- Nov. 13, 1884. F1 contains ammonia and nitrite, and is therefore nitrifying.
F2 contains ammonia but no nitrite or nitrate.
- Mar. 13, 1886. F1 contains a trace of ammonia and much nitrite.
MgCO₃ still in excess.
F2 contains much ammonia, no nitrite or nitrate.

The nitrification of F1 is, therefore, even now not complete, and is chiefly, perhaps wholly, nitrous. The quantity of liquid remaining is too small for analysis.

After the last testing, March 13, 1886, E2 was seeded with soil, and it is now, June 10, 1886, in full nitrification.

A plain solution of urea in distilled water (5 mgrms. per c.c.) behaved like F2. Soon after being made up (July 20, 1883), it developed a little ammonia, which it still contains, but no nitrite or nitrate. Most ammonia-forming substances, when dissolved in distilled water, give rise to a *little* ammonia, unless the solutions are sterilised by heat and protected from the entrance of ordinary air. The ammonia-forming ferment, however, does not cause nitrification even in presence of supply of base.

V. Nitrification of Gelatin.

The sample of gelatin used contained 19.0 per cent. of water. 250 mgrms. were dissolved in 500 c.c. water and 222 mgrms. each of crystallised magnesium sulphate, sodium phosphate, and potassium bitartrate, added to the solution. Carbonate of lime and 2 grams moist soil were also added. The solution belonged to the first series and was treated in all respects like its companions.

- Mar. 9, 1883. Made up. Total nitrogen present = 36 mgrms.
Mar. 15, 1883. No ammonia.
Mar. 26, 1883. Ammonia = 4 mgrms. nitrogen. The solution turbid, tufts of mycelium on surface.
April 1, 1883. Ammonia = 12 mgrms. nitrogen. Turbidity and flocks of mycelium much increased, odour putrid.
April 13, 1883. Ammonia = 29 mgrms. nitrogen. No odour; turbidity lessened; shows dead bacteria under microscope. No nitrite or nitrate.
June 4, 1883. NH₃ present. No nitrite or nitrate.
July 9, 1883. NH₃ absent. The solution decolorises indigo. Nitrite present.
July 23, 1883. No ammonia or nitrate. 32 mgrms. nitrogen present as nitrate.

This experiment exhibits the ordinary course of nitrification of nitrogenous organic matter under favourable circumstances. First came a putrid bacterial fermentation, during which nearly all the nitrogen was transformed into ammonia. This was succeeded by a pause until the weather became warm enough for nitrification, which set in some time between June 4 and July 9, and progressed very rapidly. Nitrite was at first found, but speedily disappeared, and on July 23, 88 per cent. of the nitrogen of the gelatine existed as nitrate.

A plain solution of gelatin was also made up March 9, 1883, in boiling distilled water in a long-necked stoppered flask. The stopper was removed occasionally when tests were made, so that slight contamination from the air was quite possible. In a few days the solution began to grow turbid, developed a putrid odour, and gave a green colour with Nessler solution. After a little while, the ordinary ammonia colour was given with Nessler reagent, but the production of ammonia soon ceased. The turbidity decreased, and the solution remained without further change until August 21, 1884, on which date it was nearly clear, but still contained ammonia and retained the putrid odour. No nitrite or nitrate was present, and in order to see whether the addition of a salifiable base would produce nitrification a little magnesium carbonate was put in. The solution still (March 13, 1886) contains ammonia, but no nitrite or nitrate, thus supporting the soil-ferment theory of nitrification.

VI. *Nitrification of Urine.*

Four experiments with diluted urine, I record here because they present two instances of nitrification through accidental fertilisation of the solutions. The peculiar behaviour of these solutions, far from being opposed to the ferment theory of nitrification, is quite in harmony with it.

E1 and E2 were made up June 11, 1883. 500 c.c. ammonia-free water with 2 c.c. urine were placed in two litre flasks, which were plunged in boiling water for 30 minutes; the necks of the flasks were plugged with cotton-wool while steam was issuing. When cold, a little unsterilised calcium carbonate, in fine powder, was added to each. E1 was seeded with soil. E2 was not seeded. Both flasks were covered with paper caps, and kept in the dark.

In 28 days E1 contained 20—24 mgrms. N as ammonia, and nitrification had commenced.

In 50 days 17 mgrms. N were present as nitrite, and oxidation to nitrate was completed during the next month.

E2, on the contrary, although it developed ammonia nearly as fast as E1, showed no sign of nitrification in 50 days. But after 113 days,

20 mgrms. N were present as nitrite, which took more than two months longer to pass into nitrate. There are three possible sources of contamination in this experiment; the unsterilised calcium carbonate, the entrance of air and dust during the 7 or 8 removals of the cotton-wool plug for testing purposes, and the use of pipettes for testing which were washed simply with distilled water instead of boiling water. The next pair of experiments fixes the responsibility upon the last circumstance, since the two former possible sources of contamination were present without causing nitrification, but when the third source was introduced, nitrification followed.

E3 and E4, made up November 10, 1883. Each consisted of 1 c.c. urine in 100 c.c. water, with a little calcium carbonate. The liquids were not sterilised by heat, and were placed in stoppered bottles, half full. E3 was seeded with five drops of the accidentally contaminated solution E2. E4 was not seeded.

E3 showed signs of nitrification in 37 days, and the nitrite present gradually increased until on the 155th day it amounted to 12 mgrms. N, which passed into nitrate before the next testing made on the 225th day.

The stopper of E4 *was not removed during the first 155 days*, at the end of which time neither nitrite nor nitrate could be detected. The stopper was replaced and *not removed again until the 225th day*, when there was still no sign of nitrification. At the third removal of the stopper, however, on the 283rd day, 8 mgrms. N were found as nitrite, which had not increased at the fourth testing made on the 368th day. Up to this time, therefore, the very slight accidental contamination had caused a very slow, incomplete, and purely nitrous fermentation. The addition of *a centigram of soil* on this date, caused rapid and complete conversion into nitrate.

VII. *Nitrification of Ammonium Salts in Artificial Solutions.*

The first solutions made up included strong and weak solutions of ammonium chloride, an intermediate solution of ammonium chloride not fertilised by soil nor sterilised by heat, and a solution of ammonium oxalate.

[Strong and Weak Solutions of Ammonium Chloride.

The weak solution (A1) contained 400 mgrms. of the salt per litre; the strong solution (A2) 2000 mgrms. per litre. Mr. Warington's weak solutions contain generally 80 mgrms. NH_4Cl per litre, and when his second report was published (1879), the strongest solution nitrified contained 640 mgrms. per litre. He states in his last report (December, 1884) that he has since completely nitrified

solutions containing 1280 mgrms. per litre, and that solutions containing as much as 8000 mgrms. are in process of nitrification. My solutions were made up on March 9, 1883; to 500 c.c. of each were added 222 mgrms. potassium bitartrate; 222 mgrms. $MgSO_4$ cryst.; 222 mgrms. Na_2HSO_4 cryst.; a teaspoonful of calcium carbonate; and 2 grams of moist soil. The progress of the solutions (kept in stoppered bottles, half-full, in a cupboard at the ordinary temperature of the laboratory) is shown in the following table:—

March 9, 1883. Made up. A1 contained 52.3 mgrms. N, and A2 contained 262 mgrms. N. Tested and Nesslerised at intervals, neither of them showed diminution in ammonia, or presence of nitrite or nitrate up to June 4, 1883. Between this date and July 9, 1883, both began to nitrify. The subsequent course of the weaker solution A1 was as follows:—

July 9, 1883. Ammonia = 33 mgrms. N. Decolorises indigo.

„ 23, 1883. No nitrite. Nitrate = 23 mgrms. N.

Aug. 16, 1883. Ammonia = 15 mgrms. N. Nitrite = 9 mgrms. N.

Nitrate = 28 mgrms. N. (by diff.).

Oct. 18, 1883. No ammonia. No nitrite. Nitrate = $47\frac{1}{2}$ mgrms. N.

Nitrite was doubtless present between June 4 and July 23, but it was not tested for; at any rate it had been entirely converted into nitrate by the latter date, between which and August 16 a fresh formation of nitrite took place, and the solution was completely nitrified into nitrate during the long vacation.

The progress of the strong solution (A2), only a third of whose nitrogen was oxidised to nitrite when A1 had run its complete course, is shown by the estimations of nitrite made at the following dates:—

July 9, 1883. Nitrite present.

„ 23, 1883. Nitrite = 70 mgrms. N.

Aug. 16, 1883. „ = 88 „ N. Ammonia estimated at 165 mgrms. N.

Oct. 18, 1883. „ = 87 „ N. Ammonia estimated at 144 mgrms. N.

Feb. 7, 1884. Nitrite = 86 mgrms. N.

„ 27, 1884. „ = 86 „ N.

Apr. 7, 1884. „ = 35 „ N. Nitrite and nitrate estimated at 115 mgrms. N.

June 24, 1884. „ = 35 „ N.

Aug. 19, 1884. „ = 175 „ N. No ammonia.

Nov. 11, 1884. „ = 175 „ N.

Mar. 18, 1886. „ = 86 „ N. Nitrite and nitrate estimated at 236 mgrms. N.

June 5, 1886. „ None.

The completion of nitrification in this solution has only taken place since I commenced preparing this paper for publication, and altogether the process has occupied *more than two years and nine months*. But the solution and residue remaining in the bottle now contain so much of the nitrifying ferment that 250 c.c. of strong ammonium chloride solution (1 gram per litre), added bodily, has commenced to nitrify in *five days*.

*Unfertilised Solutions of Ammonium Chloride, sterilised by Heat,
and not sterilised.*

A solution (A3b—March 9, 1883) of ammonium chloride like A1, but with the soil omitted, underwent a tardy and for a time entirely nitrous fermentation; a second solution (A4b) made up November 10, 1883, also unseeded, nitrified completely to nitrite after some time, and the nitrite has persisted ever since. Both of these solutions were unsterilised by heat and were kept in ordinary stoppered bottles, half-full. The nitrification of A4b was much slower than that of a similar solution (A5) made up at the same time, and seeded with five drops only of a nitrifying solution (A2), although both nitrified to nitrite only. I have succeeded in ascertaining definitely that the standard ammonium chloride* employed in making up A3b and A4b was itself contaminated with a trace of the nitrous ferment; it had been made up two years before, and *all* the solutions in which it was used *which were not sterilised*, eventually nitrified. On the other hand, two *boiled* solutions (AB1, AB2) in flasks with cotton-wool plugs and paper caps, which were made up as checks on April 14, 1884, and not opened until March 18, 1886, have remained without the formation of any nitrate or nitrite; they still contain plenty of ammonia.

Nitrification of Ammonium Oxalate.

A solution of the first series (A9, March 9, 1883) contained 250 mgrms. of the crystallised salt. It nitrified to nitrate even more rapidly than the corresponding solution of ammonium chloride made up on the same day. I have since ascertained (p. 648) that the oxalic acid is destroyed long before nitrification sets in. By taking advantage of this fact, ammonium oxalate can be completely nitrified with half the proportion of salifiable base necessary when ammonium chloride is used, and sodium or potassium oxalate may be used in place of the salifiable base usually employed.

* See note, p. 654.

Nitrification of Ammonium Chloride; Organic Carbon added in the form of Potassium Oxalate only.

On July 20, 1883, nine solutions were made up without an alkaline tartrate; in eight of these, potassium oxalate was substituted; the ninth, in which ethylamine was to be nitrified instead of ammonia, contained neither tartrate nor oxalate. The history of five of the solutions, containing respectively ethylamine, ammonium thiocyanate, thiocarbamide, urea, and urea (not seeded), has already been given. The remaining four were devoted to ammonium chloride.

Each of these four solutions contained in 100 c.c. ammonia-free distilled water—

63	mgrms. ammonium chloride = 16.5 mgrms. N.
20	„ MgSO ₄ cryst.
20	„ Na ₂ HSO ₄ cryst.
26.3	„ potassium oxalate (K ₂ C ₂ O ₄).

Three of them, A11, A12*b*, and A13*c*, were rendered alkaline by magnesium carbonate, and

A11 was fertilised with 1 or 2 mgrms. soil;

A12*b* was not fertilised;

A13*c* received 1 or 2 mgrms. soil and a few drops of chloroform.

The remaining solution,

A10, was rendered alkaline by 17.7 mgrms. KHO instead of the magnesium carbonate; it was fertilised with 1 or 2 mgrms. soil.

The four solutions were kept in 6-oz. stoppered reagent bottles in a cupboard in a warm room, and were examined at frequent intervals.

Three and a half months after making up, viz., on November 10, 1883, as no nitrite or nitrate had formed in any of them, half of each solution was removed to a wide-mouthed bottle, a crystal of Rochelle salt dissolved in each of these halves, and the bottles covered with loose paper caps. These tartrated halves of the solutions were examined at intervals up to April 7, 1884, when evaporation and frequent testings had reduced them to a very small bulk; they were then thrown away. On that date, nearly eight months after being made up, and five months after addition of tartrate, neither of them had commenced to nitrify. A13*c*, from which the chloroform had of course disappeared long before, had grown a patch of mould, but the other three were quite free from mould.

The untartrated halves of the solutions, in the stoppered bottles, were also free from moulds and from nitrite or nitrate on April 7, 1884, and they were not again examined until August 21, 1884, 13 months after being made up. On this date, one only of the four had undergone partial nitrification; this was A11, which had always been con-

sidered the most favourable solution, since it contained magnesium carbonate instead of caustic potash, and was seeded with soil. The $MgCO_3$ deposit had all disappeared, and the stopper jumped out when loosened, from accumulation of carbon dioxide gas. Ammonia was still present, but the reaction of the liquid, originally alkaline, was now faintly acid. 10 mgrms. nitrogen were present as nitrite, so that nitrification had evidently proceeded until all the available base was saturated. Some more $MgCO_3$ was added at this date, and the solution again examined on November 13, 1884, when all the ammonia had disappeared, and $14\frac{1}{2}$ mgrms. nitrogen were present as nitrite, out of the $16\frac{1}{2}$ mgrms. originally added as ammonia. The nitrite has persisted without alteration for another 16 months, so that the fermentation in this solution was purely nitrous throughout.

A10, the caustic potash solution, was re-seeded with a few milligrams of soil on August 14, 1884, and by November 13, 1884, this also had nitrified entirely to nitrite, and the nitrite still persists without diminution (March 19, 1886).

To A12*b*, the unseeded solution, a little fresh $MgCO_3$ was added on August 21, 1884, but up to the present date no nitrification whatever has taken place, and the solution gives a deep brown precipitate with Nessler's test. Here we have another instance of a non-fertilised but also non-sterilised solution, refusing to nitrify for two years and four months, although exposed to occasional chances of contamination from the air.

To A13*c*, the fertilised but chloroformed solution, some more soil was added August 21, 1884. On November 13, 1884, the odour of chloroform was very faint, but no nitrification had occurred. To-day (March 19, 1886) there is no odour of chloroform, but still there is no nitrification, and the solution gives an abundant brown precipitate with Nessler test. Of these four solutions then—

A11, with $MgCO_3$ as base, underwent a complete nitrous fermentation without re-seeding, but after a long delay.

A10, with KHO as base, underwent a complete nitrous fermentation, but only after re-seeding.

A12*b*, with $MgCO_3$ as base, but not fertilised, has altogether refused to nitrify.

A13*c*, with $MgCO_3$ as base, fertilised and chloroformed, has altogether refused to nitrify, even after re-seeding.

These solutions, therefore, strongly support the ferment theory of nitrification.

As regards the substitution of an oxalate for a tartrate, it remains for me to add that in four out of the eight solutions to which it was added, the oxalate had completely disappeared one month afterwards

(August 16, 1883). Three out of these four solutions had been seeded with soil. The two solutions in which the oxalate persisted unaltered for some months, were the *unfertilised* solution A12b and the *chloroformed* solution A13c.

These facts suggested to me that the oxalate was decomposed by the agency of soil ferments, and hence that its "organic carbon" exercised no influence on the nitrification. I have recently verified this. Solutions of alkaline oxalates are rapidly attacked by soil ferments; they become opalescent with bacteria, the oxalate is destroyed in a few days, and an alkaline carbonate is found in its place. This change is entirely prevented by boiling, and to a great extent by chloroform. It follows from this that an alkaline oxalate may be used as *salifiable base* in nitrification experiments. For example, 100 mgrms. ammonium oxalate crystals were completely nitrified with 170 mgrms. sodium oxalate as salifiable base. The oxalate was entirely destroyed by the 5th day; nitrification commenced between the 21st and 26th day; three-fourths of the nitrogen was present as nitrite on the 65th day, and nitrification to nitrate was finished before the 80th day.

Nitrification in boiled, filtered, transparent solutions of Ammonium Chloride.

Three solutions were made up on November 10, 1883, with the especial object of studying the influence of nitrification on the transparency of a solution and of observing the formation or non-formation of a deposit or surface growth. Sodium bicarbonate was, therefore, used as the salifiable base instead of calcium or magnesium carbonates; the solution was filtered twice through Swedish paper, boiled, and protected from the entrance of unfiltered air; and fertilisation was effected in one case by only 1 or 2 mgrms. soil, and in the other case by five drops of an apparently clear solution undergoing the nitrous fermentation (A2). The third solution was not fertilised and was not opened for six months after making up. Each solution contained in 100 c.c. ammonia-free distilled water—

63 mgrms.	NH ₄ Cl =	16·5 mgrms. N.
40	„	Rochelle salt cryst.
10	„	MgSO ₄ cryst.
10	„	Na ₂ HPO ₄ cryst.
60	„	NaHCO ₃ .

These solutions were filtered through double Swedish filters into narrow-necked 200 c.c. flasks, previously cleansed with boiling pure sulphuric acid. The solutions were sterilised by boiling, the necks of

the flasks were plugged with cotton-wool while steam was issuing, and, on cooling, paper caps were tied over the mouths. Of course some ammonia was lost during the boiling, and also subsequently by evaporation through the cotton-wool plugs, but plenty remained intact and underwent nitrification in due course.

The flasks were locked in a bureau in a living room, and were examined as to transparency, &c., from time to time. The cotton-wool plugs of two of them, A6 and A7, were removed on the cooling of the solutions for the introduction of the ferment, and at intervals afterwards in order to mark by testing the commencement of nitrification; the plug and cap of A8*b*, the unfertilised solution, remained untouched from November 10, 1883, to April 14, 1884. The following observations were made at the dates specified:—

Nov. 20, 1883. The solutions A6 (fertilised with a mgrm. of soil) and A7 (fertilised with five drops of a nitrifying solution) are both opalescent. There is no sign of nitrification. The opalescence of A6 is seen under the microscope ($\frac{1}{10}$ " objective) to be caused by bacteria, which exhibit a slow oscillating motion, a few rotifers are also present in active motion.

Dec. 3, 1883. A6 has become clearer; A7 still turbid.

Dec. 9, 1883. A6 still clearer; dead bacteria are settling down in minute patches. A7 still turbid. No nitrification in either.

Jan. 24, 1884. A6 clear, with deposit of dead bacteria. Nitrification has commenced.

A7 nearly clear, with deposit of dead bacteria; three or four tufts of mould have commenced to grow from the bottom. Nitrification has commenced.

Feb. 15, 1884. A6, appearance unaltered. Contains nitrite.

A7 not quite clear. Clouds of mould increased. Contains nitrite.

April 9, 1884. A6 still contains ammonia. Nitrogen as nitrite = $1\frac{1}{2}$ mgrm. Nitrate also present, but no exact estimation could be made.

A7 still contains ammonia. Nitrogen as nitrite = 2 mgrms. Doubtful whether any nitrate present.

Up to this date, therefore, A6 had undergone a partial fermentation into nitrite and nitrate, and A7 a partial fermentation into nitrite. The unfertilised solution A8*b*, opened for the first time April 14, 1884, was *absolutely* bright and clear, and contained not a trace of nitrite or nitrate. Compared with this solution, the clearness

of A6 and A7 was seen to be far from perfect; A6 was the clearer of the two, but was faintly opalescent.

June 24, 1884. A6 and A7 still contained ammonia; nitrite diminished to $\frac{1}{2}$ to $1\frac{1}{4}$ mgrm. N respectively.

The unfertilised solution A8b did not remain absolutely bright after being opened on April 9. It soon developed a delicate opalescence, which remained on June 24, 1884. There was then no sign of nitrification, however, so that the first temporary exposure to the air, although it had certainly introduced germs into the solution, had not inoculated it with the nitrifying ferment.

Aug. 21, 1884. By this time A8b had begun to nitrify, so that the second exposure or testing on June 24 was fatal to it. Ammonia was still present. The N as nitrite was 2·8 mgrms., and a very careful estimation (after oxidation with permanganate) of the total N as nitrate gave 2·77 mgrms. Evidently, therefore, the fermentation was *purely* nitrous. The opalescence previously noticed had ended in a slight flocculent deposit.

A6. The nitrite has now disappeared, but ammonia is still present.

A7. The nitrite has increased to 5 mgrms. N, but ammonia is still present.

Nitrification in A6 and A7 being arrested by saturation of the sodium bicarbonate, more of this salt was added to each solution on this date. By November 13, 1884, A6 had nitrified completely to nitrate; A7 afterwards nitrified completely to *nitrite*; in A8b, the formation of nitrite has proceeded more slowly, and even now a little ammonia is unnitrified.

Of these solutions, therefore, the only one that has, in 32 months, completely nitrified to nitrate is the one seeded with soil; the solution seeded with a nitrous fermenting solution, has nitrified completely to nitrite; and the unseeded solution, which remained absolutely intact so long as it was not exposed to contamination, has partially nitrified to nitrite. These results are in exact accordance with Mr. Warington's conclusions, and are scarcely explicable by any other theory than that of ferment introduced from without.

The difficulty in producing nitrification in otherwise clear tartrated solutions will be seen from the above description to be very great. The organisms which render so many organic infusions opalescent after even a momentary exposure to unfiltered air, are immensely more abundant than the nitric ferment, and very much easier of cultivation; and they not only attack the organic matter and render the solutions turbid, but they reduce and sometimes destroy any nitrate that may be present in the liquid. Unless ordinary bacterial germs are rigorously excluded, therefore, the presence of organic matter of this sort must be prejudicial to nitrification.

This exclusion may possibly be accomplished by employing a very pure cultivation of the nitrifying organism, and by permitting the access of filtered air only. But a much easier method of avoiding the multiplication of these bacteria is to exclude Rochelle salt or any similar organic matter from the solutions; and this may be done either by relying on the organic carbon in the soil used for seeding, or by using, as a nitrifying medium, well water to which ammonia has been added. *The organic matter of soil and of well water, being the residue of bacterial fermentations, is impervious to the attack of the bacteria in question, and does not encourage their multiplication.* Hence such solutions remain clear, although seeded with soil and exposed to unfiltered air.

VIII. *Is Organic Carbon essential to Nitrification?*

This is a vital question, for could it be positively shown that nitrification will take place in the absolute absence of organic carbon, either the theory of an organised nitrifying ferment would fall to the ground or we should have to admit two processes of nitrification, one depending on, and the other independent of, a nitrifying organism. There is no point, however, in the whole study which is surrounded with more difficulties than this one. For granting that we succeed in excluding accidental organic matter from the distilled water, the ammonium chloride, the salifiable base, and the other minerals employed, we have still to deal with the air, perhaps filtered through a cotton-wool plug, which in itself may be a source of contamination. Even supposing these difficulties overcome, there remains the organic-matter introduced with the seed, which may be either a few particles of soil or a few drops of a nitrifying solution containing added organic matter.

I therefore discuss the subject more with the view of throwing light on the practical requirements of the process of nitrification as regards organic carbon than with any intention of making the presence or absence of carbon a crux of the organism theory of nitrification. This theory is susceptible of other and easier lines of proof, and to my mind is supported by overwhelming evidence.

In nearly all of Mr. Warington's published experiments, either an alkaline tartrate (in a few cases glucose) has been added to the solutions with a view of supplying carbonaceous aliment for the nitrifying organism, or else a substance has been chosen for nitrification which in itself contained an abundant supply of organic carbon (asparagine, milk, rape cake, urine, &c.). There are, however, two experiments, recorded in his 1879 paper, in which nitrification occurred in the presence of very little carbon. These solutions contained ammonium

chloride, potassium sulphate, calcium phosphate and carbonate; some organic carbon was, however, added, for they were seeded with a small fragment of mushroom mycelium, and with 1 c.c. of a shaken up liquid which had been seeded with $1\frac{1}{4}$ grams of soil and had received 50 mgrms. potassium bitartrate as well. One of these (kept in the dark) nitrified completely to nitrate in nine months; the other, in the light, nitrified partially to nitrite. These I believe are the only recorded experiments in which the proportion of organic carbon to ammoniacal nitrogen was less than 3 : 10, exclusive of the carbon in the seed, and in most of them it was much greater. In his third (1884) report, Mr. Warrington says that "probably tartrate 100 per cent. of the ammonium salt is sufficient for every purpose," and that "urine apparently contains quite enough organic carbon for its own nitrification."

Amongst the experiments already described will be found—

One (B3) in which 31 mgrms. ethylamine hydrochloride were partially nitrified into nitrate after twice seeding with a few milligrams soil;

One (G1) in which 35 mgrms. ammonium thiocyanate with 20 mgrms. oxalic acid crystals were completely nitrified into nitrate after twice seeding with a few milligrams soil;

One (F1) in which 20 mgrms. urea with 20 mgrms. cryst. oxalic acid were completely nitrified to nitrate after twice seeding with a few milligrams soil;

One (A11) in which 63 mgrms. ammonium chloride with 26·3 mgrms. potassium oxalate were completely nitrified to nitrite after *once* seeding with 1 or 2 mgrms. soil;

One (A10) in which 63 mgrms. ammonium chloride with 26·3 mgrms. potassium oxalate were completely nitrified to nitrite after twice seeding with 1 or 2 mgrms. soil.

I now know that the oxalate in these experiments had nothing to do with the nitrification, since it was destroyed long before nitrification set in.

The experiments with natural waters, presently to be described, will show that the organic carbon in well water is sufficient for the nitrification of added ammonia in quantity at least equal to the saturating power of the calcium carbonate present for the nitrous or nitric acid produced. The series of experiments which now follows, however, is more to the point, and pretty clearly exhibits the practical relation of organic carbon to nitrification.

Six solutions were made up on February 9 and 14, 1884. All contained ammonium chloride equal to 40 mgrms. NH_3 per litre (33 mgrms. N). They were carefully filtered after being made up, but were not sterilised by heat. A14b measured 500 c.c., and was con-

tained in a litre flask, the neck of which was plugged with cotton-wool; the others measured 100 c.c. each, and were contained in 6-oz. stoppered reagent bottles. The following table shows the arrangement adopted:—

Feb. 9, 1884.	A18.	Complete solution, containing per litre 126 mgrms. NH_4Cl , 200 mgrms. NaHCO_3 , minerals (40 mgrms. MgSO_4 cryst., and 40 mgrms. K_3PO_4) 40 mgrms. Rochelle salt; seeded with a few milligrams soil.
„	„	A17. <i>Rochelle salt only omitted.</i>
„	„	A16b. <i>Soil only omitted.</i>
„	„	A19. <i>Rochelle salt only omitted; seeded with several grams of soil.</i>
„	„	A15b. <i>Rochelle salt and soil omitted.</i>
Feb. 4, 1884	A14b.	<i>Rochelle salt, soil, minerals, and salifiable base omitted.</i>

Five of these solutions, including the two unseeded ones, developed nitrite or nitrate sooner or later; but they exhibited marked differences in rapidity and extent of nitrification.

A19 nitrified completely to nitrate in less than 50 days.

A17 also rapidly nitrified; half the nitrogen was present as nitrate in 30 days; a little nitrite was formed and afterwards disappeared.

A18, differing from the last only in containing Rochelle salt, nitrified more slowly. In 30 days, less NH_3 had nitrified, and the nitrite present was considerable; during the summer, it passed completely into nitrate.

A16b, with Rochelle salt but no soil, underwent at first a slow and entirely nitrous fermentation; 14 mgrms. of nitrogen were present as nitrite even on November 13, 1884, but some time after that date conversion into nitrate took place.

A15b, with no tartrate or soil, and therefore without added organic matter of any sort, nitrified like the last, but still less readily. $12\frac{1}{2}$ mgrms. nitrogen were present as nitrite on August 17, 1884; this increased to $15\frac{1}{2}$ mgrms. on November 11, 1884, and has remained without further oxidation ever since. The clearness of the solution has never been visibly impaired.

A14b, without minerals, organic matter, or salifiable base, was tested five times at intervals from February 4, 1884, to August 21, 1884, but developed no nitrite or nitrate. A little sodium bicarbonate was then added, and a trace of nitrite was found on November 1, 1884, which did not increase. Seventeen months later, although the trace of nitrite was still present, 10 c.c. of the solution would not decolorise three drops of standard permanganate or three drops of indigotin (1 c.c. = 0.035 mgrm. N).

As regards the source of contamination in the three unseeded but unsterilised solutions, A14*b*, A15*b*, A16*b*, I have lately succeeded in proving decisively that the standard solution of ammonium chloride used, *which was not boiled*, actually contained the nitrous ferment.* The source of inoculation being known, it is not a matter of surprise that fermentation in these solutions should have taken place, but the differences in their behaviour are very significant. A14*b* with *no added organic matter or minerals*, develops only a trace of nitrite, although kept for 17 months after addition of a salifiable base. On the other hand A15*b*, *with minerals but no added organic matter*, undergoes *a complete but purely nitrous fermentation*; and A16*b*, for the first nine months at any rate, follows precisely the same course, although tartrate was purposely added. The merest traces of organic matter, therefore, such as may be furnished accidentally by occasional exposure to the dust of the air, are sufficient for complete nitrous fermentation of the quantities of ammonia used in these experiments; but it does not appear that the process is equally independent of purposely added minerals.

A17, again, shows that the organic matter of 1 or 2 mgrms. of soil suffices for the requirement of a complete *nitric* fermentation; and the addition of tartrate to A18 was not attended with the smallest advantage.

Moreover, unless aërial bacteria can be rigidly excluded, and nitrification carried on with *pure* cultivations of the nitric ferment instead of soil, the presence of a tartrate or any similar organic body is distinctly prejudicial to nitrification. This will appear clearly from the experiments detailed in the next section.

IX. *Nitrification of Ammonium Salts in Natural Waters.*

It is a matter of common knowledge that the ammonia and nitrogenous organic matter which find their way, say in the form of sewage, into our rivers and wells, ultimately give rise to the formation of nitrates; and the proportion of "free ammonia" and "nitrogen as nitrite and nitrate" have had their place in our analytical schemes as measures of sewage pollution "present" and "past." But few investigations, however, have taken place as to the exact manner in which the transformation of ammonia into nitrite or nitrate is effected; and such as have been made have proceeded upon purely chemical lines, the rapid aëration and large surface exposure which

* This standard solution, made up more than four years ago, now *contains* a trace of nitrite sufficient to give an orange colour with metaphenylenediamine; and by adding a little of it to *sterilised* well water, I have recently been able to effect complete conversion of the ammonia into nitrite..

moving water undergoes being tacitly or openly inferred to be sufficient causes of nitrification.

As soon as I had repeated some of Mr. Warington's experiments which strongly supported the theory of a special nitrifying organism in the soil, I commenced (November, 1883) a series of experiments with the view of ascertaining whether natural waters oxidise ammonia because of their contact with soil and the ferment contained therein, or because they themselves contain this ferment which they have obtained from the soil, or (according to then current theories) simply because they offer the ammonia the means of free and thorough atmospheric contact. The experiments were continued in 1884, but (until within the last two months) no new solutions were started after April of that year, although those then in operation have been under observation ever since in order to ascertain the completion or non-completion of nitrification in some of them.

Some months after my first experiments on waters were commenced, the late Dr. Angus Smith's last Report to the Local Government Board was published, and although he mentions some facts as to denitrification which he had observed some time before, and with which I had then become familiar from my own experiments, he does not appear to regard the oxidation of ammonia in waters in any other light than a purely chemical one. In his previous Report, indeed (1882), he says: "Putrefaction and oxidation are two well-known modes of destroying organic bodies at ordinary temperatures. The second is not proved to be connected with organisms." In the last Report he says: "Animal or vegetable matter containing nitrogen produces nitrates by oxidation with and without organisms." He illustrates the latter position by quoting experiments on the oxidation of ammonia by permanganates, and by persalts of iron, and seems to regard it as the more important process, for he says that it is "an action of the oxygen without the intervention of organisms probably carried out to a great extent in nature." Whilst fully admitting that ammonia may be oxidised by the very powerful reagents named, it is much to be doubted whether a purely chemical oxidation of ammonia ever takes place in nature when organic matter decays in contact with air, water, or soil. As regards the ordinary oxidation of nitrogenous organic matter following putrefaction, Warington's experiments with rape cake, asparagine, milk, urine, &c., show that the presence of the nitrifying ferment of the soil is a *sine qua non* of nitrification; and the experiments which follow show the same statement to be true of the ammonia contained in natural waters.

My experiments were made by adding known quantities of ammonium chloride to waters under various conditions; and it occurred to me that if waters possessed the power of readily nitrifying ammonia

added in this way, they would form very suitable media for the study of nitrification and for the culture of the nitrifying ferment, the organic matter and minerals necessary for the nutrition of the ferment, as well as the salifiable carbonate of lime, being present in such forms as not to interfere with the clearness of the liquid. A still greater advantage is that well waters have no tendency to encourage the growth of the bacteria which multiply in and render turbid most organic infusions during the first week or two of their exposure to the air. The germs of these bacteria are present in all well waters, but they do not multiply because of the absence of *suitable* organic matter. There is one drawback to the use of well water for experiments on nitrification. In most cases it already contains nitrate. In my experiments the proportion of nitrogen as nitrate was therefore very carefully determined beforehand, and the progress of the nitrification of the added ammonium salt was ascertained by—

Testing at intervals with Nessler reagent for the disappearance of the added ammonia ;

Testing with metaphenylenediamine for the appearance of nitrite, and estimating the quantity in some cases by standard potassium permanganate ;

Determining by means of standard indigotin (Warington's improved process) any increase over the original nitrate present,—in the presence of nitrite *and* nitrate, the nitrite was first estimated with permanganate, and the total nitrogen then present as nitrate estimated with indigo.

The merest traces (if any) of free ammonia and nitrite were present in the waters with which I worked ; and even the organic nitrogen present was in altogether insignificant proportion to the ammonia salt added, and to the ammonia actually nitrified ; it did not exceed $\frac{1}{100}$ th part of the added ammonia.

The standard solutions employed were of the following strength :—

Potassium permanganate..	1 c.c. = 0.072 mgrm. N as nitrite.
Indigotin.....	1 „ = 0.03847 „ nitrate.
and	1 „ = 0.055 „ „
(Diluted to $\frac{1}{2}$, $\frac{1}{4}$, &c., according to circumstances.)	

Ammonium chloride, 1 c.c. = 1 mgrm. NH_3 .

Experiments with River Water.

The earliest experiments showed the rapidity and ease with which relatively large quantities of ammonium chloride could be nitrified in natural waters. Three dark glass bottles were half filled with 500 c.c.

each of water from the River Avon, which irrigates the water meadows of the whole valley, and is frequently analysed in the College of Agriculture Laboratory.*

To each bottle was added 25 c.c. standard ammonium chloride = 20.6 mgrm. N.

α Received no addition beyond the ammonium chloride.

β A crystal of Rochelle salt.

γ " " and a centigram of soil.

When made up on November 21, 1883, 10 c.c. of each of these solutions decolorised 0.53 c.c. indigotin (1 c.c. = 0.055 mgrm. N), and contained, therefore 0.204 grain N per gallon as nitrate.† The bottles were simply corked, and were kept in the dark in a bureau by the side of a fire-place in a living room.

β and γ clouded in a few days; α remained clear.

Tested on December 17, 1883, 26 days after making up, 10 c.c. α decolorised 7.4 c.c. indigotin. A little ammonia was present, but no nitrite.

Nitrogen originally present as nitrate	= 0.204 grain per gallon.
" in added ammonia.	= 2.747 " "
	2.951
" as nitrate on Dec. 17, 1883	= 2.849

2.6 grains of nitrogen in NH_4Cl require for nitrification 18.5 grains per gallon of calcium carbonate, whereas the water contained only 15 grains; doubtless the magnesia, potash, and traces of other bases in the water supplied a grain or two more of salifiable base.

* Analyses made at various times show the composition of this water to be as under:—

Total solid matter varies from	19.98 to 21.32	grains per gallon.
Free ammonia	0	trace.
" Albuminoid " ammonia	} 0.02	" 0.08 part per million.
varies from		
Nitrogen as nitrate varies from	0.09	" 0.46 grain per gallon.
" nitrite	0	" trace.
CO_2	6.4	" 12.3 grains "
Calcium carbonate	14.58	" 15.26 " "
Loss on ignition	2.50	" 3.85 " "
Chlorine	0.92	" 1.05 " "
Calcium sulphate	0	" trace.

† It will be understood that the quantities of water taken for analysis, and the dilution of the indigotin, were varied as is necessary in working the process; but for the sake of uniformity all the results are calculated for 10 c.c. water and for indigotin, of which 1 c.c. = 0.055 mgrm. nitrogen, the strongest solution used.

Not only had β and γ *not* nitrified in the same time, but γ (soil and Rochelle salt) had lost every trace of the nitrate it originally contained, and β had lost the greater portion.

Both of these solutions eventually nitrified, but the addition of the Rochelle salt, which determined denitrification, brings them more appropriately under that head, to be considered by-and-bye. At this stage they showed me that I could with advantage eliminate Rochelle salt from my experiments, and rely on the organic carbon of the water only.

Experiments with Laboratory Well Water, commenced December 19, 1883.

A series of experiments was next made with the water of the Laboratory Well, an unpolluted but very shallow well sunk in alluvial gravel, and situated about 400 yards from the River Avon.

This well water contains—

Total solid matter	23·1 grains per gallon.
Chlorine	1·0 " "
Free ammonia	none.
Nitrogen as nitrate	0·444 " "
" nitrite	none.
CO ₂	6—13 " "
Sulphates	none.
Calcium carbonate	15·4 " "

2500 c.c. of this water were placed in a half-gallon "Winchester quart" and 100 c.c. standard ammonium chloride added (= 100 mgrms. NH₃ = 82·4 mgrms. N). The water was titrated with indigotin after this addition, when 10 c.c. decolorised 1·0 c.c. indigotin.

Nitrogen as nitrate	0·427 grain per gallon.
Nitrogen added as ammonium chloride	2·217 grains "
	<hr style="width: 10%; margin-left: 0;"/> 2·644 " "

This was divided between 16 flasks and bottles, as in the following table:—

With no added Organic Matter.

- L16. Unsterilised.
- L4. Chloroformed.
- L1. Boiled, and not opened or tested until April 14, 1884.
- L3 Boiled, and then seeded with a few milligrams soil.

- L14. Boiled, then seeded with five drops of nitrifying solution (A2).
 L2. Boiled, then seeded with five drops unboiled well water.

With added Organic Matter.

- L7. Rochelle salt only added.
 L8. Cane-sugar only added.
 L9. Glycerol only added.
 L10. Sodium acetate only added.
 L5. Rochelle salt added ; boiled, and not opened or tested until April 14, 1884.
 L6. Rochelle salt added ; boiled, then seeded with soil.
 L11. Rochelle salt and a little phenol added. Seeded with soil.
 L13. Salicylic acid added ; seeded with soil.
 L12. Rochelle salt and a little chloroform added. Seeded with soil.

L 1, 2, 3, 5, 6, 15, the *boiled* solutions, were kept in flasks, half full, the necks of which were plugged with cotton-wool, and a paper cap tied over the mouths. The seed, if any, was added during a momentary withdrawal of the plug, after cooling.

The remaining solutions were kept in stoppered reagent bottles, half full, except L16, which formed a layer of about 2 inches depth at the bottom of a stoppered Winchester quart.

Of these 15 solutions five, viz., the two boiled and unseeded solutions, L1 and L5, the two chloroformed solutions, L4 and L12, and the solution with phenol, L11, absolutely refused to nitrify, although tested at intervals for more than two years after being made up. Filtered air had access to these waters through the cotton-wool plugs all that time.

Nitrification, to a greater or less extent, took place in three or four of the solutions containing added organic matter, but since in all these solutions partial or complete denitrification of the water was first produced, they are better considered under the section devoted to denitrification (p. 667).

The remaining four solutions, free from added organic matter, all nitrified completely to nitrite or nitrate, but at different rates.

L15, seeded with five drops of a strong solution in active nitrification, underwent almost complete oxidation to nitrate in less than two months ; for on February 25, 1884, a trace only of nitrite was present, and nearly 3 grains per gallon of N existed as nitrate.

L3, seeded with a few milligrams of soil, completely nitrified to nitrate in about the same time as L15. Nitrite was present as late as February 4, 1884.

L16, the unboiled well water with ammonium chloride only added, completely nitrified to nitrite in less than 54 days, but the nitrite has

since remained without any further alteration for more than two years. The following table shows the dates of testing:—

Dec. 19, 1883,	10 c.c. consume	1.1 c.c. indigotin.	
Jan. 24, 1884,	10	1.5	”
Feb. 4, ”	10	2.6	”
” 25, ”	10	2.6	”
Mar. 15, ”	10	2.6	”
Apr. 17, ”	10	2.6	”
Aug. 21, ”	10	2.6	”

} Nitrite present.

} Nitrite = 1.96 grains N per gallon.

Nov. 11, 1884, nitrite undiminished.

Aug. 14, 1885, ” ”

Mar. 27, 1886, ” ” A little ammonia still present.

Nitrogen as nitrite and nitrate = 2.11 grains per gallon.

2.22 grains of added nitrogen (as NH_4Cl) require 15.8 grains per gallon CaCO_3 for complete nitrification, which is rather more than that contained in the water; hence a little ammonia escaped nitrification. The persistence of the nitrite for over two years is a very striking result, and points to an essential difference between the nitrifying power of this well water and that of the soil and nitrifying solution used in L3 and L15.

L2, boiled and seeded with 5—10 drops of the unboiled well water, also nitrified, but very slowly, and, as in the last case, nitrite only was produced. The formation of nitrite, instead of being complete in less than 54 days, had then made little progress and was not finished until two months later. The nitrite (1.96 grain N per gallon) has persisted for over two years.

Four more experiments were made with this well water, in order to see whether filtration would remove the nitrous ferment which it appeared to contain, and to again compare its behaviour with a soil-seeded water.

Each of the four solutions, L17, 18, 19, 20, consisted of 500 c.c. laboratory well water + 20 c.c. standard ammonium chloride (= 20 mgrms. NH_3 = 63 mgrms. NH_4Cl). They were treated as follows:—

L17 and 18 were filtered through two Swedish filters into cleansed and sterilised 1500 c.c. flasks, which were plugged with cotton-wool. But whereas L17 was opened and tested at short intervals, L18 was *not opened for 40 days after being made up.*

L19 was similar to the two preceding, but was seeded with a few milligrams soil.

L20 consisted of the *unfiltered* mixture, and was placed in a stop-

pered bottle to allow of accurate determinations of nitrite from time to time.

The subsequent course of these solutions is shown in the following table:—

Made up 1884.	Description.	Consumed by 10 c.c. of each water.				c.c. Permanganate: August 26, 1884.
		c.c. Indigotin.				
		Feb. 4 & 9	Feb. 25.	Mar. 20.		
Feb. 4 ..	L17. Filtered	1·4	1·7	2·9	4·0 = 1·96 grains N per gallon as nitrite.	
„ 9 ..	L18. Filtered and un- opened until March 20	1·4	—	2·9	4·0 = 1·96 grains N per gallon as nitrite.	
„ 4 ..	L19. Filtered and seed- ed with soil	1·4	1·6	1·8	3·5 = 1·72 grains N per gallon as nitrite.	
„ 4 ..	L20. Unfiltered	1·4	1·4	3·4	3·4 = 1·67 grains N per gallon as nitrite.	

Nitrogen added as ammonium chloride = 2·22 grains per gallon.

L17, 18, 19, being in open flasks, were subject to evaporation. Careful estimations were made in L20 at intervals for two years, without showing the slightest further change. The latest determinations give—

Nitrogen as nitrite	1·67 grains per gallon.
„ nitrite and nitrate....	1·96 „ „ *
„ nitrate.....	0·29 by difference.

As there was about 0·54 grain per gallon of nitrogen as nitrate in the original water, it will be seen that the fermentation was purely nitrous.

L17 and L18 followed precisely the same course, showing that filtration did not remove the nitrous ferment, although it appears to have weakened it; the unfiltered solution had made more progress in nitrification on March 20 than the two filtered ones. The period of incubation in these solutions was under 21 days; temperature 60—70° F.

L19 presents an apparent anomaly. Although seeded with soil, it nitrified less rapidly than the rest, and to nitrite only. But all these bottles were kept in diffused daylight, and L19 slowly grew a powdery green alga, the spores of which were introduced with the soil. By the

* Ammonia is still present, because the salifiable base is exhausted.

continued growth of this alga, all the nitrite originally formed was gradually destroyed in the course of a year or so.

The experiments hitherto described show that whereas distilled water, even after the addition of a salifiable base, will not nitrify added ammonium chloride (A14*b*), well or river water fertilised with a particle of soil or a drop of nitrifying solution supplies everything necessary for the nitrification of added ammonium chloride to the full extent of the saturating power of the calcium carbonate present; and that nitrification in these waters, as in the artificial solutions used by Warington, is entirely prevented by boiling or by the addition of an antiseptic. But they further appear to show that river water itself is sufficiently contaminated with this soil ferment to produce a complete nitric fermentation of added ammonia; and that a well water, in five separate experiments, possessed sufficient nitrifying power to produce a complete nitrous fermentation. Also, that whilst this nitrifying power is perhaps weakened by filtration of the water through Swedish paper, it is not entirely removed.

Since Warington's experiments (1884 Report) prove that the nitrifying organism is confined to the *surface* soil, the question as to how far different natural waters become contaminated with the ferment is one that can only be settled by experiment. *A priori*, we should suppose that all surface waters, and all waters liable to accidental contamination with soil, must possess this ferment; but it is possible that they do not all contain sufficient for the practical requirements of nitrification, and even that some protected deep well waters may be quite free from it. Quite recently, therefore, I have re-tested the well and river waters already alluded to, and have examined several other natural waters, under the following rigorous conditions:—

A flask, holding about 350 c.c., is carefully cleansed, and distilled water is then boiled in it, and a cotton-wool plug inserted in the neck. After a few minutes' boiling the plug is removed for a moment and the distilled water replaced by 5 c.c. standard ammonium chloride (= 5 mgrms. NH_3). The plug is again inserted, the ammonium chloride boiled, and the flask covered with a paper cap and allowed to cool. When cold it is taken to the water supply, the cap and plug carefully removed, and about 300 c.c. water placed in the flask, either directly from a pump or tap, or by means of a sterilised beaker. The plug is then replaced, the paper cap tied over the mouth, and the flask is placed in a warm place in the dark, and *not opened until a sufficient period has elapsed to allow of the commencement of nitrification*. If nitrification has commenced, nitrite will then in nearly all cases be present, and will give a yellow, orange, amber, or red colour with the metapenylenediamine test, according to the amount. Should

complete nitrification have taken place, the ammonia will have disappeared; and should a partial but *purely nitric* fermentation have occurred, more indigo will be consumed than was the case with the original water. (In experiments with well waters unseeded with soil, I have hitherto invariably found *nitrite* present during the greater part of the nitrifying period.)

The following table exhibits the results obtained with six river and shallow well waters examined at Downton, and seven miscellaneous waters examined at Bristol. The samples altogether comprise shallow and deep well waters, pure waters, and waters with "present" and with "past" sewage contamination, the water of a "level" in a coal mining district, river water, and rain water. A summary of the composition of each water is given, and the result of the metaphenylenediamine test at the conclusion of the period allowed for incubation, and afterwards. The Bristol samples were kept at a temperature of 80—85° F., excepting the boiled or blank solution. This one belonged to a series made up May 7—20, 1886, but unfortunately they were slightly over-heated during a portion of the incubating period; the temperature rose to a few degrees over 100 F. on two or three days, and as a consequence all except one refused to nitrify.* That they were really sterilised by the heat is proved by the fact that duplicate flasks, made up as soon as the overheating was discovered, *all* began nitrifying in less than 18 days. These sterilised flasks, therefore, strengthen the evidence afforded by the boiled or blank solution. The Downton waters were incubated at the ordinary temperature, and with one exception commenced nitrification in less than 18 days; and this commenced nitrifying between the 18th and 23rd day.

The "rain water caught during a shower in a sterilised beaker" was of course added to some *boiled* well water, since of itself it did not contain the salifiable base and other impurities essential to nitrification.

The shorter of the two intervals mentioned under each water is the date of *first opening* and testing the flasks; a second testing was made after another few days in order to ascertain the relative rapidity of nitrification in the different waters. The colour with metaphenylenediamine on the first testing is no guide, since the nitrite *increases* during the first period of nitrification and *decreases* during the second, and it is necessary to know whether the nitrite is in the increasing or decreasing phase. The second testing accomplishes this. Thus with the Downton waters we have (see p. 666)—

* This one began to nitrify *before* the over-heating took place, and the nitrification has made no further progress since. A second testing, made 40 days after the first, still shows no nitrification in any of the other over-heated solutions.

Experiments on the Nitrifying Power

	Tests made		
	Grains per gallon.		
	Total solids.	Chlorine equal to sodium chloride.	Nitrogen as nitrate.
<i>Bristol Waters. Incubated at 80—85° F.</i>			
A1. Well 56 feet deep, in Coal-measures. End of pump tube 25 feet from surface. Water 10 feet from surface. Well imperfectly closed with iron plate, water raised by iron pump. Pure and clear	18·5	2·38	0·04
A2. <i>The same water boiled after addition of NH₄Cl</i>	"	"	"
A3. <i>The same water, a few drops lead acetate added.</i>	"	"	"
A4. <i>The same water, accidentally overheated while incubating.</i>	"	"	"
B. Well at least 40 feet deep, in Coal-measures. Completely closed in and water raised by pump. Much polluted with recent sewage	68·9	16·2	0·03
C. Well about 70 feet deep, in Coal-measures. Over 50 feet to surface of water. Open, and water raised by bucket. Contains oxidised sewage	49·7	7·28	2·07
D. Water of old level driven under Kingswood Hill. Sample taken at the outflow, foot of Warmley Hill. Forms the water supply of the immediate neighbourhood . . .	42·7	3·22	none or trace
E. Bristol Waterworks Co.'s water taken from their mains..	17·5	1·4	0·03
F. Rain water from excavated and bricked cistern, raised by closed pump under cover	4·2	0·56	trace
G. Rain water, caught in a sterilised beaker during a shower. (<i>Added to boiled well water A.</i>)	—	trace	none
<i>Downton Waters. Incubated at 55—65° F.</i>			
H. River water; sample taken halfway across the Avon. Clear, with minute flocks suspended matter	19·8	1·5	0·20
II. Shallow well (15 feet) in alluvial gravel. Water raised by horse gear and pumps, and delivered at the end of tinned lead pipe 300 feet long. Pure and clear . . .	23·6	1·9	0·27
12. The same water filtered through Lipscombe's charcoal filter in common use	—	—	—
J. Laboratory well, very shallow, in alluvial gravel. Water raised by closed pump inside the laboratory. Pure and clear	21·3	1·6	0·37
K. Shallow well in alluvial gravel, completely closed in and water raised by pump. Contaminated with sewage three years ago. Since purified. Clear	29·3	2·5	0·37
L. Shallow well in alluvial gravel. Water raised by pump. Subject to constant contamination from farmyard sewage	34·2	4·90	0·76

* These unnitrified waters consumed the same amount of indigotin as at first.

of Rain, River, and Well Waters.

with original water.			Tests made after incubation.		
c.c. Indigo consumed by 10 c.c. of water.	Ammonia (colour with Nessler).	Nitrite (colour with meta-phenylenediamine).	Interval after making up.	Nitrite (colour with meta-phenylenediamine).	Ammonia (colour with Nessler test).
0·4	trace	none	19 days	deep amber	much NH ₃ present.
"	"	"	42 "	none*	" " "
"	"	"	42 "	" *	" " "
"	"	"	42 "	" *	" " "
0·3	yellow ppt.	"	19 "	yellow	NH ₃ gone.
24·0	distinct trace	"	19 "	pale yellow	much NH ₃ present.
0·1 ^p	faint trace	"	19 "	red	" " "
0·4	none	"	19 "	yellow	" " "
—	amber	"	19 "	faint yellow	" " "
—	trace	"	21 "	none*	" " "
0·8	faint trace	"	18 "	yellow	much NH ₃ present.
			29 "	very deep orange	much less NH ₃ .
1·1	none	"	18 "	very faint yellow	much NH ₃ present.
			29 "	bright orange	much NH ₃ present.
—	"	"	18 "	yellow	much less NH ₃ .
			29 "	deep orange	much NH ₃ present.
1·5	"	"	18 "	none*	much NH ₃ present.
			23 "	faint yellow	much NH ₃ present.
			29 "	light orange	" " "
			18 "	bright yellow	" " "
1·5	"	"	29 "	bright orange	little NH ₃ present.
			18 "	yellow	much NH ₃ present.
3·1	0·26 per million	"	29 "	deep orange	much NH ₃ present.

Indigotin used at Bristol, 1 c.c. = 0·01236 mgrm. N ; at Downton 1 c.c. = 0·035 mgrm. N.

Depth of colour on 1st testing.	Depth of colour on 2nd testing.	Relative rapidity of nitrification.
1. K, well water.	1. H.	1. K, well water.
2. H, river water.	2. I2.	2. H, river water.
3. I2, well water filtered.	3. L.	3. I2, well water filtered.
4. L, well water.	4. K, well water.	4. L, well water.
5. I1, well water unfiltered.	5. I1.	5. I1, well water unfiltered.
6. J, laboratory well water.	6. J.	6. J, laboratory well water.

All except K were in the *ascending* stage of nitrite reaction at the 1st testing, and preserved the same order at the 2nd testing; K entered the *descending* stage before the 2nd testing, and hence is the most rapidly nitrifying water of the series. The Nessler test confirms this classification; tested again after 44 days, nitrification to nitrate was complete in K and H, the rest still contained varying quantities of nitrite.

The Bristol waters compare with each other as follows:—

Depth of colour on 1st testing.	Depth of colour on 2nd testing.	Order of nitrification.
1. D, level water.	1. E.	1. B, polluted well water.
2. A, well water.	2. A.	2. D, level water.
3. E, Bristol Waterworks.	3. C.	3. A, pure well water.
4. B, well water.	4. D, level water.	4. E, Bristol waterworks.
5. C, well water.	5. F.	5. C, well water.
6. F, rain water.	6. B, no colour.	6. F, rain water.

B contained neither nitrite nor ammonia when tested again on the 39th day; D contained nitrite but no ammonia; the remaining four still contained ammonia and much nitrite.

The sterilised solutions, it will be seen, have all refused to nitrify added ammonia. The rainwater caught from the clouds in a sterilised beaker is also destitute of nitrifying power.

With this one exception, all the natural waters examined possess the nitrifying power in a greater or less degree. The polluted well waters appeared to be most potent, then the surface waters, and last the pure and well protected wells. Very deep well waters may perhaps be either free altogether from the nitrifying organism, or contain it in such small quantities as to require a very lengthened period of incubation. It is curious that filtration through a charcoal filter should have increased the nitrifying power of the well water I, yet this is undoubtedly the case.

In other experiments, I found that the river water commences to

nitrify the added ammonium chloride in 7—9 days, the commencement of nitrification being marked by the presence of a trace of nitrite; enough nitrite to give a bright orange colour with metapenylenediamine was present even on the 42nd day, but nitrification to nitrate was complete on the 46th day. Reinforced by the addition of 1 per cent. of soil, this water commenced nitrifying on the 3rd—4th day; very little nitrite was formed, which began to diminish after the 12th day; on the 30th day nitrification to nitrate was complete. With much soil and a thin layer of water no nitrite, or a mere trace, was formed.

The laboratory well water, on the other hand, has never commenced to nitrify before the 18th—22nd day; much nitrite is formed, and, as has been shown above, this water two years ago was unable to carry the oxidation of ammonia further than the stage of nitrite. If 150 c.c. of the boiled water be seeded with 5—10 drops of a weak nitrifying solution, about the same period of incubation is observed (18—22 days), so that the quantity of ferment in 150 c.c. of the unboiled water may be taken roughly as equal to that in a few drops of such a nitrifying solution. These experiments were made at the ordinary temperature and with 5 c.c. standard NH_4Cl (=5 mgrms. NH) to 150 c.c. of water.

In the purest well waters, there is a *very slight* flocculent deposit during nitrification; there is more deposit in the rapidly nitrifying waters. Nitrification in filtered well water, or in boiled well water seeded with a nitrifying solution free from fermentable organic matter, would probably furnish a deposit well suited for microscopic observation of the nitrifying organism, called by Schloesing and Müntz, *Micrococcus nitrificans*. In this country, I believe its existence has been inferred rather than made the subject of ocular demonstration.

X.—Denitrification, and the Influence of Organic Matter on Nitrification.

As already mentioned, the first three experimental solutions for observing nitrification in river water contained—

Nov. 21, 1883.

α ..	River water + ammonium chloride.
β ..	„ + „ and Rochelle salt.
γ ..	„ + „ Rochelle salt, and soil.

The very great nitrifying power of ordinary river water without any addition was unsuspected by me at the time, and the additions of Rochelle salt, Rochelle salt and soil, to β and γ respectively, were made with the view of favouring the process of nitrification. Great, therefore, was my surprise on examining the waters 26 days afterwards, to find that whereas α had almost completely nitrified the

added ammonia to nitrate, β and γ had not commenced to nitrify, but on the contrary every trace of nitrate originally present in γ was destroyed, and most of that in β . α had remained practically clear throughout; β and γ had developed a slight turbidity (greatest in γ) a few days after making up. The subsequent course of these waters was as follows :—

β , which originally contained 0.204 grain per gallon as nitrate, contained about half this quantity 26 days after making up, and still less after another 34 days; in 30 days more there was a barely perceptible increase; in another month the indigo consumed had doubled, and between this testing, April 16, 1884, and August 21, 1884, complete conversion of the added ammonia into nitrate had taken place, about 2.8 grains per gallon of N being then present in that state. (Nitrite was present on April 16.) The solution has remained until now without further change.

The nitrate originally present in γ was *totally* destroyed during the first month, and there was a considerable flocculent deposit. Not until three months later was there any perceptible renitrification, but between April 16, 1884, and August 21, 1884, about 2 grains per gallon of ammonia was converted into nitrite, and this has since suffered complete conversion into nitrate.

Mr. Warington has observed in his last paper (1884) that denitrification precedes nitrification in certain cases. Under the head of "Reduction of Nitrates by Soil," he says: "I wish simply to call attention to the curious fact that when 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. * * The reduction of the nitrates now in question is always accompanied by turbidity of the fluid."

No doubt this is strictly true as regards the solutions with which Mr. Warington experimented; these were made from substances like asparagine, urine, rape cake, milk, &c., or consisted of artificial solutions of ammonium salts to which an alkaline tartrate, cane-sugar, or glucose had been added. I shall, however, show that the presence of easily fermentable organic matter is a necessary condition of rapid denitrification; in the absence of this, soil has no tendency to reduce nitrates already formed, and indeed if it had it would not be easy to understand the formation and persistence of nitrates in arable fields and in drainage waters.

The addition of Rochelle salt to a recently nitrified solution sometimes causes a rapid and total fermentative destruction of the nitrate.

To test this point, I took the seven artificial solutions of my first series, which had undergone complete nitrification to nitrate, viz. :—

B1.	Ethylamine hydrochloride nitrified to nitrate.		
B2.	" "	"	"
C1.	Potassium thiocyanate	"	"
F1.	Gelatin	"	"
E1.	Urine	"	"
A9.	Ammonium oxalate	"	"
A1.	" chloride	"	"

On December 18, 1883, all these solutions had remained for three or four months after complete nitrification without any further change; they were all in bottles or flasks about half full and closely stoppered or corked. On this date, a crystal of Rochelle salt was dropped into each bottle, and A1 (only) was heated for an hour in the steam-oven after this addition. When examined again on February 7, 1884, the nitrate had totally disappeared from all *except* A1; no nitrite was present in any case, and scarcely any ammonia except in F1, B1, and B2. They were, however, thick with a ropy growth of mould, so that the nitrogen of the nitrate or of the ammonia at first formed by its reduction, had been partly consumed in feeding these growths. C1 contained a black deposit and smelt strongly of sulphuretted hydrogen. A1, the sterilised solution, had remained perfectly clear, and the nitrate was intact at this date; but a few days after the stopper was removed for testing, turbidity of the solution set in, followed by a very active bacterial fermentation, with evolution of bubbles of gas. After 13 days, the solution was examined for nitrate and nitrite, and was perfectly free from them; ammonia was however present. Under the microscope, large, very active bacteria were easily seen with a $\frac{1}{4}$ " objective to be swarming in the liquid, singly and in chains of 2—8 or 10.*

Alternation of Nitrification and Denitrification.

F1, the gelatin solution which contained most ammonia after denitrification, was kept for further observation. On February 7, 1884, shortly after denitrification, it was thick with mycelium and had a putrid odour, but by April 7, 1884, it had become clear, the odour had disappeared, and a considerable amount of renitrification had taken place. By August 14, 1884, it contained no ammonia or nitrite, but 20 mgrms. N as nitrate, or about $\frac{3}{4}$ of the quantity nitrified the first time. Addition of a few crystals of sodium acetate on that date again brought about a rapid and total destruction of this nitrate, but by November 13, 1884, a *third* nitrification had commenced, which has since made considerable progress. How far this alternation of nitri-

* Probably the *Bacterium denitrificans*, α or β , of Gayon and Dupetit.

fication and denitrification could be carried, I do not know, but it is noteworthy that the available nitrogen diminishes after each reduction, most of it being evolved as nitrogen gas. This experiment well illustrates the relation of fermentable organic matter to denitrification.

As to the ferments themselves which bring about destruction of nitrates, they may exist in the soil, or the water, or may be derived from added impurity such as sewage, or even from the air. The particular ferment encouraged will depend on the fermentable organic matter employed, on the different species which gain access to the liquid, and on the quality of the liquid as a nutritive medium. Since nitrates are not only the most suitable nitrogenous food of green plants, but are also well adapted for the nourishment of many lower organisms, the presence of suitable organic matter in water is almost sure to encourage the growth of *some* organism or other which will sooner or later effect a destruction of nitrate. Thus a slowly growing mould or a green alga such as that already mentioned (p. 662), may feed on a nitrate and gradually destroy it, but this species of denitrification is easily distinguishable from that brought about by the bacterial ferments above mentioned. The former process may take weeks, months, or years to completely destroy the nitrate, which disappears only as fast as it is assimilated; the latter process is complete in a few days, is always accompanied by turbidity of the liquid, frequently by evolution of gas, and is marked by reduction of the nitrate to nitrite, ammonia, or nitrogen and nitrogen oxide.

In order to exhibit more clearly the antagonism between fermentable organic matter and nitrification, I will next put on record all those bottles of the Laboratory Well Water Series (p. 7) which received the addition of organic matter as well as of ammonium chloride.

(In all these cases, the original N as nitrate = 0.427 grain per gallon: the N added as NH_4Cl = 2.22 grains per gallon.)

L5. *Boiled after the addition of Rochelle Salt.*—Made up December 19, 1883. Tested on April 14, and again on August 21, not the slightest nitrification or denitrification had taken place. The addition of Rochelle salt to well waters causes after a time the separation of beautifully transparent and sometimes large crystals of calcium tartrate. It is plain from this experiment that organic matter such as Rochelle salt does not *per se* cause denitrification. Another water boiled after the addition of Rochelle salt and *soil* gave the same result.

L12. *Chloroformed after addition of Rochelle Salt and Soil.*—The history of this solution is identical with that of the preceding.

Chloroform absolutely prevented either nitrification or denitrification in the presence of soil ferments favourable to both processes.

L7. *Rochelle Salt added. Not boiled or filtered.*—This was contained in a stoppered bottle, half full. The indigo consumed by 10 c.c. at various dates was as follows:—

Dec. 19, 1883	1·2 c.c. (made up).	} Nitrite present.
Jan. 24, 1884	0·9 "	
Feb. 25, "	0·9 "	
Apr. 1, "	1·2 "	
" 16, "	1·6 "	
Aug. 21, 1884	No nitrite and very little ammonia. Nitrification to nitrate as complete as possible.	

This is an example of what I find generally takes place in good waters. Addition of Rochelle salt produces an early and partial reduction of the nitrate to nitrite; if ammonia is present this is presently succeeded by complete nitrification to nitrate, nitrite being first formed.

L6. *Boiled after addition of Rochelle Salt; afterwards fertilised with Soil.*—The prejudicial effect of organic matter on nitrification is well marked in this experiment, for the commencement was very slow, and the fermentation was purely nitrous. In three months, the indigo consumed had barely increased, but a complete conversion of ammonia into nitrite afterwards took place, and the nitrite has persisted. Comparing this with L3 (the conditions being precisely similar except that one had Rochelle salt and the other had not), we see that the *soil alone* would have nitrified the ammonia to *nitrate* long before the commencement of even the *nitrous* fermentation in presence of Rochelle salt; indeed I have met with no instance in which after the addition of *soil alone* to a well water containing ammonia, the fermentation has stopped at the nitrous stage.

L11. *Rochelle Salt, Soil, and a little Phenol added.*—The presence of phenol interferes with the indigo process. Nevertheless, by means of the diphenylamine test, I have been able to ascertain that denitrification was nearly or quite completed in spite of the phenol, and that not the slightest nitrification has since taken place up to the present day. Abundance of ammonia is still present. There is nothing paradoxical about this. Phenol is fatal to many ferments, and amongst others to the nitric ferment, but there undoubtedly are bacteria and other organisms, which it is powerless in small doses to kill.

L13. *Salicylic Acid only added.*—The salicylic acid was added to

this bottle as an *antiseptic*, in order to study its influence on nitrification. The result was curious. Small dots or patches of mould soon began to grow at many points on the sides and bottom of the bottle, and in two months' time a gradual and nearly complete denitrification had occurred. However, after the destruction of all the salicylic acid by the mould (shown by cessation of the ferric chloride reaction), nitrification of the added ammonia set in and proceeded to a very considerable extent. (Salicylic acid interferes to some extent with the indigo process.)

Here we have a mould growing at the expense of a popular antiseptic and destroying it, and thus paving the way for nitrification of added ammonia.

L9. *Glycerol added*.—In this case, a very slow but finally complete destruction of the nitrate occurred, with a gradually increasing flocculent deposit. In the course of this reduction, a little nitrite was at one time produced. No nitrification of the added ammonia has taken place.

L10. *Crystallised Sodium Acetate added*.—I was a good deal surprised to find that a month after making up, this solution was totally destitute of nitrite or nitrate. Acetate of soda, in fact, brought about denitrification quicker and more completely than the tartrate, sugar, or glycerol, which were tried simultaneously under identical conditions with the same water; and up to the present date not the slightest renitrification has taken place. Sodium acetate, in fact, is easily fermentable in presence of a nitrate; it does not interfere with the indigo or permanganate processes, and hence is suitable for experiments on denitrification.

L8. *Cane-sugar added*.—Cane-sugar brought about a bacterial turbidity, during which the nitrate was reduced to nitrite, and subsequently a gradually increasing flocculent deposit with total denitrification occurred. Since then a mould has grown, but no nitrification has taken place.

I have now brought forward enough instances to show that what I may call fermentable organic matter, added to a water containing a little nitrate and also well fitted to nitrify ammonium chloride, undergoes a fermentation which seldom fails to destroy the nitrate already existing, and always retards nitrification of the added ammonia. If the solutions are kept in stoppered bottles (half full), nitrification of the ammonia *may* be prevented for an indefinite time, but if in open bottles or flasks, it does eventually take place. (I have no reason to suppose that the nitrifying ferment is destroyed during the denitrifica-

tion, unless in exceptional cases.) It may be thought that the exclusion of air from the liquid is the essential feature which determines denitrification, and at the same time retards nitrification. This is not the case, for rapid and complete destruction of nitrate will take place in flasks half full and freely open to the air in the case of polluted waters supplied with fermentable organic matter. In an experiment on this point, when sodium acetate was added to the water, the nitrate (1·1 grain N per gallon) was destroyed in 10 days. A little ammonia was formed from the nitrogenous matter of the sewage, and this nitrified during the summer.

The vigour and rapidity with which denitrification takes place after the addition of Rochelle salt, sugar, or sodium acetate to a well water, are closely connected with the condition of the water as regards contamination. I will give an extreme instance.

From the very large quantity of nitrate and (*for the district*) of chlorides contained in a certain well water, I strongly suspected the propinquity of a cesspool, sufficiently far removed, however, to allow of complete nitrification of all sewage finding its way into the well by percolation through the intervening gravelly soil. At any rate the water was condemned, and the following experiments made with it:—

1. A crystal of Rochelle salt was added to a portion contained in a stone jar half full. In a few days the nitrate was absolutely destroyed, the liquid was thick with bacteria, growths of mould rapidly followed the bacteria, not the slightest renitrification occurred for *at least eight months*, and at the end of that time (August 21, 1884) the water was *thick* with a ropy mould, had a very peculiar greyish-pink colour, and had developed an overpowering odour, exactly resembling that of sewage. Ammonia was present. During the 18 months since that date renitrification of this ammonia has taken place, and the odour has disappeared. The jar has been corked all the time.

2. As soon as I had noticed the very energetic denitrifying powers of this water, I procured, on February 20, 1884, a fresh sample, filled a 200 c.c. flask with it, added 140 mgrms. Rochelle salt, adapted a gas delivery-tube and graduated receiver to the neck of the flask, and placed it on a warm shelf in diffused daylight. The water clouded on the third day, gas began to be evolved on the fourth, increasing in amount up to the eighth, when 10 c.c. had collected. The fermentation was now practically over, the swarms of bacteria*

* I examined these bacteria with the microscope, and made a drawing of them. The recent publication of Gayon and Dupetit's memoir enables me to recognise them as *Bacterium denitrificans*, isolated by them in successive cultivations of a drop of sewage in artificial media.

died down, and the water again became comparatively clear. It gave not the slightest reaction with the diphenylamine test, but smelt *strongly of sulphuretted hydrogen*, and gave a black precipitate with lead acetate. The gas had all the characters of nitrogen.

This is the most energetic denitrification I have witnessed in a well water not containing unoxidised sewage (no free ammonia); not only was the nitrate totally destroyed, but the small quantity of sulphate was reduced to H_2S . It impressed me very much at the time, and caused me to make some experiments with a view of devising a mode of testing waters based on their power of denitrification in presence of suitable organic matter.

Shortly afterwards the late Dr. Angus Smith's last report appeared in print, and in it he proposed a method of examining waters very similar in principle. It is based on his observation that sugar, when added to certain waters, undergoes a bacterial fermentation with evolution of hydrogen gas. He had also observed, in 1881, that the addition of excreta to a solution of nitre, causes an evolution of nitrogen equivalent to the whole of the nitre employed. Gayon and Dupetit have observed that when sewage is added to chicken broth and other organic infusions to which nitre has been added, a strong denitrifying fermentation is set up, resulting in the evolution of gaseous nitrogen.

The experiment described above, and others which it is not necessary to detail, led me to recognise that sewage, soils, and most waters contain organisms which are capable of provoking denitrification, and that the one circumstance necessary to determine this action is the presence of fermentable organic matter—not sugar alone, but probably the great majority of organic compounds found in plants and animals, and including such simple substances as acetates, and even oxalates.

Dr. Angus Smith's test for the bacterial activity of a water was to add cane-sugar, and observe the activity of the fermentation produced, and the quantity of hydrogen gas evolved in a given time. He remarks on the irregularity of some of the results as regards production of gas, and this irregularity may, I think, be explained by the relation of nitrates to this fermentation. The waters examined would contain very variable quantities of nitrate, some none at all, and its presence or absence greatly influences the result. In the first place a water exempt from nitrate is very unfavourable to the fermentation, because ferments of this class are greatly encouraged by a large supply of nitric or ammoniacal food. In the second place, if nitrate is present it may be decomposed in various ways, and may either prevent the evolution of hydrogen or substitute that of nitrogen and oxides of nitrogen.

It is possible, therefore, that an improvement on Dr. Angus Smith's proposed mode of examining waters may be made by adding a little nitrate to waters deficient in it, together with appropriate organic matter, and observing the extent and kind of denitrification produced. Denitrification to a greater or less extent will always occur, whereas evolution of hydrogen is an exceptional circumstance. I have worked a little in this direction and will summarise my results.

The addition of Rochelle salt, sugar, acetate of soda, or even an oxalate, to any natural water, is followed after 3 or 4 days by a perceptible clouding, and if the water contained nitrate, this clouding will be found by the metaphenylenediamine test to be *coincident* with the production of a trace of *nitrite*. The air itself, if it gain access to the water, invariably brings germs which produce this incipient reduction. The cloudiness produced in good waters will be longer delayed than that in polluted waters, will be much slighter, and will disappear after a few days, leaving a very slight deposit of dead bacteria. Unless a very small quantity of nitrate was present the reduction to nitrite will not be complete, and the nitrite will eventually renitrify to nitrate. Unless the entering air be carefully filtered, however, a growth of mould may be set up which will hinder this reoxidation and even produce a further reduction.

With more doubtful waters, especially those which contain several grains per gallon of added or original nitrate, the denitrification will proceed farther. Turbidity will be produced in 2 or 3 days, and coincident with this appearance will be that of a trace of *nitrite*. The nitrite, however, will rapidly increase, and in the course of 3 or 4 days more nearly all the nitrate may be reduced to nitrite, so that 10 c.c. of the water will decolorise several cubic centimetres of permanganate, and the metaphenylenediamine test will give an immediate deep orange colour and then a precipitate. During this fermentation, which takes place whether the bottles be full or half full, open or corked, little or no gas will be evolved, and not a trace of ammonia will be produced. Oxalates seem to be equally efficacious with the other substances named in producing this purely nitrous reduction of nitrate; at first, of course, a precipitate of calcium oxalate is caused, but the water is perfectly clear the next day, and gives no reaction for nitrite; on the 3rd or 4th day a fresh turbidity is seen and is signalled by the appearance of nitrite. With these waters the deposit is flocculent, and much more considerable than with the best waters; but the nitrite, after its rapid formation, persists at any rate for some time, although, if there is but little of it, it may soon be used up at the expense of various growths. In corked bottles, half full, a mould will generally be encouraged, and ammonia will be afterwards found.

Other waters again quickly become clouded, and set up a fermentation which ends in total destruction of the nitrate, and is accompanied by evolution of nitrogen gas. If the water contains 4 to 5 grains per gallon nitrogen as nitrate, this will be completely destroyed in about 10 days from the first noticeable turbidity, and minute bubbles of gas will escape during the entire progress of the fermentation; an example of this has already been given. If the water contains very little nitrate originally, perhaps only a few bubbles of gas will be evolved, but the complete destruction of the nitrate instead of the production and persistence of nitrite will mark off this fermentation from the previous one. If, on the other hand, a considerable quantity of nitre, as well as of fermentable organic matter, be added to the water (say as much as 2 grams per litre of each), the fermentation and evolution of gas will go on for, at any rate, 4 or 5 months in a suitable apparatus. I have employed for this purpose an inverted Florence flask, full of water, and corked, with a bent tube of fine bore passing through the cork to allow the escape of liquid as fast as gas accumulates.

Cane-sugar, sodium acetate, and Rochelle salt, employed in parallel experiments with the same water, all provoked this same fermentation; the rapidity was in the order named. When the last two substances are used, an alkaline carbonate is the result. I cannot say whether oxalates will support this fermentation. When the fermentation is very rapid and the water contains nitrogenous organic matter, ammonia is usually found to be present at the conclusion; and the denitrified solution, in the case of bad waters, very easily lends itself to rank growths of various sorts which speedily render the whole liquid thick or ropy. Should hydrogen sulphide, however, be produced by reduction of a sulphate during the fermentation, these growths do not take place. The ammonia formed renitrifies after a considerable time if the liquid be exposed to the air.

Bearing in mind Dr. Angus Smith's observation, that excreta added to solution of nitre produces *this* destructive fermentation, and Gayon and Dupetit's experiments, in which sewage added to nitrated fowl broth and similar decoctions produced the same result, there is some presumption that a well water found to act in this energetic manner may do so because of sewage contamination.

This is confirmed by the fact that the addition of a few drops of fresh sewage to a good well water will cause it to take on this action. Although the gaseous fermentation of nitre *will* take place in bottles only half full of water, and even in vessels fully exposed to air, yet I have little doubt that the exclusion of air is favourable to it. Day-light seems to exert little influence. A circumstance that may prove fatal to such a test is the fact that, according to Dehérain and Maquenne, all soils contain a bacterium which provokes the butyric

fermentation of sugar, and in the presence of nitre a gaseous denitrification is produced very similar to that caused by sewage. So far as my experiments have gone, however, neither good waters nor good waters with even the addition of a *little* soil, will produce this result under ordinary circumstances. In Dehérain and Maquenne's experiments much soil appears to have been used, and they state that the exclusion of air is essential.*

The conclusions arrived at in this paper suggest one or two remarks bearing upon the chemistry of waters and water analysis.

In the first place, what is the nature of the organic matter contained in potable waters?

The soil is the abode of many ferments, some of them having opposed functions, but all lying in wait for suitable conditions which shall encourage one species for a little time until it has done its work and has brought about an alteration favourable in turn to the encouragement of another species. From the soil, these ferments pass into the waters, from which they are not completely removed even by filtration, and the nitric ferment—certainly one of the most subtle of them all—seems little affected by this process. The addition of any ordinary organic matter instantly excites activity in one or other of these ferments, and the effect is soon visible to the eye by the impaired clearness of the water, and to chemical tests by the effect produced on the nitrate of the water.

Now this organic matter need not be by any means that commonly regarded as putrefiable, or even like sugar, of a nature long recognised as easily fermentable. On the contrary, these soil and water ferments do not spare such simple organic compounds as acetates and oxalates, and they attack even such unlikely ones as ethylamine, cyanides, and thiocyanates. The broadest answer then, that I can find to the question asked above, is, that the organic matter of potable waters can be only such organic matter as is nonfermentable, or at any rate not rapidly or easily fermentable; and the conception of rapidly or easily fermentable organic matter must be enlarged so as to include a great number of substances of diverse natures. What two compounds, for example, could exhibit a greater contrast than gelatin and potassium thiocyanate? yet the one is as readily broken down by soil ferments as the other. Indeed, the difficulty is to make out a list of known compounds which are *not* fermentable, especially if they are to be at the same time not fatal to organic life (antiseptics).

The following remarks on the occurrence of inorganic nitrogen in waters apply to well waters more especially; river waters and the water supplies of large towns represent a blend of many conditions.

* Gayon and Dupetit have recently shown that the hydrogen evolved in the butyric fermentation does not reduce nitrates present in the solution.

The normal condition of an unpolluted well water I should state thus (having regard only to the points directly raised in this paper):—Perfectly clear; ammonia and nitrite absent, or present in barely measurable quantities; nitrate always present, but in strictly limited amount.

Ammonia may be present as the result of a putrefactive fermentation of nitrogenous organic matter actually in progress; in which case the water will be more or less cloudy. It may be persisting without sensible alteration during the variable period of inaction of the nitrifying ferment which follows the dying down of the putrefactive ferments—in which case the water may be clear. It may be in process of active nitrification, and a large quantity may disappear *in a few days* with a corresponding increase in the nitrate; this is especially liable to occur in the summer, and should a week elapse between two analyses of the same sample of water some very striking differences in the results would be manifest, although each analysis might be perfectly correct. I am within the mark in stating that a well water may completely nitrify 1 grain per gallon of ammoniacal nitrogen within a week; but it must be remembered that this will not take place immediately after the addition of the ammonia. Even if the presence of the ammonia is not associated with a putrefactive fermentation which delays nitrification, a variable period of quiescence will take place—in fact the *rapid* conversion of ammonia only takes place when nitrification has reached its height.

Finally, ammonia may be present in the stagnant waters supporting confervoid growths, as a bye-product of the reduction of nitrate by various organisms. Ammonia formed by reduction is not of frequent occurrence in well water, however, unless it is accompanied by ammonia resulting from putrefaction.

Nitrite.—"Nitrogen as nitrite and nitrate" has long held a place in water analysts' reports, but nitrite alone is seldom tested for, and still less frequently estimated. Although nitrite is very easily formed by both oxidising and reducing fermentations, it is very rarely present in natural waters except in very minute traces. It will sometimes persist in quantity, and for long periods, in artificially fermented solutions and in water contained in vessels, but in contact with any large quantity of fresh soil it is very rapidly oxidised. It may exist in a water because the conditions do not favour complete nitrification of free ammonia, and in this case the water may be clear; or because of a bacterial reduction of the nitrate, caused by an influx of almost any organic matter; in this case the water is *not* clear.

The reason why nitrite formed by reduction is not often found in well waters is that in most cases the organic matter provoking the reduction consists of sewage, and, as we have seen, sewage contains

bacteria which speedily destroy both nitrate and nitrite, with liberation of nitrogen gas.

If well waters are kept out of contact with soil, nothing is easier than to produce nitrite by nitrification of artificially added ammonia; but the low temperature, extreme dilution of the ammonia, and general contact with soil and air, combine to produce a purely nitric fermentation in most wells and rivers.

An experiment with the recently polluted well water B (p. 664), illustrates this point. This water contained sufficient free ammonia to give an orange *precipitate* with the Nessler test; about 300 c.c. of it was therefore placed in a plugged flask, without any added ammonium chloride, in order to compare the nitrification of the sewage ammonia under the artificial conditions of these experiments with that which takes place *in situ* in the well. Placed in the flask on May 24, 1886, and incubated with the rest of the Bristol waters at 80—85° F., the free ammonia disappeared in less than 22 days, but enough nitrite was formed to give a bright yellow or orange reaction with metaphenylenediamine; 10 days later the nitrite also had disappeared. In the well itself, the water never developed sufficient nitrite to give a recognisable colour with metaphenylenediamine.

Nitrate.—An excessive quantity of nitrate in water is very generally regarded with suspicion; I am not aware, however, that the *absence* of nitrate has been pointed out as a ground of condemnation. Clean rain water and the water of mountain streams often contain but a trace of nitrate; well and river waters *must* however contain more than a trace unless some cause has brought about the destruction of previously existing nitrate. This cause is the access of fermentable organic matter to the water, and in most cases the fermentable organic matter is derived from sewage. When, therefore, a water contains enough mineral matter to demonstrate its percolation through soil, and at the same time is free from nitrate, or contains only a trace barely recognisable by diphenylamine, the occurrence of a destructive fermentation may be inferred. These cases are not uncommon amongst well waters, and the water is generally not perfectly clear—the well water B (p. 664) is an example.

XI. *Period of Incubation of the Nitrifying Organism.*

In all published experiments, including my own, a period of apparent inaction follows the addition of a little soil or nitrifying liquid to a solution prepared for nitrification; this period varies from a few days to weeks or even months, according to the strength of the ammoniacal solution, the quantity of seed used, and other circumstances. At one time I thought that by excluding the tartrate or other fer-

mentable organic matter which encourages the rival denitrifying ferments, I should be able to suppress the incubating period altogether. This can actually be done provided enough seed be used; but to get sufficient or sufficiently active seed it is generally necessary to resort to a first cultivation, during which a true incubating period, or period of growth of the nitrifying organism, is observed. The following examples will explain my meaning. Although the well and river waters used were free from fermentable organic matter, it will be remembered that 150 c.c. laboratory well water required 21 days of incubation before it commenced to nitrify 5 mgrms. NH_3 ; river water required 7—9 days;* river water + 1 per cent. of soil required 4—5 days; and even a solution containing 150 c.c. distilled water, 5 c.c. NH_4Cl , a few milligrams of K_3PO_4 , and 5 per cent. of soil, only commenced nitrifying after 3—4 days. These are periods of true incubation: the less nitrifying ferment there is present, the longer does it take to multiply to an extent sufficient to attack the relatively enormous proportion of ammonia presented to it. But once the ferment is sufficiently developed, it will produce nitrification in a fresh solution *without* incubation, *provided* fermentable organic matter be absent (or perhaps if all reducing organisms can be *rigorously* excluded). When the nitrified solution last-mentioned, for instance, was poured off the 5 grams of soil and replaced by 150 c.c. fresh NH_4Cl of the same strength, nitrification commenced in *less than 24 hours*, and lasted 20 days instead of 30. When this was over, the solution was poured away, the wet soil divided into two equal portions, and each of them covered with 50 c.c. of NH_4Cl solution of half the former strength. The bottles were also heated to 80—85°. *To one of them a little sterilised solution of Rochelle salt was added.* The one without tartrate commenced nitrifying in *less than seven hours*, and finished in nine days. Here the suppression of incubation was complete. The tartrated solution, on the other hand, although it showed a trace of nitrite in seven hours, was completely free from nitrite and nitrate on the third day, and nitrification of the added ammonia did not commence for some days later. This represents a period of false incubation; the trace of nitrite at first found proceeded from *reduction* of the trace of nitrate left adhering to the soil from the previous nitrification, and on the third day this reduction had ended in destruction.

The commencement of nitrification in these experiments was taken to be coincident with the first recognisable trace of nitrite (metaphenylenediamine test). I proved by daily testings in a separate

* Thorough aëration of the river water, caused by allowing it to fall through the air in a minute stream several times every day, did not perceptibly shorten this period of incubation.

solution that *soil alone* causes no reduction of nitrate to nitrite; so that the nitrite observed, except in the tartrated solution, really arose from oxidation of the added ammonia.

Even strong solutions of ammonium chloride will commence nitrifying without any appreciable period of incubation if the conditions indicated above are fulfilled. Thus 700 c.c. distilled water containing 267.5 mgrms. ammonium chloride with no addition but 500 mgrms. calcium carbonate and 333 mgrms. washed soil taken from a recently nitrified solution, in three days developed enough nitrite for easy detection.

Appendix.—In a memoir published since the above paper was in manuscript, MM. Gayon and Dupetit (*Recherches sur la réduction des nitrates par les infiniment petits*, Nancy, 1886) examine certain cases of denitrification in an exhaustive manner. After recognising that many different species of microbes will effect the reduction of nitrate to nitrite, and that the presence of fermentable organic matter of any description is the circumstance which determines denitrification under ordinary conditions, they devote the remainder of the treatise to a minute study of two species of microbes, *Bacterium denitrificans*, α and β , which reduce nitrates with the liberation of nitrogen gas, and sometimes nitrous oxide. Both of these organisms were isolated for study by successive cultivations of a drop of sewage in artificial media. In liquids free from nitrate, they behave as aërobic bacteria, and multiply only on the surface of the liquid, forming a zooglœa impermeable to air. It is even possible to cultivate them in liquids containing nitrate without any destruction of the latter, provided a continuous and thorough aëration of the liquid is maintained. But in nitrated liquids protected from the air, or only partially exposed to it, they live at the expense of the oxygen of the nitrate, and effect the complete oxidation to carbon dioxide of the carbon contained in the organic matter introduced into the liquid. And it is only when this organic matter is nitrogenous that any ammonia is formed during the process. Nitrite is at first formed, but is speedily destroyed. By calculation and experiment, MM. Gayon and Dupetit show that the heat produced by oxidation of the organic carbon, *minus* that absorbed by destruction of the nitrate, is a positive quantity, sufficient in fact to raise the temperature of the fermenting liquid several degrees above that of the surrounding air.
