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INVESTIGATIONS UPON NITRIFICATION AND THE NITRIFYING ORGANISM.[1]

THE nitrogen of organic substances is, for the most part, liberated during decay in the form of ammonia or ammoniacal compounds; and these substances yield, by oxidation, nitrous acid and finally nitric acid, which, in turn, in the form of nitrates, feeds the living plant, and thus begins again the cycle of transformation.

The oxidation of the nitrogen of ammonia, and its ultimate conversion into nitric acid, is called nitrification. This change is especially active in soils near the surface, where nitrates are formed abundantly from percolating waters which contain much nitrogenous matter.

This phase of nitrification, the formation of nitrates in porous soil, has been attentively studied: but less attention has been given to the process of nitrification as it goes on in surface waters, such as streams and ponds; and it is to this side of the question, namely, nitrification as it occurs in natural waters, that our study has been chiefly directed.

Some eighty samples of water, selected from the two hundred and forty coming each month to the laboratory of the State Board of Health, were examined at intervals of from two to seven days for ammonia, nitrites, and nitrates. These samples were received from all parts of the State, and included all classes of surface water, rivers, ponds, and reservoirs. They were examined repeatedly during the months of June, July, and August, 1888.

The results may be briefly stated as follows. The organic matter in suspension decays in about seven days, as is shown by the increase in "free ammonia." In about fourteen days this "free ammonia" has disappeared, and nitrite has taken its place, reaching a maximum in about twenty-one days. Later the nitrite too disappears, and in twenty-eight days or more all the nitrogen has been converted into the form of nitrate. When the suspended matter is removed by filtration through paper, or by precipitation with alumina, no change occurs unless free ammonia were present at the outset.

These changes were so universal, and so independent of the character of the water and of its condition of aeration, that it seemed important to avail ourselves of the unusual opportunity offered by the close proximity of the chemical and biological laboratories of the State Board of Health, to carry on a series of chemical and bacteriological investigations on solutions of known composition. Accordingly, we began a series of experiments covering a period of nearly two years, in which the daily and weekly changes caused by the growth of bacteria were watched from both the chemical and the bacteriological standpoint, in order to determine the sequence and rate of such changes. Other points came up in the course of the work, as will appear from the following pages.

It has long been known that the first step — the decomposition of nitrogenous matter, and consequent production of ammonia — is due to the vital activity of bacteria. The early experiments of Schwann and Schultze (1839), and the later and thoroughly conclusive work of Pasteur, showed that putrefaction of organic matter is brought about solely by the small vegetable organisms known as bacteria. Even after this fact became generally known, it was some time before the importance of the complete range of this discovery was suspected. It was still maintained that the process of nitrification proper — the oxidation of ammonia to nitric acid — was of a purely chemical nature, although the burden of proof was soon thrown on those who upheld this view. The close dependence of nitrification upon a rather narrow range of temperature, the cessation of the process on the addition of antiseptics, the operation of "seeding" one solution with another, the impossibility of effecting rapid nitrification by chemicals, the analogous phenomena of putrefaction, — all pointed clearly to the fact that nitrification depends on the presence of living organisms.

The first conclusive proof that such was the case, however, came from the work of Schlœsing and Muntz in 1877 (Comptes Rendus, 1877, Tome 84, p. 301). The work of these observers rendered it practically certain that living organisms of some kind are the true agents of nitrification. "It now remains for us," they said, "to discover and isolate the nitrifying organisms." Schlœsing and Muntz, in their subsequent investigations, believed that they had succeeded in making this discovery; but, in view of the facts of modern bacteriology, we are unfortunately unable to assign much value to this part of their work. It is not easy to satisfy one's self that Schlœsing and Muntz ever worked with really "pure cultures" of isolated species. While the work of these investigators established beyond all question the fact that nitrification, like the analogous phenomena of fermentation and putrefaction, is caused by living organisms, it left entirely open the precise nature of these organisms.

The first experiments with species of bacteria isolated by modern methods, and therefore undoubtedly pure cultivations, are those recorded by Heræus (Zeitschr. für Hygiene, I., 1886, p. 193). Heræus experimented with fourteen well-known species of bacteria, and with about as many others freshly isolated by himself from water and soil. He cultivated these in an ammoniacal solution, and obtained in the case of several familiar species good qualitative tests for nitrous acid. Among these species were *Bacillus prodigiosus*,

[1] Edwin O. Jordan and Ellen H. Richards, in report on water supply and sewerage to the State Board of Health of Massachusetts. (The series of experiments detailed in this paper were planned and carried out jointly by the authors, the bacteriological portion of the work being done by Mr. Jordan, and the chemical portion by Mrs. Richards.)

the Finkler-Prior bacillus, the bacillus of typhoid-fever, the anthrax bacillus, and others. Heræus concludes that all these organisms possess oxidizing powers, since they are thus apparently able to oxidize ammonia to nitrous acid.

The work of Adametz (Untersuchungen über die niederen Pilze der Ackerkrume. Inaug. Diss., Leipzig, 1886) and Frank (Forschungen auf dem Gebiete der Agriculturphysik. X, 56), on the other hand, did much to offset this positive result reached by Heræus. They found, as other investigators had found before them, that the introduction of a small quantity of garden soil into an ammoniacal solution would produce rapid nitrification. The various species of bacteria, however, which they isolated from this same soil, and introduced as pure cultures into sterilized ammoniacal solutions, refused to nitrify. In no case was more than a trace of nitric acid observed. Frank was so influenced by his continued negative results that at a later date he went so far as to deny that living organisms had anything whatever to do with nitrification. This sceptical attitude seemed for a time to be fully justified by the experiments of Celli and Zucco. It was soon, however, demonstrated by several skilful investigators that nitrification could not be accounted for by purely chemical influences. There was, nevertheless, no cessation in the publication of negative results. The work of Heræus was extended and elaborated by P. F. Frankland and by Warington. Frankland (Jour. Chem. Soc., April, 1888, Vol. LIII., No. CCCV., p. 373) failed entirely to obtain any evidence of oxidation of nitrogen by individual species of bacteria, and on this point came into direct conflict with Heræus. To use his own words: —

"The [ammoniacal] solutions were examined after forty days' growth, but in no case was anything more than a faint indication of nitrous acid obtainable with sulphanilic acid, phenol, and ammonia.

"It is worthy of notice that Heræus had experimented with three of the micro-organisms which we have had under observation, viz., *B. subtilis*, *B. prodigiosus*, and *B. ramosus*. On growing these in sterilized urine, he found that *B. subtilis* alone gave no nitrous acid reaction, whilst the other two gave distinct reactions for nitrites; from this he concludes that *B. prodigiosus* and *B. ramosus* possess oxidizing powers, and that *B. subtilis* does not. My experiments, however, conclusively prove that both *B. ramosus* and *B. prodigiosus* exert a reducing action, whilst *B. subtilis* does not; and therefore that the nitrous acid reactions which he obtained in the case of the two former organisms must obviously have been due to the reduction of the nitrate in the urine, and not to oxidation of ammoniacal nitrogen, as he supposes. That nitric nitrogen is an invariable constituent of human urine has been shown by Warington (Trans. Chem. Soc., 1884, p. 669), and has in fact been long known." Frankland summarizes his results as follows: "8. None of the organisms under examination were found capable of oxidizing ammoniacal nitrogen to nitrous or nitric acids, when introduced into a nutritive solution containing ammonium chloride."

This emphatically negative result with pure cultures of single species was directly confirmed by Warington, who wrote: "It seems to me very clear that not one of the investigators who have experimented with isolated species of bacteria has obtained in his solutions more than a trace of nitrous or nitric acid; no one has obtained an amount that could be determined quantitatively. Another point which generally appears is that every organism tried gives nearly the same result. . . . The statement of Heræus that seven of the organisms examined commenced the nitrification of a twenty per cent urine solution in one day is apparently due to a mistake. My own experiments show that a urine solution of that strength cannot be nitrified by soil without the addition of gypsum; the commencement of nitrification in a strong solution is also extremely slow. The nitrous acid which so speedily appeared in his solutions was due to the reduction by the organs of the nitrates naturally present in the urine" (Journ. Chem. Soc., August, 1888, Vol. LIII., p. 727). Of his own experiments, he says: "A distinct reaction with diphenylamine was in some cases obtained, but this did not appear to grow in amount, although in such cases the examination was specially prolonged. The amount of nitric or nitrous nitrogen in the solutions did not apparently in any case exceed one per million, and all of this could not be attributed to the action of the organism, as the unseeded solutions in the incubator also gave some reaction with diphenylamine. When we have discounted the trace of nitrites probably obtained from the atmosphere, there is clearly very little left that can be attributed to the action of the organism. The question whether any part of the nitrate or nitrite present was produced by the organism, I am unable to decide; but it is quite clear that none of the organisms examined possessed any nitrifying power in any way comparable with that possessed by soil. An organism which nitrifies as soil nitrifies has yet to be isolated."

There are thus several views which are held regarding the action of individual species of bacteria on nitrogenous solutions: —

1. That there is a group of bacteria capable of oxidizing ammonia to nitric acid, and another and separate group able to reduce nitrates to nitrites in the presence of organic matter. Both kinds are widely and abundantly distributed. Attendant circumstances determine whether the reducing or the oxidizing group will gain the upper hand (Heræus).

2. That all kinds of bacteria, under favorable circumstances, are capable of producing nitric acid, and that the same organisms in the presence of organic matter are capable of reducing nitrates (Celli and Zucco, Leone).

3. (*a*) That different species of bacteria vary greatly in their ability to reduce nitrates; and (*b*) that there is no reliable evidence that any individual species is able to oxidize ammonia either to nitric or nitrous acid (Warington, Frankland).

Such is a brief sketch of the divergent opinions upon nitrification which were held at the time we began our work in the autumn of 1888. It seemed to us important to approach the subject from all sides, and we have worked accordingly not only with pure cultivations of bacteria, but also with various sands, soils, and waters containing mixtures of several kinds. We have considered it of fundamental importance to determine the distribution of the nitrifying organism, and, if possible, to ascertain the relative frequency with which it occurs over a wide area. The question, for instance, naturally arose, is the nitrifying organism present in the Boston city water as delivered from the tap in the laboratories of the Massachusetts Institute of Technology, since this is the water used in making up our solutions. To this question we are able to give a decided affirmative. Ammoniacal solutions carefully made with tap water always nitrify. Moreover, ammoniacal solutions which have been sterilized and then inoculated with a cubic centimetre of fresh tap water always nitrify. Repeated experiments show that the nitrifying organism is invariably present in this water. When, however, ammoniacal solutions were inocu-

lated from the separate colonies appearing on a gelatine plate culture of this water, in every instance there has been obtained only a negative result. To this matter of inoculation with pure cultures of bacteria we shall recur presently.

In many of our early experiments upon nitrification we used a mixture of one cubic centimetre of fresh urine with two litres of tap water. This mixture was found to yield, when freshly made, about .5000 free ammonia, .2000 albuminoid ammonia, .0002 nitrites, and .0250 nitrates, in 100,000. This nitrogenous solution was allowed to stand at the temperature of the room (21°–23° C.), and was tested from time to time for nitrites and nitrates. The method used for the determination of nitrites has been Griess's naphthalamine method. This method is sufficiently delicate to detect the presence of one part of nitrogen as nitrite in one thousand millions. The method for determining nitrites is a modified form of the phenolsulphonic method of Grandval and Lejoux.

If the nitrogenous solution be first sterilized and then inoculated with fresh tap water, the same course is followed, with the exception that the period of incubation is considerably lengthened. If seeded with sand from a sewage filter tank, or with garden soil, the whole process is materially quickened, and may even be wholly completed in thirty days.

Not only is the nitrifying organism present in Boston tap water, as the above experiments clearly demonstrate, but it appears to be equally common in water from all parts of the State of Massachusetts. So far as our experience has gone, any natural water, containing the ordinary amount of free or albuminoid ammonia, contains also the nitrifying organism, as is shown by our long series of tests. In these natural waters the nitrifying organism seems to be under wholly normal conditions, and to be abundantly able to effect the oxidation of the small quantities of nitrogen usually present in these waters. Waters that contain high albuminoid ammonia, in cases where this ammonia comes from the nitrogen in infusoria, algæ, etc., go through the same changes as those which contain free ammonia, but more slowly. The organisms in time die, the bacteria set free the nitrogen of their bodies, forming free ammonia, and then in turn nitrites and nitrates.

It might, perhaps, be reasonably expected that, since the nitrifying organism is undoubtedly present in all these waters, an examination of gelatine plate cultures of these waters would reveal some particular kind or kinds of colonies common to all, and in that way aid in sifting out the nitrifying organisms. Our experience has shown, however, that such a hope is unfounded. So far as the inspection of gelatine plate cultures enables us to judge, no one kind of colony is common to all these waters. This fact, on the surface, seemed to favor the view that the power of nitrification was not the property of any particular organism, but was very likely possessed in common by a number of kindred species.

The other line of bacteriological work — the inoculation of nitrogenous solutions with pure cultures of isolated bacteria — has been followed up from the outset, and was begun with full confidence in ultimate success. It is unnecessary to give a detailed account of our experiments in this direction. It is sufficient to say that the nitrogenous solutions have, from beginning to end, failed to nitrify. Nitrogenous solutions of various sorts have been used, pepsin solutions, peptone solutions, ammonium chloride solutions, Frankland's solution (Zeitschr. für Hygiene, Bd. VI., 376), etc., all with the same unfailingly negative result. A large number of species of bacteria have been used for inoculation, not only well-known species like *B. prodigiosus*, *B. megaterium*, *Proteus*, etc., but many species freshly isolated from water, sewage, the sand of nitrifying filter tanks, and similar favorable situations for the nitrifying organism. The experiments have been always prolonged for several months, and in some cases for more than a year. Conditions of temperature, amount of surface exposed to the air, etc., have been varied in many directions. Nitrogenous solutions containing a single species of bacterium have been poured upon sterilized sand, and allowed to settle in such a way as to imitate closely the conditions obtaining in filter tanks. In all, more than one hundred and fifty experiments have been made, covering a period of two years. In every case, without a single exception, there was not the slightest evidence of nitrification by any single species.

There still remains a plausible explanation of this striking succession of negative results. It might be that, although any one species working alone was not able to effect nitrification, a number of different species working together might be able to produce the desired result. This was certainly not an unreasonable supposition, judging from analogous fermentative processes; co-operation and combination might perhaps effect more than individual and independent action. Several experiments were accordingly made with a view of determining this point. Here again the results were invariably negative. Ammoniacal solutions, inoculated with mixtures of several species under pure cultivation, always failed to nitrify. In one experiment, for example, a nitrogenous solution, found by experience to nitrify rapidly and completely when seeded with garden soil, was inoculated with a mixture of six different species of bacteria. These six species were all isolated from soils and waters known to contain the nitrifying organism. An examination of the solution from time to time, by the method of gelatine plate culture, showed a vigorous growth on the part of all the species, but there was at no time the slightest evidence of nitrification, although the experiment continued for upwards of five months.

In the course of our experiments we have found it necessary to guard against two possible sources of error. We noticed at the outset a tendency in all our solutions, whether inoculated with pure cultures, or entirely free from bacteria, to show an increasing quantity of nitrogen as nitrite. This increase of nitrite in standing solutions is shown in the following instance. A nitrogenous solution, placed in a flask stopped with cotton wool, was sterilized in the usual way, and allowed to stand in the laboratory. At first no nitrogen in the form of nitrite was present, but after one month .003 parts per 100,000 had appeared, and at the end of three months .008 parts of nitrite were present. In some cases a much larger amount than this appeared, although no bacteria were in the flasks. In all these instances nitrite was undoubtedly absorbed from the air of the laboratory. Sterilized distilled water was found to absorb nitrite with the same rapidity as did our nitrogenous solutions, in one case absorbing .0015 in a few days. If the solutions were protected from the free access of air, no increase of nitrite was noted, and there was also no increase if they were removed to a room in which little or no gas was burned. In rooms in which much gas is burned it is obvious that, with the present refined methods for detecting nitrites, this absorption from the air, unless guarded against, may lead to erroneous conclusions. This fact of nitrite absorption from the air has been already noticed by Warington and other observers.

A second possibility of misinterpretation lies in the reduction of the nitrates that may be present in the solution. This reduction takes place even when the quantity of nitrate and organic nitrogen is small, although more slowly than is the case in the presence of considerable quantities of organic nitrogen. In one example there were no nitrites and .036 nitrates present at the beginning of the experiment in the sterilized solution. On inoculation with a certain bacterial species, afterward found to possess a reducing action, the quantity of nitrogen as nitrite increased in a short time to .0256, while the nitrate diminished to .015. On another occasion, with .036 initial nitrate, the nitrites rose from nothing to .021, and the nitrates disappeared proportionally. If larger amounts of nitrate are present, the increase of nitrite is more striking. Certainly this reducing action of many species of bacteria will go far to explain such results as those reached by Heræus (*loc. cit.*).

An interesting experience, and one very significant in the light of our further investigations, should here be mentioned. A nitrogenous solution prepared in the usual way was inoculated with a certain species, — *Bacillus ubiquitus*, — and examined from time to time, both chemically and bacterially. The solution, on standing for several months, nitrified completely, and the gelatine plate culture showed the presence of a pure culture of *B. ubiquitus*. We naturally concluded that we had discovered a nitrifying organism; but repeated inoculations with a culture of this same organism, both from the flask that had nitrified and from the original growth in a test-tube, gave a negative result. No better success was had with the same organism freshly isolated from water or soil. No explanation of this perplexing occurrence could be given at the time, but subsequent events made it probable that our assumed pure culture was not a pure culture at all, but a mixture of the nitrifying organism and *B. ubiquitus*. Whether the nitrifying organism was introduced from the air, or, as seems more likely, accompanied the first inoculation with *B. ubiquitus*, is unknown. Possibly some of the investigators who have claimed a positive result with species of bacteria grown on gelatine may have been misled in a similar way.

There was, as has been intimated, one possible explanation of our failure to reach consistent positive results by the use of species of bacteria isolated by the method of gelatine plate culture. It might be that the nitrifying organism did not grow on gelatine. Everything seemed to point in this direction, and the belief was further strengthened by a very significant fact observed about this time. We had known for some time that in the history of the filter tanks at the Lawrence experiment station speedy nitrification was always coincident with a marked decline in the numbers of bacteria. The effluents discharged from the filter tanks, although high in nitrates, were low in bacteria; and, moreover, the more complete the nitrification, the fewer were the bacteria in the effluent.

We also observed, that, in an ammoniacal solution which is seeded with ordinary pond water containing several species of bacteria, there is during the first few days a rapid multiplication of the contained germs. Nitrification, however, does not as a rule begin until from ten to fourteen days have elapsed. By the time nitrification begins, the numbers of bacteria, as shown by gelatine plate cultures, have begun to decline; and, while the nitrogen in the form of nitrites in the solution is increasing, the numbers of bacteria are steadily diminishing. Thus, in one instance, an ammoniacal solution, four days after its inoculation with a cubic centimetre of Cochituate water, contained 3,762,000 bacteria per cubic centimetre. Nitrification had not yet begun. When the first signs of increasing nitrites appeared, the numbers of bacteria had sunk to 19,200; and when the nitrites reached their maximum, the bacteria, shown by gelatine plate cultures, were only 9,454. It was certainly difficult to understand why nitrification, a process apparently dependent upon the life and activity of bacteria, should seem to flourish best under conditions in which bacteria were perishing. If, however, it were assumed that the nitrifying organism could not grow in the usual gelatine media, all the perplexing results above recorded could be more easily explained. Under these circumstances it was natural for us to make such an assumption.

There was, of course, the possibility that the nitrifying organism, by its growth on gelatine, had lost its peculiar property; but it did not seem to us likely that so fundamental a property could be parted with in so short a time. However that might be, we determined to test the other hypothesis first, since we believed it to be the more probable of the two. Accordingly, experiments were begun to attempt to isolate the nitrifying organism by the method of dilution. This is the method that was commonly used by investigators in bacteriology before the invention of solid culture media. It has, as is well known, serious practical as well as theoretical drawbacks. In our practice a small portion of an actively nitrifying solution is transferred on the loop of a sterilized platinum needle to a sterilized ammoniacal solution, and when nitrification is thus induced in the second solution a fresh transfer is made from this to a third, and so on. Rigid precautions have been taken to avoid the introduction of foreign germs.

Hardly were these experiments well under way, before our interest in this method of procedure was stimulated by the publication of communications by Percy F. Frankland and Grace Frankland, and by Robert Warington (Chemical News, Vol. LXI., p. 135).

The Franklands, having reached a conclusion similar to our own regarding the behavior of the nitrifying organism in gelatine, had also attempted to isolate the nitrifying organism by the dilution method, and had succeeded in this attempt. They state, in their abstract of the paper read before the Royal Society, that, "after a very large number of experiments had been made in this direction, the authors at length succeeded in obtaining an attenuation consisting of about $\frac{1}{1000000}$ of the original nitrifying solution employed, which not only nitrified, but, on inoculation into gelatine-peptone, refused to grow, and was seen under the microscope to consist of numerous characteristic bacilli, hardly longer than broad, which may be described as bacillo-cocci."

Warington's communication entirely confirms that of the Franklands, in so far as it relates to their earlier and negative results. He had not, however, at the time of writing, succeeded in isolating the nitrifying organism.

A paper by Winogradsky followed soon after. He appears to have discovered independently a nitrifying organism, and attributes his success largely to his microscopic examinations of the nitrifying solutions, and to his use of solutions devoid of organic matter. The following is the composition of the liquid adopted by him: ammonium sulphate, 1 gram; potassium phosphate, 1 gram; water from the lake (at Zurich) 1,000 grams. Each portion of 100 cubic centimetres received in addition .5 to 1 gram of basic magnesium carbonate, suspended in distilled water. Winogradsky found that this layer of magnesium carbonate at the bottom of each flask

afforded an excellent gathering place for flocks of the nitrifying organism. The "nitric ferment" does not, as the Franklands had already shown, grow well upon ordinary gelatine plate cultures; and this is probably the cause of the failure of all previous experimenters to isolate the special ferment.

Before receiving Winogradsky's paper, in the spring of 1890, we had been using in our work, at the suggestion of Mr. Allen Hazen, an ammoniacal solution of the following composition: ammonium chloride (resublimed), 1.907 grams; sodium carbonate, 3.7842 grams; sodium phosphate, .2 grams; potassium sulphate, .2 grams. These salts were dissolved in such a quantity of re-distilled water that the solution contained 100 parts of nitrogen per 100,000, and two equivalents of alkali. Ten cubic centimetres of this solution were mixed with one litre of re-distilled water, and then inoculated as desired. The flasks used have been made chemically clean by boiling with potassium permanganate, and the water used has been twice distilled. The other rigid precautions absolutely necessary in all work of this character have always been taken. The solutions thus prepared have contained from .0001 to .0010 parts per 100,000 of albuminoid ammonia.

Proceeding with this solution by the method of dilution, we at length succeeded in isolating a nitrifying organism. A flask was first inoculated with a few grains of sand from Tank No. 13, at the Lawrence Experiment Station, and when nitrification was at its height in this solution, a small portion was transferred from this to a second flask, and so on. After a large number of unsuccessful attempts, two solutions were finally obtained which nitrified well, but gave no growth upon ordinary gelatine plate cultures, although the plates were allowed to stand for seven days. Microscopic examination of these solutions showed them to be inhabited by a particular form of bacillus, and apparently by that alone. These bacilli are short, of a slightly oval shape, and vary from 1.1 μ to 1.7 μ in length; they are about .8 μ to .9 μ broad. They are grouped very characteristically in irregular clumps, and are held together by a jelly-like material. Each aggregation is indeed a typical zoöglœa. The aggregations of bacteria were found chiefly on the bottom of the flasks, as was also the case with the organism described by Winogradsky. These masses of zoöglœa, obtained as a pure culture from a nitrifying solution, resemble significantly the zoöglœa discharged in considerable quantities from the filter tanks at Lawrence. The bacilli stain with some difficulty with the usual aniline dyes. We have not observed independent movement. Owing to the lack of the usual means of diagnosis, it is difficult to determine in a short time whether this species is the same as the one described by the Franklands and by Winogradsky. On one important point there appears to be a difference between our results and those reached by the above-mentioned investigators. The organism discovered by them oxidizes ammonia to nitrite, but carries it no further. Our own flasks give complete oxidation to nitrate. Whether this be due to a difference of conditions, a difference in the virility of the organisms, or a specific difference in the bacteria, we are not at present prepared to say. The short time at our disposal has made it impossible to settle this and many other questions to our own satisfaction. We are not even prepared to say that there may not have been a mixture of two or more species in our flasks, all agreeing closely in morphological characters, and in giving no growth on gelatine, but differing in important physiological respects. Further investigation is necessary to settle this and other important points regarding the relations of this organism to the process of nitrification.

Whether or not we accept the views of Winogradsky, it is certainly worthy of remark, as he observes, that an organism should exist, which, without chlorophyll and in the apparent absence of organic nitrogen and of organic carbon, should be able to multiply and thrive upon wholly inorganic compounds. It may be well doubted, we think, whether this is really the case. It seems more reasonable to suppose that exceedingly minute quantities of organic nitrogen and carbon are actually present, and escape detection by our present methods of chemical analysis, although in reality sufficient to nourish generations of bacteria.

Our own experience, as well as that of previous investigators, seems to be a warning against a too confiding use of the gelatine plate culture in bacteriological work, since in this instance such confidence has left us for a long time in ignorance of a common and widespread as well as highly important organism.

THE PARASITE OF QUARTAN AGUE.

IN the *Zeitschrift für Hygiene* (x. 137) appears the first of a series of papers by Camillo Golgi, demonstrating by means of photography the development of the parasite found in malarious fevers. This paper, of which an abstract appears in the *British Medical Journal*, deals with the *amœba malariæ febris quartanæ*, the form found in the quartan type. In 1880 Laveran stated that these parasites are present in every case of malaria, and in no other condition, and that they are probably the cause of the disease. His observations have been confirmed by pathologists in all parts of the world, and at the present time the weight of proof seems to be in favor of his contention. In his paper Golgi claims to have been the first to demonstrate that the different forms described as occurring in the blood are simply modifications of one form, and, further, that these metamorphoses follow each other according to a fixed law. This development takes place within, and leads to the destruction of the red blood corpuscles.

At first the amœba-like parasite is small and non-pigmented; it increases in size at the expense of the substance of the blood corpuscles, becomes pigmented, and, after passing through a series of metamorphoses, finally ends in a process of segmentation. This process of segmentation takes place at the same time as, or a short time before, the onset of the febrile paroxysm, and has for its object the formation of a new generation of the parasites. The pigment granules stored up in the body of the parasite take no part in this process of segmentation, and hence, on its completion, escape into the blood plasma, where they are seized upon by the white blood corpuscles and cells of the liver, spleen, etc.

The new brood of parasites at once pass into fresh red blood corpuscles, and so commences anew the cycle of metamorphoses leading up to the next paroxysm of fever. The period of time which elapses between the entrance of the parasites into the red blood corpuscles and their segmentation is exactly three days, and hence arises the periodicity of the quartan type of malarial fever. During the first and second days the parasite passes through the various phases of its development within the blood corpuscles, on the third day segmentation takes place, the new brood is set free and fever results; in other words, the period of apyrexia corresponds with the endoglobular growth of the parasite.

Golgi states that a knowledge of these developmental stages is of immense practical importance for the purpose of diagnosis, by which an almost mathematical degree of accuracy can be arrived at, and that it is no exaggeration to say that by the simple microscopic examination of a few preparations of blood the physician is in a position to tell when the last attack of fever occurred, to foretell the time of the next attack, and further, to recognize what type of malarial fever he is dealing with. The simple quartan fever is explained by Golgi as resulting from the development the blood of one set of the parasites, which ripen every three days, while the double and triple quartan fevers are caused by the