ART. LXIV.—*Note on the Ferment-theory of Nitrification;* by F. H. STORER, Harvard University.

THE results of the following experiments bear so immediately upon the recent observations of Schlösing* and Warington,† noticed in the April number of this Journal, that I am led to publish them by themselves, out of their legitimate connection with other experiments upon which I have been for some time engaged. My experiments were made for the purpose of testing the action of certain oxidizing agents on ammonium compounds, and they are, perhaps, not the less interesting as a contribution to the ferment-theory of nitrification, inasmuch as they were undertaken without any reference to the observations of Schlösing, and not at all with the view of supporting or even of testing his experiment.

For my first trial, eleven common glass bottles of greenish tint, each of 500 cc. capacity, and fitted with corks carrying inlet and outlet tubes, were charged as follows: No. 1 with distilled water that had been carefully freed‡ from nitrates, nitrites, and ammonium compounds; No. 2 with a solution of ammonium chloride, containing $\frac{2}{10}$ milligram of NH_3 to the centimeter; No. 3 same as No. 2, with the addition of a quantity of ferric hydrate; No. 4 same as No. 2, with the addition of ferrous hydrate; No. 5 same as No. 3, with the addition of charcoal made from white sugar; No. 6 ammonium chloride, black oxide of manganese and sugar charcoal; No. 7 leached peat and pure water; No. 8 leached peat and ammonium chloride; No. 9 leached peat and ferric hydrate; No. 10 leached peat, ferric hydrate, and ammonium chloride; No. 11 pure water.

Pure water was used in each case to dissolve or suspend the

* Comptes Rendus, lxxxiv, 301.
† Journal of London Chem Soc., 1878, i, 44.
‡ In the manner described in the note on page 182 of vol. xii of this Journal.

chemicals, and the absence of nitrates and nitrites was proved by testing each of the solutions and mixtures with iodo-starch, at the beginning of the experiment.

The bottles were about half filled with liquid, i. e., each of them contained about 250 cc. of the solution or mixture allotted to it.

The "leached peat" was prepared from some bog-meadow mud from the Bussey farm, which had been kept in barrels in a dry store-room for three or four years. This thoroughly air-dried substance was percolated with pure water until the filtrate gave no reaction for nitrites or nitrates.

The ferric and ferrous hydrates were used in the recently precipitated condition; they were made from the corresponding chlorides by precipitating with ammonia-water, in the cold.

The bottles were connected with one another, in the order indicated, with short pieces of caoutchouc tubing in such manner that by aspirating at No. 1 air could be made to bubble through the water in each member of the series. The corks of the bottles and the caoutchouc connectors were covered with shellac in alcohol so that the entire apparatus was completely air-tight. That is to say, none of the solutions had the least connection with the external air, excepting as it was purposely admitted at the inlet-tube. To purify the air that was drawn into the apparatus, it was made to pass through a long tube loosely filled with clean cotton wool; through two sets of Liebig bulb-tubes filled with a tolerably strong solution of yellow prussiate of potash, to remove ozone; through two sets of similar bulbs charged with potash-lye to remove nitrates and nitrites; through a dry bottle, to catch liquid drawn forward from the potash bulbs; and through a large drying tube charged for two-thirds its length with calcium chloride and one-third with soda-lime.

By means of a Bunsen filter-pump a rapid current of air from out-of doors was drawn incessantly night and day, through the series of bottles, for a fortnight, in September, 1877, in the direction from No. 11 to No. 1; at the end of which time the contents of each bottle were tested for nitrites and nitrates, by boiling with cadmium, distilling with acetic acid, and mixing iodo-zinc starch and acid with the distillate. 100 cc. of liquid were taken for each of the tests, and fifteen minutes were allowed in which to watch for the appearance of the blue coloration. Nothing could be more distinct and decisive than the results of these tests. The solutions from bottles Nos. 8, 9 and 10 namely gave immediate and strong reactions for the nitrogen oxides, while the contents of Nos. 1, 2, 3, 4, 5, 6, and 11 gave no reaction whatsoever. No. 7 gave a reaction, but not a very strong one. The reaction seemed to be strongest in

the contents of No. 10. It appeared from these results that there had been formation either of a nitrate or nitrite in each of the bottles which contained humus, and no such formation in either of the other bottles; but the thought suggested itself, that the small amount of nitrogen oxides found in No. 7 may perhaps have been dragged over mechanically from No. 8 by the current of air.

A second series of tests was made upon Nos. 8, 9 and 10, to see which of them gave the weakest reaction, 25 cc. of liquid being taken from each bottle and diluted with pure water to the volume of 100 cc. before applying the test. It appeared again that No. 10 gave the strongest reaction and that No. 8 gave the weakest. Roughly estimated, the strength of the reactions from jars Nos. 10, 9 and 8 were to one another as 5 : 2 : 1.

It may here be said that humus was employed in these experiments for the sake of testing the old observation of Millon,* who noticed that ammonium salts are changed to nitrates when in contact with oxidizing humus, and who argued that the chemical action originated by the coming together of humus and oxygen, was communicated to the ammonium compound. In his own words: "The oxidation of the humic acid is the cause of the oxidation of the ammonia."

In so far as the foregoing experiments go, we have manifestly a striking confirmation of the accuracy of Millon's work. But on repeating the experiments with the difference that the bog-earth was henceforth exhausted with hot, strong muriatic acid, before proceeding to wash it with water, no such results as the foregoing were obtained. For example, in a second series of trials, arranged like the first, the contents of bottles Nos. 1 to 6 were identically the same as before, only that the amount of liquid was now 150 cc. instead of 250 cc. No. 7 contained the purified peat mixed with water; No. 8 was the same as 7 plus a quantity of gypsum; No. 9 was the same as 8 plus some ammonium chloride; No. 10 contained water alone; No. 11 purified peat plus ammonium chloride and ferric hydrate; No. 12 well-washed cotton rags plus ammonium chloride and ferric hydrate; No. 13 purified peat plus ammonium chloride; No. 14 pure water; No. 15 pure water, the same namely, which had already done service (as No. 11), in the first series of experiments.

The weather (October 13, 1877), being too cold to permit the keeping open of a window, the current of air, purified as before, was drawn from the cold-air-box of the hot-air furnace used for warming the laboratory. The current of air passed this time in the direction from No. 1 to No. 15; it was maintained constantly

* Kopp and Will's Jahresbericht der Chemie, 1860, xiii, 101 and 1864, xvii, 158.

during ten days, and the contents of the bottles were then tested as before for nitrites and nitrates. But no reaction was obtained in either instance, with the exception of No. 12 (cotton rags, etc.), which gave a faint coloration of a not very satisfactory character. After the application of the test those of the bottles which still contained a sufficiency of liquid were re-attached to the aspirator and air was drawn through them continually during another week, when the test for nitrites and nitrates was again applied. But in no case was there any reaction, with the exception of bottle No. 8 whose contents gave a faint coloration.

Inasmuch as warm, and, at times, hot summer weather had prevailed during the term of the first series of experiments, while the out-door air was decidedly cool and that of the laboratory by no means very warm during the time allotted to the second series, a third set of trials was undertaken in December, 1877, in which the contents of the bottles were heated artificially. That is to say, the bottles were immersed in a large water-bath, which was heated to from 70° to 80° C. during the progress of the experiment.

In this third series, there were eight bottles containing the purified peat, first by itself; then admixed with gypsum; with gypsum and ammonium chloride; and with ammonium chloride and ferric hydrate. One bottle contained washed cotton rags, ammonium chloride, and ferric hydrate as before. The water-bath was heated during working hours for a fortnight, perhaps a hundred hours in all, and during this time air was drawn from the furnace-box and purified as before. The contents of the bottles were then tested for nitrites and nitrates, but no reaction was obtained in either case. To make sure that the absence of the reaction was not due to any interference caused by the presence of the peat or the chemicals, a fresh portion of liquid was taken from each of the bottles, enough nitrate of potash to amount to 0·001 gram of N_2O_5 was added, and the test for nitrites and nitrates was applied in the usual way: reactions were now obtained immediately in every instance.

In the light of the facts observed by Schlösing, the natural inference from the results of these experiments is that the formation of nitrates or nitrites in bottles Nos. 7 to 10 of the first series of experiments was due to the presence of living organisms which the peat had harbored, and that the absence of nitrification in the other series of experiments is to be attributed to the destruction of the ferment-germs by the hot acid with which the peat employed in these experiments had been treated. It is to be observed, moreover, that the formation of nitrogen oxides in the bottles Nos. 7 to 10 is in nowise out of

accord with the important fact observed by Warington, that darkness* is essential to the action of the nitrifying germs, for although my bottles were not shielded from diffused daylight, the mixtures of peat and water which they contained were practically dark-colored muds, not ill-fitted to shelter the germs from the light.

It is possible of course that the colder weather which prevailed during the later trials may have had an influence upon their results; but if this be so, the fact must be counted as an additional argument in favor of the ferment-theory. Moreover, it may fairly be inferred that if mere oxidation be all that is needed to induce nitrification, the heating of the liquids by day in the third series of experiments would have been sufficient; though both the strong heat and the cooling of the bottles by night would have tended to prevent the growth of living organisms.

In the interest of the ferment-theory, it would have been well to control the negative results above given by trials with mixtures of the purified peat and carbonate of lime, for peat which has been treated with muriatic acid has always a slight acid reaction, due I suppose to free humic acid, no matter how thoroughly it may have been washed with water, and it is to be supposed that this acid peat, devoid withal of phosphatic and other saline matters, is not favorable for the growth of the ferment. But as was said before, my experiments were made to test the oxidizing action of certain chemicals, not to cultivate living organisms.

It may be added that I have not as yet found any evidence

* The following statement has a certain interest for analysts as bearing upon the stability of dilute solutions of ammonium chloride. In February, 1877, it was noticed by my assistant, Mr. Lewis, that a solution of ammonium chloride which had been prepared ten or twelve months previously for use in connection with Nessler's test, by dissolving the salt at the rate of 3·15 grams to the liter, now gave a strong reaction for nitrites, although freshly-prepared solutions, made in the same way from like materials gave no reaction. The old solution was contained in a glass-stoppered bottle which was about half filled by it, and it had been kept most of the time in a dark cupboard.

Acting on the supposition that the change of the ammonium salt to a nitrite had been caused by the growth of some fungus in the liquid and that such fungus might perhaps be present in other bottles in the neighborhood, I collected as many different specimens of moulds as could be found growing in the various saline solutions kept in the laboratory, and, after rinsing with pure water, placed them in a series of half-gallon bottles, into which had been poured from half to quarter of a liter of dilute ammonium chloride, such as is used for Nesslerising. A quantity of mother of vinegar also was placed in a similar bottle. This series of bottles was left to stand during early autumn, and winter in a green-house, in a rather strong light, and the solutions were tested from time to time, at intervals of three or four weeks, but no reaction for nitrites or nitrates could ever be obtained from either of the bottles. The experiments of Warington show that these trials were vitiated by the strong light to which the bottles were exposed. It is not altogether unlikely that different results would have been reached if the bottles had been kept in the dark.

that solutions of ammonium compounds can be oxidized to nitrites or nitrates by means either of ferric oxide, of black oxide of manganese or of gypsum. I can say with Millon,* that, in spite of all that has been written in favor of the oxidation of ammonium by ferric oxide, "I owe it to truth to state that though the most varied attempts have been made to oxidize ammonia in the cold (i. e. in the wet way), by peroxide of iron, they have all proved unavailing."

I am indebted to my assistant, Mr. D. S. Lewis, for his careful attention to the details of these experiments.

Bussey Institution, Jamaica Plain, Mass., April, 1878.