residue obtained from the distillate was considerable in quantity and gave about 1.5 grams of a yellow platinum compound, which showed on ignition a percentage of 43.52 and 43.55 of platinum in two determinations. The platinum in ammonium chloroplatinate amounts to 43.92 per cent.

These experiments showed, then, about .5 per cent. of nitrogen as ammonia in the deposit—the main point of this communication.

Attempts to show the presence of primary amines by the isocyanide reaction failed, and nothing but the odor seemed to indicate the presence of amines of any kind. A deposit formed in an iron (not galvanized) pipe under similar conditions had little or no odor.

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Direct Nitration of the Paraffins. R. A. Worstall.

[Am. Chem. Journal, March, '98; Vol. 20, No. 3.]

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Evolution of Free Nitrogen in Bacterial Fermentations. A Preliminary Paper on the Composition of the Gas Evolved in Bacterial Fermentations. By Sevfrance Burrage and A. Hugh Bryan.

During the study of certain species of bacteria in the bacteriological laboratory at Purdue last year, Miss Clara Cunningham found one that produced an enormous amount of gas in fermentation tubes. In fact, the evolution of gas was so rapid and profuse as to attract immediate attention as something extraordinary. The bacillus responsible for this had been separated from sugar beet. It was thought to be of sufficient interest to have the gas analyzed, which was done. The gas was found to be made up of $CO_2$, H, O and a residual gas which was presumed to be nitrogen. But the occurrence of free nitrogen in this way and in this comparatively large proportion is rare and unusual, and it raised the question whether this could really be nitrogen. No positive test had been made. Every other possible gas had been shown to be present or absent, and nitrogen and argon were all the possibilities remaining. This seemed to be sufficient proof for the chemist, but the bacteriologist wanted a positive proof for nitrogen, which was made, and the nitrogen was found.

In looking up the literature on the subject very little was learned. Nitrogen had been found in a few cases, but no positive tests given. And in some of these cases, on account of the small amount of the nitrogen

present, it was thought possible that it might have come from air getting in during the analysis. In several of our analyses the large percentage of nitrogen found would have excluded this possibility. In one case, in our laboratory, the fermentation took place in a fermentation solution which had been made up in the usual way except that no peptone was used. Curiously enough, the percentage of nitrogen in this instance was larger than we found in any other.

A paragraph from an article "On a Pure Cultivation of a Bacillus fermenting Bran Infusions," by J. T. Wood and W. H. Wilcox, B. Sc., will show in a general way how unusual this occurrence of nitrogen is: "A remarkable fact in this fermentation is the evolution of free nitrogen, which seems to be rare, except in the case of putrefactive organisms. As in the vast number of fermentative decompositions due to bacteria, almost the only gases found are carbonic anhydride ($CO_2$), hydrogen, $H_2S$ and marsh gas."*

In our laboratory this year Miss Lillian Snyder found that a species of bacillus associated with the pear blight, produced a considerable quantity of gas. The analysis of this again showed nitrogen.

The question was raised as to whether the occurrence of nitrogen in these gaseous products of fermentation is really as rare as had been thought. An extensive series of experiments bearing on this subject are in progress, the results of which we hope will to some degree settle this point. So far our results with the same germs and in the same solutions do not give the same proportions of gases. Whether this is a normal variation or due to some causes unknown to us, we cannot say.

The following table shows the results of analyses:

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*Journal of the Society of Chemical Industry, June 30, 1897, p. 512.

| BACILLUS AND FERMENTATION FLUID.* | $CO_2$ | O | CO | $CH_4$ | H | N |
|---|---|---|---|---|---|---|
| Y.† Smith's Fluid, +2% Sucrose | 67 | .5 | none | none | 20.6 | 11.9 |
| Y. Same as above | 61.8 | .69 | none | none | 27.5 | 10.01 |
| Y. Smith's Fluid, +2% Glucose | 38.7 | 3.6 | none | none | 36.5 | 21.2 |
| Y. Smith's Fluid, *no peptone*, +2% Sucrose | 37.6 | 7.2 | none | none | 21.6 | 33.4 |
| Y. Smith's Fluid, + 5% Sucrose, +3% $CaCl_2$ | 44.2 | 1.9 | none | none | 33.6 | 10.2 |
| Y. Smith's Fluid, + wort + .7% Acid | 53.2 | .9 | none | none | 31.7 | 14. |
| Y. Smith's Fluid | 45 | .83 | none | none | 26.3 | 26.8 |
| Y. Smith's Fluid | 58.5 | none | none | ..... | 31.7 | 9.8 |
| Y. Smith's Fluid | 43.2 | none | none | ..... | 40.7 | 16.2 |
| Y. Smith's Fluid, +2% beet juice | 72.7 | 1.02 | none | ..... | 19.7 | 6.4 |
| X††. Smith's Fluid | 50 | .2 | none | ..... | 32 | 18 |

The method of systematic analysis in Hempel's "Gas Analysis" was followed. The gas was transferred from the fermentation tube to a beaker inverted over water and from this to the measuring pipette. Five minutes was allowed for the absorption of the gases and another five minutes before the readings were made. Analyses were conducted where the temperature remained practically constant. The gas was passed into the solutions until no further diminution of volume was noticed.

Carbon dioxide was absorbed by a solution of potassium hydroxide, oxygen by phosphorus and carbon monoxide by an ammoniacal solution of cuprous chloride. Hydrogen was estimated in two ways: (1) By absorption by palladium and (2) by explosion with oxygen. When the latter method was used the gas, after explosion, was passed into potassium hydroxide to make sure of the presence or absence of marsh gas in the

* Fermentation fluid is the common one known as "Smith's" 10 gr. peptone, 5 gr. sodium chloride, 1000 c. c. of water. The additions to this are marked in table.

† Y. Bacillus separated from sugar beet by Miss Clara Cunningham.

†† X. Bacillus separated from pear tree tissue by Miss Lillian Snyder.

original sample of gas. Pure oxygen was used in the explosion, and the excess after it could be absorbed and a residue, if any, measured. In all cases there was a residue of from one to five cubic centimeters, hardly enough for proof of the presence of nitrogen.

In order to prove the presence of nitrogen in the residue, a large fermentation tube was procured and a culture made in this, using the necessary precautions. Smith's fermentation fluid, +1% of glucose, was used. The gases, $CO_2$, CO, H and O, were absorbed as described before. The residue, amounting to some 20 c. c., was kept in a pipette over water; 10 c. c. of this residue was transferred to a eudiometer provided with platinum electrodes and mixed with 22 c. c. of oxygen prepared from the electrolysis of water. This mixture was sparked. In the course of three hours the volume showed some signs of diminution, and after six hours the volume had been reduced to less than half. The current was turned off and the gas allowed to stand for 24 hours, with no practical change in results.

The contraction in volume of the gas when sparked with oxygen showed the presence of nitrogen in the formation of an oxide of nitrogen which was soluble in water.

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### Micro-Organisms in Flour. By Carleton G. Ferris.

Flours have been studied from the chemical standpoint with considerable care, but comparatively little has been done as regards investigation from the bacteriological standpoint. Although the chemical side of the question has been considered the most important, as it undoubtedly is, there are certain changes occurring in dough made from chemically pure flour which cannot be attributed to the chemical side of the question. For example, bread made from a flour which chemically contained a proper quantity of gluten, etc., may be spoiled. The point might be raised by some that the bad bread was not the result of using a certain flour, but that the micro-organisms present were found in the water used and in the surrounding air, or possibly from an impure yeast. Experiment has proven, however, that bad bread can be obtained even when sterilized, distilled water and pure yeast are used. As the growth of bacteria does not commence in the dough until nearly all fermentation has ceased, it is reasonable to assume that the changes in the dough and in the