III. OBSERVATIONS ON THE ALBUMINOID AMMONIA TEST.

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SOME time ago in this laboratory it was found that effluents of fair and even very good quality were yielding albuminoid ammonia figures of 1.0 to 2.0 parts per 100,000, while pure drinking water gave figures varying from 0.02 to 0.1.

The albuminoid ammonia figures of good effluents generally approximate to 0.15, while in the case of pure waters the figures are generally less than 0.005.

It was evident that the technique involved some very considerable error, which quite vitiated the value of the test, and it was necessary to make an investigation to ascertain the cause of these abnormally high figures.

The distilling apparatus seemed to be quite above suspicion, as the corks were securely covered with tin-foil and there was no difficulty in obtaining ammonia-free water. Furthermore, the free and saline ammonia figures were normal.

Some impurity in the chemicals used in the albuminoid ammonia determination was therefore suspected. It had been observed that the permanganate-alkali mixture was very difficult to free from ammonia, but after two days' boiling it was always apparently possible to free the mixture by a single distillation in the apparatus.

The great difficulty experienced suggested however that possibly the mixture was not after all free from nitrogenous material and that by diluting and redistilling with ammonia-free water further yields of ammonia might be obtained.

It was resolved to test this possibility by experiment. Potassium permanganate and caustic soda in the usual proportion of $\frac{1}{2}$ g. to 10 g. respectively were boiled together in a small flask with ammonia-free distilled water for two days. The mixture was then poured into a litre of ammonia-free water in the distilling apparatus and distillation was carried out. Large amounts of ammonia were generated, *e.g.* 0.0001 g. When the ammonia ceased to come over, the ammonia-free distillate was collected and the contents of the flask were concentrated to a small bulk. The ammonia-free distillate was next

poured back and the distillation recommenced. As much ammonia was again produced as in the first distillation. This could often be repeated for several days; sometimes however the generation of ammonia ceased after a few distillations. Taking 0.0001 g. as a typical yield from *one* distillation, as 500 cc. of a drinking water are used in the albuminoid ammonia determination, this would correspond to the enormous error of 0.02 parts per 100,000. Occasionally a single distillation yielded even larger amounts, and the error was correspondingly increased.

It was evident that this was at any rate one source of error. The permanganate-alkali mixture could apparently be cleared of ammonia by a single distillation, but when added to the water being analysed the dilution and boiling would lead to the production of more ammonia, sufficient in amount to give altogether inaccurate results.

Prolonged boiling rarely led to the purification of the mixture, and it was concluded that a stable nitrogenous impurity was present which only decomposed with extreme slowness. The condition favourable to decomposition was great dilution, while concentration stopped the process entirely.

Through the kindness of Prof. Harden, a sample of permanganate-alkali mixture was obtained from a laboratory in which *normal* albuminoid figures were being obtained. When 50 cc. of this solution (containing $\frac{1}{2}$ g. of permanganate and 10 g. of alkali) was distilled with ammonia-free water, only 0.000005 g. ammonia was generated in each distillation. This corresponded to the small error in the albuminoid-ammonia figure of a water of 0.001 parts per 100,000.

It was thought that this mixture could be employed to ascertain whether the impurity existed in the permanganate or the soda in the contaminated mixture which was yielding the high results.

Accordingly 50 cc. of the pure mixture was boiled with $\frac{1}{2}$ g. of the suspected permanganate for two days and the solution was then distilled with ammonia-free water in the ammonia apparatus. Large amounts of ammonia were generated, *e.g.* 0.0001 g. ammonia in each distillation. This pointed to the existence of a nitrogenous impurity in the permanganate.

The pure mixture was next separately heated with 10 g. of the suspected soda and distilled as before. During each distillation only small amounts of ammonia were produced, *e.g.* 0.000005 g.

The impurity was thus chiefly concentrated in the permanganate.

Experiments were next carried out with the same brand of permanganate and soda as constituted the *pure* mixture. A solution was prepared in this laboratory, and after one day's boiling it generally yielded an error of about 0.001 to 0.002 parts per 100,000 in an albuminoid ammonia determination for a water. On one occasion the error was 0.003.

Some control experiments were also carried out to ascertain the amount of albuminoid ammonia generated from strychnine when decomposed by the permanganate-alkali. The alkaloid yields half its nitrogen content as albu-

minoid ammonia. The results as would be expected were about 10 % high, since if 0.0001 g. were produced from the strychnine this would be increased to about 0.00011 owing to the trace of nitrogenous impurity in the permanganate.

For ordinary work this would be sufficiently accurate, but for very important work it would be advisable to make a control estimation, under the same conditions of dilution and rate of boiling, of the ammonia yielded in one distillation of the permanganate-alkali mixture with ammonia-free water. This figure could then be subtracted from the figure obtained for the water or sewage effluent which was being analysed.

When fresh supplies of chemicals come into the laboratory, tests should be made before using them in routine work to ascertain whether the organic impurity is present in unusually large amount.

The presence of this oxidisable impurity may be one explanation of the observed decomposition of about 5 % of the permanganate in the control distilled water test carried out in the determination of the Tidy figure or oxygen absorbed in 4 hours at 27° C.: *e.g.*

Initial titration 10 cc. *N*/80 permanganate, equals 14.9 cc. standard thiosulphate.

(10 cc. permanganate,
10 cc. 25 % H_2SO_4 ,
100 cc. distilled water.)

Titration after 4 hours' incubation at 27° C. equals 14.2 cc. thiosulphate.

SUMMARY.

1. Potassium permanganate may contain a stable nitrogenous impurity which cannot as a rule be removed by prolonged boiling with alkali.

2. The impurity is not decomposed at all in concentrated alkaline solution, but gradually decomposes when the solution is considerably diluted. Consequently permanganate-alkali mixture can apparently be freed from ammonia by boiling with water, but when the resulting concentrated solution is again diluted and distilled, ammonia may once more be liberated in large quantities. When the mixture apparently freed from ammonia is boiled with the water, the albuminoid ammonia figure of which is being determined, the yield of ammonia is thus greatly augmented by that liberated from the permanganate.

3. The error involved may be so great as to vitiate the value of the albuminoid ammonia test altogether.

4. In the case of a purer brand of permanganate the error is reduced to about 0.002 parts albuminoid ammonia per 100,000.

5. It is essential in routine work to test fresh supplies of chemicals to ensure that the impurity is not present in excessive amount and in very accurate work a control experiment should be made during each determination of albuminoid ammonia.