

It is evident that almost all the adrenaline solutions underwent considerable deterioration on storage and this could not be correlated with factors which are known to facilitate oxidation of adrenaline, e.g., exposure to light, alkalinity of glass containers, pH of the solutions, etc. Thus, in most instances recorded in Table II, the pH of the solutions remained more or less at the optimum level and yet the physiological activity was found to be reduced to nearly 25 per cent. in less than a year's time (Sample No. 6). Only in one instance (Sample No. 9), the pH was on the alkaline side and according to expectation, was found, inactive. The colour and transparency of the solutions, on which considerable emphasis is commonly laid by the official Pharmacopœias and by the medical practitioners in general, were found to be no safe criteria in judging the potency of adrenaline. Sample No. 10, which remained perfectly clear during storage, was found to contain only 55.5 per cent. of its original strength, whereas Sample No. 8, which was slightly brownish and turbid (though no definite precipitate was noted), preserved its activity intact even after three years' storage in the laboratory.

Such findings have naturally focussed attention to the important question whether the factor or factors responsible for the deterioration of adrenaline solutions were not intrinsic in the adrenaline powders themselves from which solutions are being made. Both synthetic and natural adrenaline powders are used by the manufacturers in preparing Liq. Adrenaline Hydrochloride of the Pharmacopœias. Work is in progress in this laboratory in connection with a comprehensive study of the physical (including crystallography and optical rotation), chemical (Folin, Cannon and Denis method<sup>5</sup> and persulphate method<sup>6</sup>), and biological behaviour of as many brands of synthetic and natural gland powders as can be secured in the Calcutta market. Preliminary analysis of our data appears to corroborate, to a certain extent, the observations of Bose and Ganguli.<sup>7</sup>

It seems clear that adrenaline solutions deteriorate on storage. While all the factors responsible are yet imperfectly known, it seems desirable to enjoin on all manufacturers that the *date of manufacture* and the *date of expiry* of potency (Approx. 1 year) be stated on the label of all adrenaline preparations intended for parenteral administrations:

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<sup>1</sup> *Indian Information*, March 1940, 6.

<sup>2</sup> *Triennial Report, B.S. Laboratory*, 1941, No. 1, 13.

<sup>3</sup> Burn, *Biological Standardisation*, Oxford University Publication, 1937, 115.

<sup>4</sup> —, U.S.P XI, 1936, 207.

<sup>5</sup> Folin, Cannon and Denis, *J. Biol. Chem.*, 1913, **13**, 477.

<sup>6</sup> Barker, Eastland and Evers, *Biochem. J.*, 1932, **26**, 2129.

<sup>7</sup> Bose and Ganguli, *Curr. Sci.*, 1942, **11**, 281.

#### FURTHER STUDIES ON THE ROLE OF PROTOZOA IN THE ACTIVATED SLUDGE PROCESS

THE ciliate protozoa as a group were found much more efficient in sludge formation and clarification of sewage than any single strain or combination of bacteria that were tried and, among the Ciliata studied, *Vorticella* gave much the best results.<sup>1</sup> It was further observed that the members of the family *Vorticellidae* and those of the closely allied family *Epistylidae* play a special role in the flocculation of sewage colloids, in the process of nitrification as also in the conservation of nitrogen and other fertilising elements in the sludge.<sup>2,3</sup> More recently, Reynoldson<sup>4</sup> in England has also confirmed the special significance of *Vorticella*.

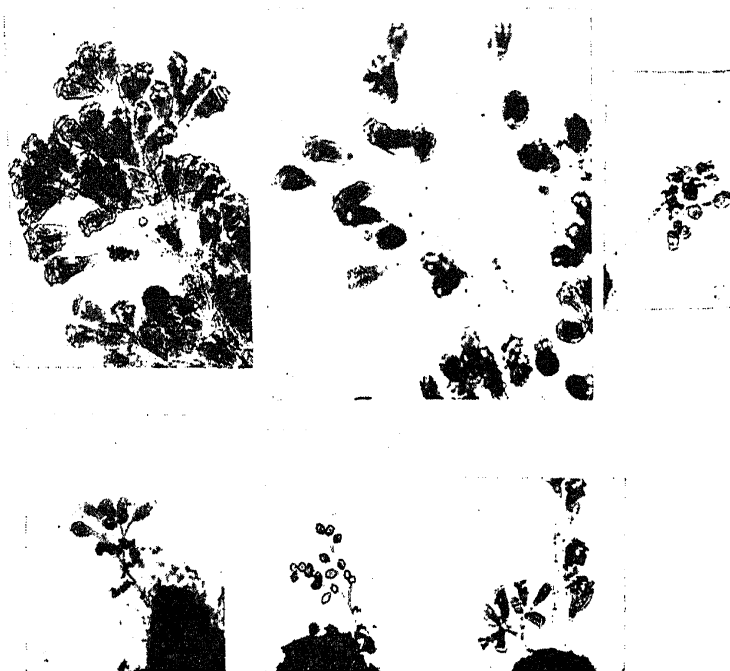
Further investigation into the protozoal activity in the activated sludge tank has revealed the occurrence and development of a number of species of *Epistylis*; not less than half a dozen species of this genus<sup>5</sup> have been found to flourish in the aeration chambers, forming fluffy masses on the sides of the aeration tanks which treat domestic sewage (Figs.). These forms have also been found to occur in small groups or bunches in activated sludge during aeration, at least 16,000 active individuals per ml.

Extensive experiments have been carried out with these forms of protozoa, by inoculating them in different concentrations into experimental bottles containing varying quantities of raw and sterilised sewage (up to 10 litres) and bubbling air through the suspensions. Comparing on numerical basis, it was found that *Epistylis* was at least twice as efficient as *Vorticella* in clotting and sludge formation, and much more efficient in bringing about the attendant oxidation changes as also in conserving the fertilising ingredients in the sludge. The detailed results will be published elsewhere.

Examination of activated sludge from the different installations in India<sup>6</sup> has shown that *Epistylis* sp. and *Vorticella* sp. are invariably associated with them. It is of further interest to observe that in the sludge obtained from the aeration tanks at Tuticorin (S. India) in addition to these organisms, *Zoothamnium* sp. (belonging to the family *Vorticellidae*) was also present. The occurrence of *Zoothamnium* in the sludge from Tuticorin is probably traceable to the sea water used there for flushing purposes. Apart from the special significance of the occurrence of *Zoothamnium* in activated sludge in Tuticorin, it is of considerable scientific interest to observe that it is for the very first time that the existence of this organism in India, Burma or Ceylon is being reported.<sup>7</sup>

The peritrichous ciliate protozoa are therefore most important in the activated sludge process in their relation to sludge formation, to the attendant oxidation changes as also to the concentration and conservation of the fertilising ingredients in the sludge. Among the Peri-

tricha, the members of the families, *Epistylidae* and *Vorticellidae* are of the greatest importance, more especially the species of *Epistylis* which



Photomicrographs of the different species of *Epistylis* isolate from the aeration tanks at the Indian Institute of Science Bangalore  $\times 60$ .

have been found to thrive best in the aeration tanks throughout the year.

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<sup>1</sup> Pillai, S. C., *Cur. Sci.*, 1941, **10**, 84.

<sup>2</sup> —, *Indian Med. Gazette*, 1942, **77**, 118.

<sup>3</sup> —, *Proc. 29th Indian Science Congress*, 1942, Part III, p. 196.

<sup>4</sup> Reynoldson, T. B., *Nature*, 1942, **149**, 608.

<sup>5</sup> Identification of one of the species has been kindly confirmed by the late Dr. B. L. Bhatia of Lahore as *Epistylis articulata* Fromentel (Private communication dated 18th June 1941); the other species are being further identified; Photomicrographs of the organisms were taken with the kind assistance of Dr. B. R. Seshachar, Central College, Bangalore.

<sup>6</sup> Samples of sludge from the different Activated Sludge Plants in the country were obtained through the kind courtesy of Dr. Gilbert J. Fowler, Representative of Messrs. Activated Sludge Ltd., in India and the East.

<sup>7</sup> Bhatia, B. L., "The Fauna of British India, including Ceylon and Burma: Protozoa: Ciliophora," 1936. (Taylor and Francis Ltd., London.)