definitely the most marked in these teeth. It is believed that these preliminary findings point to the use of controlled applications of fluorine-containing compounds as a means of preventing dental caries.

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The Fresh-Water Annelid, Tubifex, as a Pharmacological Test Object.

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A study of the regeneration of the fresh-water annelid, Tubifex, necessitated an evaluation of the factors conducing to its survival in the laboratory. Other workers[1, 2] have observed its satisfactory survival in tap-water. Our own experience has shown that it will not survive in the tap-water of New Orleans. The significant differences between the tap-water of New Orleans and the compatible tap-water of other cities appear to be the higher concentration of hydroxyl ion (pH 9-10) and the higher concentration of available chlorine of the water of this city. The chlorine content of New Orleans tap-water is stabilized at a minimum of 0.7 ppm by the addition of ammonium salts.

To investigate the influence of the reaction of the medium on the survival of Tubifex, solutions buffered with phosphate to cover the range from pH 6 to pH 8.4 were prepared. In the original solutions where the concentration of phosphate ion was 0.04 M there was no survival. The criterion of toxicity was death of all animals at the end of the arbitrarily chosen period of 18 hours. To ascertain at what concentrations of phosphate ion survival would result, a graded series of concentrations of phosphate ion for separate concentrations of hydroxyl ion from pH 4.6 to pH 8.4 were prepared. It was observed that Tubifex does survive in aqueous media if the phosphate ion concentration is low enough and also that the toxicity of phosphate ion is increased if the hydroxyl ion is increased. The toxicity seems to be a conjunctive result of phosphate ion with hydroxyl ion rather than an intrinsic toxicity of hydroxyl ion. This interpretation is strengthened by the survival of Tubifex in aqueous media from

1 Stone, R., *J. Morph.*, 1932, **53**, 389.

2 Stone, R., *J. Morph.*, 1933, **54**, 303.

which phosphate ion was excluded in the range pH 5.0 to 9.5. Boiled tap-water was shown to retain its alkaline reaction even when it was kept in contact with living animals as long as 18 hours, its final pH being 8.5.

The influence of residual active chlorine on the suitability of an aqueous medium for Tubifex was tested by subjecting the animals to varying concentrations of hypochlorous acid at pH 5.5. At concentrations of elementary chlorine (in hypochlorous acid) in excess of 0.6 ppm (0.0000085 molar) no worms survived. It should be pointed out that ordinary distilled water may contain appreciable quantities of active chlorine.[3]

The possibility that the chloramines,[4] produced in tap-water by the addition of ammonium ion to stabilize the concentration of residual chlorine were either more or less toxic than hypochlorous acid was tested by subjecting the animals to concentrations of chloramines at the previously utilized pH 5.5. The lethal concentration of chloramines in conjunction with non-reacted chlorine was found to be identical with that of hypochlorous acid, 0.6 ppm. The toxicity of the component ions of the ammonium sulphate used for producing chloramines was also measured. The requirements of the experimental situations were such that it was never necessary to add more ammonium ion than one-fortieth of the lethal concentration for that ion nor more sulphate ion than one-seven-hundredth of the lethal concentration of sulphate ion. The lethal concentrations of ammonium ion as ammonia nitrogen and sulphate ion are 20 and 390 ppm, respectively. It should be observed that the chloramine present at the experimental pH was largely dichloramine.[4]

The significant implication of the foregoing experiments is the extreme sensitivity of Tubifex to small quantities of residual chlorine and also of phosphate ion. It is conceivable that related forms and possibly even tissue slices are similarly sensitive, perhaps not always to the degree of actual destruction of the test-object but certainly to the degree of a possible significant modification of experimental results. It would appear that it might be routinely desirable to eliminate chlorine from a presumably good sample of distilled water prepared from tap-water known to be high in chlorine or chloramine content.

The phosphate ion concentrations of the stations where these animals have been collected were always lower than those concentrations which have been shown to be compatible with survival of Tubifex even under more average conjunctive conditions of pH.

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[3] Anderson, A. K., and Zipkin, I., *J. Lab. and Clin. Med.*, 1939, **24**, 1209.

[4] Chapin, R. M., *J. A. C. S.*, 1929, **51**, 2112.