appropriate tests, nor as amino acids, nor as protein with proper tests for these substances. With bromine water they give a yellow precipitate, and a blue color with the Folin-Macallum Reagent.

Polyneuritis was induced in pigeons by putting them on a diet of polished rice and water for about two weeks. In birds suffering with the acute symptoms (*i. e.*, retracted neck muscles and paralyzed legs and drooping wings), these acute symptoms were relieved by injecting minute doses of three milligrams of the crystals dissolved in water and dilute alcohol, into the pectoral muscles. Within an hour these pigeons were able to stand erect and showed normal movement of the head. This was repeated with four adult pigeons each weighing on an average about 345 grams. They were placed on the restricted diet and weighed each day and the weights recorded from which, later, curves were made. Two of these pigeons were given doses of the solution of three milligrams of the vitamin crystals at intervals of three days. The curves, while showing a steady decline in weight, show a sharp upward trend after each injection of vitamin into the pectoral muscle due to the renewal of the appetite and consequent eating of more polished rice. The other pigeons kept on declining until the end of the experiment. Charts were kept of each pigeon individually.

Growth experiments were also made with guinea pigs and the crystals, but the development of scurvy complicated conditions unless special diets to prevent scurvy were used. Pigeons on the whole are much more satisfactory subjects in studying Vitamin B or the anti-beri-beri accessory.

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The use of chloretone as an anesthetic for paramecium.

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The described methods for quieting paramecia by using formaldehyde, quince seed jelly, cotton fibers, etc., are by no means satisfactory, if observations are to extend over more than a few

minutes. They are of no use at all for observing a single animal, or a particular group of animals, over a period of several hours or days. Experiments have been made with chloretone* (trichlortertiarybutyl alcohol), and have shown it to be especially valuable for quieting paramecia for long periods of time, up to 8 days, with no undesirable effects.

The paramecia used were extracted from wild cultures, and grown in hay infusion of known constant concentration. New cultures were started from time to time by adding a pipette full of the old to fresh infusions. These cultures contained a few of the smaller rotifers, many of the smaller ciliates, and a preponderance of *Paramecium caudatum*, a mixed population which seemed to be favorable for their growth and reproduction. It was found that a solution containing about 0.06 percent chloretone by weight would anesthetize paramecia in a few minutes, and keep them anesthetized for varying periods of time. The animals would recover if placed in fresh culture fluid previous to cytolysis.

The technique consisted of placing 1 drop of culture fluid, densely populated with paramecia, in a shallow glass chamber on a slide, and then adding 1 drop of 0.12 percent chloretone solution. After measuring the drops of the two fluids from their respective pipettes it was found that such a mixture contained 0.056 percent chloretone. In all subsequent tests the same two pipettes were used. The size of the drops can be adjusted so as to yield just enough fluid to fill the chamber. The latter was then sealed with a cover glass using a mixture of bee's wax and white vaseline, the consistency of which just allowed of its easy spreading at 20° C. (approximately 1 part of wax to 3 parts of vaseline). Gentle heat applied to the cover insured an air-tight seal. Vaseline alone did not give good results, since the animals always recovered within a few hours (from 2 to 24), a fact so far unexplained satisfactorily. It was noted that a few individuals are sometimes killed immediately upon the addition of the chloretone, due probably to the fact that they come in contact with the chloretone before it has been diluted with the culture fluid.

After a maximum of 1 hour the majority of the paramecia come to rest and remain so for 24 hours. During this period

* After the completion of this abstract a casual reference to the use of chloretone for "partially stupefying" paramecium by Jennings was found. Jennings, H. S., *J. Comp. Neurol. and Psychol.*, 1904, xiv, 442.

there is no movement of the animal as a whole, although the cilia in the oral groove, the undulatory membrane, the contractile and food vacuoles continue to show their usual movements. In any large group of the animals there will be several different positions of rest, so that observations on special structures, such as the gullet, contractile vacuoles, "anus," etc., or on protoplasmic streaming, become easy to make. Only rarely does a paramecium become deformed or show cytolysis at the concentration of 0.056 percent before the end of the second day.

The length of the period of anesthesia varied widely in the different chambers. In general a concentration of 0.056 percent was effective for 2 days, many of the animals then resuming locomotion for from 3 to 6 days. After recovery, all of the animals died within 24 hours. In one case the paramecia remained quiet for 8 days, recovering on the ninth day, and dying during the tenth day. Other chambers showed quiet animals up to 4, 5 and 6 days, although some of the paramecia in each chamber were active. The reason for the variation in the recovery process in the different experiments is a subject of further investigation.

In several cases there was noted an increase in the numbers of the smaller ciliates, but in only one case did the number of the paramecia increase slightly. In other words the method is not favorable for the reproduction of paramecium, although it may be for other forms. It is interesting to note that none of the smaller ciliates were ever anesthetized at the concentration of 0.056 percent.

By increasing the concentration to about 0.066 percent and using the ordinary slide and cover, the method becomes applicable to elementary class work, and has given excellent results in this laboratory. The paramecia become quiet within a maximum of 10 minutes and a large number will remain so for several hours without showing any deformity or interruption of the functions mentioned above, provided evaporation is compensated by adding 0.066 percent chloretone solution at the edge of the cover whenever necessary. A few animals will cytolyse and die within the first hour, and the whole population will be dead after a maximum of 24 hours.

Further experiments are in progress on the specific functions of paramecium by the chloretone method, and also on the application of the method to other protozoa.