time. It was difficult to stop the animals from drinking the substance, once they had tasted it. Efforts to remove the bottle were met with fierce resistance. The bottle was held tightly with both paws and even with the teeth. By reaching far up into the bottles the rats made an effort to obtain every remaining drop of the vitamin. Riboflavin (lactoflavin), Dimethyltetrahydroxyamyliso-alloxazin 0.05 per cent. solution, Winthrop Chemical Company, elicited a similar, although less marked, craving. Due to the small available amount of the vitamin preparations, it was not determined how long the craving might remain evident.

It is of general biological interest that such a powerful craving should be associated with a food stuff of the great nutritive importance of vitamin B_1 and that both smell and taste elicit marked responses. The fact that the animals showed an immediate liking for the vitamin indicates that the appetite may not depend entirely on the experience of a beneficial effect resulting from the ingestion of the vitamin. This question of the rôle played by experience and by the deeper biological factors dependent on the taste mechanism we must leave unsettled at present.

The knowledge of the existence of this craving should be helpful for work in animal behavior for use as a reward stimulus. We do not know how general this craving is in different species of animals. In the rat it would seem to be one of the strongest of all the cravings.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A METHOD OF MEASURING THE VOLUME OF AMPHIBIAN EMBRYOS

DURING the course of an investigation on the physiology of development of amphibian embryos, it became necessary to determine the volume of the eggs. Two facts make accurate determinations of volume by existing methods very difficult: (1) it is impossible to obtain accurate wet weights, from which the volume can be calculated after the specific gravity of the eggs has been determined; (2) the early stages burst if brought in contact with the air-water interface. Both these difficulties are avoided by the method described below.

The present method is an indirect, colorimetric determination and is based on the fact that a known concentration of material, when made up to different volumes, will give colorimetric readings that vary as the dilution of the solutions. The determinations are made with the aid of a glass container whose appearance in section and dimensions is shown in Fig. 1. It consists of a bulb with a narrowed mouth above and capillary entering it below. The purpose of the capillary is to permit the removal of the contents without having large air bubbles in contact with the eggs. The total volume of the container is about 4 cc. Before being used, the container is filled with water and a reference mark is made on the capillary at the upper level of the water in it when the bulb is in a vertical position. In the container shown in the figure this was 35 mm from the upper end. Thereafter, when the container is filled with fluid, the level in the capillary is adjusted to the reference mark, and thus the same volume of fluid is used in every experiment.

To make a determination the container is filled with water, the height of the fluid in the capillary adjusted, and the open end of the capillary sealed with a piece

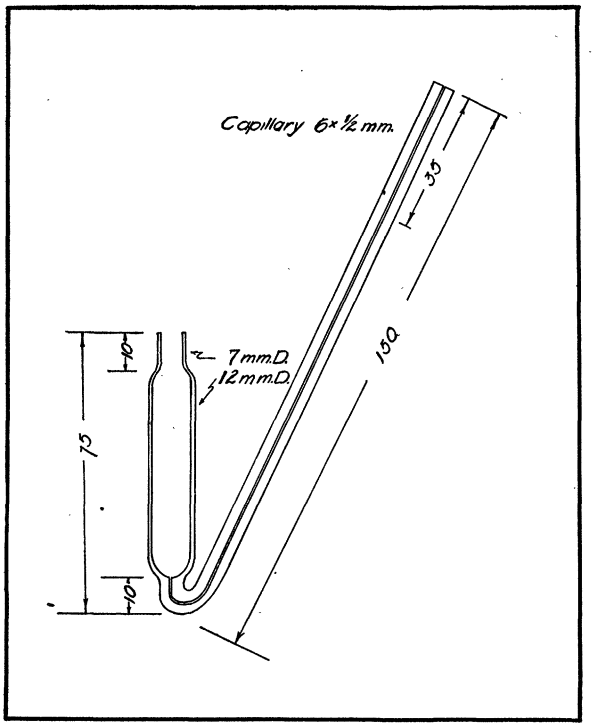

FIG. 1

of Scotch tape. It is important that the seal be airtight, otherwise the embryos may be drawn into the capillary during subsequent manipulation. The container is then immersed in a beaker of water, and the embryos, which have been removed from their membranes, are introduced into the bulb by means of a pipette. In this way they are never in contact with the surface film. Enough embryos are used to make up a volume of about 1 cc. This number varies from 200 *Rana pipiens* and 75 *Amblystoma punctatum* embryos

in the tail bud stage to 75 and 35, respectively, in the feeding stage. The container is then taken out of the beaker, and most of the water removed by pipette, care being taken not to lower the level so much as to bring the animals in contact with the surface film. A convenient amount, usually 2.5 cc, of hemoglobin solution (1 cc of blood diluted to 2 liters with water) is added to the container with the animals and then sufficient water added to bring the fluid level up to the top of the narrowed neck of the bulb. A microburette is used to measure the hemoglobin solution. The Scotch tape is removed and a length of rubber tubing with a mouthpiece is connected to the open end of the capillary. The container is then inverted over a small beaker and the contents expelled by air pressure through the capillary. This prevents bubbling, and with a little practice the embryos can be removed from the bulb without injury, even in open neural plate stages. A standard solution is then made by repeating the procedure without the embryos, the additional volume being of course made up by water. The relative concentration of hemoglobin in aliquot parts of the standard and the unknown solution containing the animals is then determined with a colorimeter by the method of Bing and Baker,[1] using Bing's[2] modified reagent.

The results are calculated by a colorimeter formula as follows:

$$\frac{U}{S} \times V = V_1$$

U = reading of unknown solution
S = reading of standard solution
V = volume of container
V_1 = volume of liquid in unknown solution

and

$$\frac{V - V_1}{\text{Number of eggs used}} = \text{volume of one egg}$$

The advantage of the indirect method is that the actual concentration of reference substance in the standard solution is of no consequence. The greatest source of error is in the reading of the colorimeter. It is possible that a reference substance with a blue color might improve the accuracy. However, tests of this method on known volumes of mercury with hemoglobin as the reference substance showed it to be accurate to within 5 per cent.

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THE CULTIVATION OF VIRUSES ON THE CHORIOALLANTOIC MEMBRANES OF CHICK EMBRYOS

DURING the studies of the cultivation of the viruses of Myxamatosis of rabbits and Vaccinia on the chorioallantoic membranes of chick embryos, modifications of the technique as described by Woodruff and Goodpasture[1] were used. A stand for holding the egg, which was found superior to those made of plasticene, was devised by one of us (Elizabeth Osterman). It was made by soldering the bowl of an ordinary teaspoon on a piece of iron pipe, 1½ inches high and 1½ inches in diameter. This is easily cleaned and sterilized as well as being heavy enough to hold the egg steadily during inoculation.

The eggs were inoculated under a hood free of air currents and kept dust free by a continuously steaming pan of water. It was not necessary, therefore, to place the eggs in warm water during inoculation.

A grinder, known as the Handee Grinder, manufactured by the Chicago Wheel and Manufacturing Company, to which had been fitted a ½ inch Carborundum dental disk was found most efficient for opening the eggs. This has three advantages: first, that the disk can be easily sterilized by immersion in alcohol between operations; second, by use of it the number of eggs which can be opened in one hour is largely increased; and third, the cost ($10.25 for the disk and drill) is much less than a dental drill.

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