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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[1] F. C. Bing and R. W. Baker, *Jour. Biol. Chem.*, 92: 589–600, 1931.
[2] F. C. Bing, *Jour. Biol. Chem.*, 95: 387–388, 1932.

## 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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[1] A. Woodruff and E. W. Goodpasture, *Am. Jour. Path.*, 7: 209–222, 1931.

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