

The lipase in some macrophages suggested the trial of macrophages in tissue culture; Dr. Charity Waymouth kindly gave me some tissue cultures of chick heart and Rous sarcoma No. 1. The macrophages of the former were slightly positive, of the latter negative although they contained fat globules. Probably the resting macrophage contains little lipase, but when actively phagocytic may elaborate a good deal.

Both Gomori's and Wachstein's histochemical methods for demonstrating lipase in tissue cells require further improvement before they can be relied upon to give consistent results and, therefore, to be of value in diagnostic work.

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June 13.

¹ Gomori, G., *Proc. Soc. Exp. Biol. N.Y.*, **58**, 362 (1945).

² Wachstein, J., *J. Exp. Med.*, **84**, 25 (1946).

³ Mark, *Arch. Path.*, **49**, 545 (1950).

⁴ Gomori, G., *Arch. Path.*, **41**, 121 (1946).

⁵ Danielli, J. F., *Nature*, **165**, 762 (1950).

Artificial Feeding of *Culicoides nubeculosus* in the Laboratory

ALTHOUGH the normal hosts of *Culicoides nubeculosus* are horses and cattle, they will readily bite man in the laboratory, and a strain has been maintained in this way; but as the numbers increased it became essential to devise a method by which they might be fed artificially. At 20° C., the temperature at which the strain is kept, the females will take their first blood meal three days after emergence, will oviposit two to three days later and require a further blood meal before they will oviposit again. The duration of the life-cycle is four to five weeks.

The method was based on that used by Bishop and Gilchrist¹ for the feeding of *Aedes aegypti*. Unlike *A. aegypti*, *C. nubeculosus* in a large container will not readily seek its food, nor can it be induced to feed through gauze. The midges were therefore enclosed

in a small tube and could be in direct contact with the membrane. The apparatus is shown in the diagram. A vacuum flask served as a constant temperature bath and was filled with water at 37° C. The membranes were made from pieces of chicken skin which were soaked in absolute alcohol for thirty minutes and washed; these could be dried for storage and became flexible again when soaked in water. Midges were withdrawn from the breeding cages into 4 in. × 1 in. tubes which were inverted over the membrane. It was found that unless the membrane was distended by blood under slight pressure, the proboscis of the insect was so short that air bubbles tended to form immediately below the membrane; the necessary pressure was obtained by filling the tube completely before closing with the rubber bung and laying the whole apparatus on its side during the experiment.

Bishop and Gilchrist have already commented on the relative merits of whole blood, defibrinated blood and plasma, and also of heparinated and citrated blood. In the present experiments, results were obtained only on the comparative merits of the human subject, heparinated ox blood and citrated ox blood. Ox blood was withdrawn from the veins and was sufficiently sterile to be stored at 4° C. for twelve days. To allow for storage, heparin was used at a dilution of 1/1,000.

All the midges used were three to four days old and of the same generation in each experiment. The total number was divided into three, one-third being offered a human subject, one-third heparinated blood and one-third citrated blood.

RELATIVE MERITS OF HUMAN BLOOD, HEPARINATED AND CITRATED OX BLOOD FOR THE FEEDING OF *C. nubeculosus*

No. of females offered food	No. which fed	No. which oviposited	Average No. eggs laid	Length of life	
84	71 (72.6%)	48 (67.6%)	380	4-7 days	Human subject
77	56 (70.1%)	38 (67.8%)	371	4-7 days	Heparinated ox blood
61	20 (32.8%)	10 (50%)	247	3-5 days	Citrated ox blood

From the results shown in the accompanying table, it will be seen that only 32.8 per cent of the midges offered citrated blood fed, whereas 70.1 and 72.6 per cent fed on heparinated blood and the human subject respectively. On several occasions 100 per cent of the females have fed when offered both heparinated and human blood.

Only 50 per cent of the females which fed on citrated blood afterwards oviposited; but 67.8 and 67.6 per cent oviposited of those fed on heparinated and human blood. The average number of eggs laid by the group fed on citrated blood was considerably less than that laid by the other two groups, and the average length of life of the females was also less. Consequently, citrated blood was regarded as being unsuitable, and it seems that heparinated blood is an adequate substitute for the human subject.

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June 21.

¹ Bishop, Ann, and Gilchrist, B., *Parasit.*, **37**, 85 (1946).

