lected urine from the subjects before and after the run. The nitroprusside test for pyruvic acid<sup>1</sup> was negative for the urine passed before exercise, but was positive for that passed after exercise. We collected urine also from eight boys after they had run the  $\frac{1}{2}$  mile or the  $\frac{1}{2}$  mile race in a track meet. In seven cases the test was positive.

Pyruvic acid 2:4-dinitrophenylhydrazone was prepared from the boys' urine by the method used for pigeons' blood by Johnson.<sup>2</sup> The melting point was 214° (uncorr.) and the mixed melting point with synthetic pyruvic acid 2:4-dinitrophenylhydrazone was 213° (uncorr.). Therefore the substance giving the positive nitroprusside test in the fresh urine was probably pyruvic acid.

In one experiment a subject ran on a treadmill to complete exhaustion in 1.70 minutes at 11.2 m.p.h. Urine was collected before and 50 minutes after the run and blood was drawn from the antecubital vein before and 5 minutes after the run. Analyses for pyruvic acid were made by Peters and Thompson's<sup>3</sup> modification of the Neuberg-Case method. The results are shown in Table I.

TABLE IPYRUVIC ACID (MG. PER 100 CC)Before runAfter runBlood<1</td>3.373.37Urine05.80

Methylglyoxal also can be detected by the use of 2:4-dinitrophenylhydrazine, but we have not seen methylglyoxal 2:4-dinitrophenylosazone in any of our experiments. Therefore, pyruvic acid but not methylglyoxal seems to be one of the variable constituents of blood and urine during hard exercise. These results are interesting because of the contention by the schools of Embden and Meyerhof that pyruvic acid is of considerably greater significance than methylglyoxal in muscular metabolism.

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

#### A PRACTICAL METHOD FOR INDUCING OVIPOSITION IN DIURNAL LEPIDOPTERA

It has long been the general impression that living butterfly eggs were difficult to obtain. During the summer of 1932, while attempting to obtain numbers of larvae for experimental purposes, the writer succeeded in finding a satisfactory method for inducing oviposition in all the species tried.

Gravid females ready to deposit eggs were obtained in the field. These could be detected by their tendency to hover about the food plant, or by their leisurely flight and inclination to pause near leaves rather than on flowers. The wings of these insects were clipped to within a fraction of an inch of the body in order to prevent fluttering, which usually resulted in rapid exhaustion. A small amount of food plant was next placed in a curved-neck bottle filled with water, and the tips of the stems were cut off under water. The leaves of the plant were placed so that they were in contact with the bottom of an ordinary Mason jar lying on its side (Fig. 1). The jar was then oriented so that the bottom or closed end was nearest to the source of light, which was a large window. After the butterfly was released in the jar, the open end was closed with netting.

<sup>1</sup>L. J. Simon and L. Piaux, Bull. Soc. Chim. Biol., 6: 477, 1924.

<sup>8</sup> R. A. Peters and R. H. S. Thompson, *Biochem. Jour.*, 28: 916, 1934.

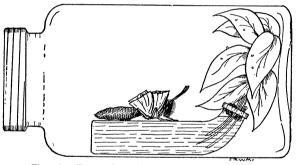


FIG. 1. Butterfly ovipositing jar. Light enters from the right.

In such close confinement with the food plant the butterfly invariably would become active, walking first in the direction of the light, up over the food plant and back again. Many individuals would deposit an egg on the plant at each trip until all the eggs were laid. Once a day the insects were removed and fed on sugar water, the fore tarsi being immersed in the solution to insure prompt feeding.

The following species were tried and all oviposited readily: Papilio ajax L. (=asterias Cram.); Pieris protodice Bdv. and Lec., Pieris rapae L.; Colias eurytheme Bdv.; Vanessa atalanta L.; Vanessa cardui L.; Basilarchia arthemis (Drury), and Basilarchia archippus (Cramer). When confined without food plant, a specimen of Harris' Checker-spot, Melitaea harrisi (Scudder), laid a single mass of more than two

<sup>&</sup>lt;sup>2</sup> R. E. Johnson, Biochem. Jour., 30: 31, 1926.

hundred eggs on a bit of cheese-cloth. In the case of *Papilio ajax*, a breeding cage was employed and a sprig of wild parsnip was placed in its best lighted corner. The result was excellent.

In order to rear larvae, the bits of leaves with attached eggs were placed on fresh food plant in tin cans covered with pieces of glass. The plant in this case was also kept in a curved-necked bottle. This type of tight container retained the moisture, which is absolutely essential for small larvae. However, they were soon transferred to large breeding cages.

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## PREPARATION OF NON-TOXIC URINE FRACTIONS FOR ASSAY OF MALE HORMONE BY THE FEMALE BITTERLING TEST<sup>1</sup>

In testing urines for hormones by means of the female bitterling (*Rhodeus amarus*)<sup>2,3,4</sup> it was found that certain urines were toxic to the fish. Sometimes the addition of as little as 45 cc of untreated urine to 4 liters of water proved fatal to the fish within 24 hours. Since this test will probably be widely used as a means of assay for male hormone, it was deemed important to attempt to remove the toxic factor or factors.

After many experiments the simple expedient of dialysis proved to be all that was necessary. It had previously been found that the ovipositor-lengthening factor was not lost in dialysis.

Two hundred cc portions of fresh urine with specific gravities ranging from 1.018 to 1.036 were placed in Cellophane bags and dialyzed against running tap water for 24 hours. The specific gravities then were 1.001 to 1.003. The urines were then boiled to destroy bacteria. Amounts equivalent to 150 cc of the original urine to four liters of water containing two bitterlings were not in the least toxic to the fish. Since employing routine dialysis we have had no deaths of the fish due to the toxicity of urines.

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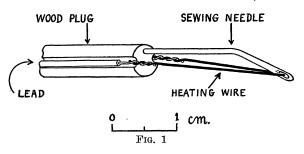
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<sup>1</sup> Aided by a grant from the Lucius N. Littauer Foundation.

- <sup>2</sup> Kleiner, Weisman and Barowsky, Jour. Amer. Med. Assoc., 104: 1318, 1935.
- <sup>3</sup> Kleiner, Weisman and Mishkind, Jour. Amer. Med. Assoc., 106: 1643, 1936.
- <sup>4</sup> Kleiner, Weisman and Mishkind, Proc. Soc. Exper. Biol. and Med., 34: 367, 1936.

## AN ELECTRICALLY HEATED NEEDLE FOR PARAFFIN EMBEDDING

THE use of needles periodically heated in a gas flame for orientating small material during the process of embedding in paraffin has several disadvantages. The constant reheating involves loss of time, and there is danger of damaging fine material, such as root tips, with needles that are too hot. The device described here has been in almost daily use in this laboratory for the past four months and can be easily made at a trifling cost.



The drawing shows the method of construction. It will be seen that a loop of heating wire is passed through the eye of a bent sewing needle, the needle being fixed into a small wooden plug. This plug, which has grooves cut in it to receive the leads, can either be long enough to serve as a handle or else it can be fitted into the end of a thin bamboo cane. In the former case it can be bound round with insulating tape to hold the leads; these are best made of light electric bell flex. The heating wire, 4 cm long, is of 40 S. W. G. nickel chromium, having a resistance of about 60 ohms per meter. It is best, but not absolutely necessary, to silversolder the wire to the leads. The current is supplied from the mains through a small transformer giving 3.3 volts, so that about one ampere flows through the heating wire. It is of course easy to adjust the length and gauge of the resistance wire to suit any small transformer that is available.

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