A Simple Fluorometer of the Duboscq Type

VARIOUS fluorometric methods are employed in determining riboflavin and thiamin. The fluorescent substance is freed, and in the final stage is compared with solutions of known concentration. Weisberg and Levin[1] recommended the use of a block-comparator, but later authors[2,3,4] employ a photo-cell connected with a galvanometer for testing the intensity of fluorescence. These photocell arrangements, however, are very expensive.

The method described below is simple and relatively inexpensive. The light-source, a U-shaped Hanovian quartz burner, is projected on to the cups of a Duboscq colorimeter by means of a flask so that each branch of the U-shaped burner illuminates one cup. The fields of the colorimeter are then matched in the usual way and the intensities compared. The advantages of this method are as follows. (1) Both cups are illuminated by the same light-source; therefore changes in the output of light cannot influence the readings. (2) In passing through the liquid the exciting light is partially absorbed; for this reason sample-holders are kept as short as possible in fluorometric work. On the other hand, only low concentrations of fluorescent matter yield a linear response. (3) When illuminating the cups through the window at the bottom, the whole length of the cup cannot be evenly illuminated. In my arrangement, however, the exciting light enters from the front. Hence (*a*) the activating light passes through only the width of the cup (the path thus being rather short); (*b*) the whole length of the cup is evenly illuminated; (*c*) since the cups are viewed through the colorimeter from above, the bright halves of the fields can be easily matched.

The light-source is a 220-volt Hanovian U-shaped burner. The light passes through a Wood's filter to a 500 ml. round-bottom flask filled with distilled water or a saturated solution of copper sulphate (to exclude the red portion of the spectrum transmitted by Wood's filter). If the colorimeter is not fitted with blackened rods, both rods are covered with black varnish and only the bases are left free. The supports of the cups of the colorimeter are copied from the original supports. A ring is soldered on to the support. Then two test-tubes with flat bottoms are fitted into the ring and sealed to it. The size of these tubes depends on the length and diameter of the rods, in our case 60 mm. and 14 mm. respectively. To avoid reflexions, a black ring of about 10 mm. height is painted on the outside of the cups. To protect the eyes an ultra-violet absorbing filter (or an ordinary photographic plate, fixed and bathed in picric acid) is inserted into the eyepiece or between rods and eyepiece.

The estimation of fluorescein is given as an example. (1) Solutions of sodium fluorescein, 0·2, 0·4, 0·6, 0·8, 1·0 and 1·2 gamma/ml., are prepared. (2) Both cups are filled with 0·2 gamma/ml. sodium fluorescein; and the rods are brought to the same position, say, 50 mm. (3) Now a piece of white paper is put up immediately in front of the cups. Then lamp, colorimeter and flask are so adjusted as to project on each cup one branch of the 'U'-lamp. To get an illumination of the whole width of the test tubes, these should be slightly out of focus. Then the paper is removed and the whole arrangement shielded against daylight by a black cloth. By small movements of the stand (but not by moving the rods) a position can be found where both halves of the field are evenly illuminated. Without moving the stand, both rods are now brought to, say, 30 mm.; then again both halves of the field should be equal. Between 0·2 and 1·0 gamma/ml. the relation between scale readings and concentration is a linear one. (4) When determining the concentration of an unknown solution of fluorescein, the unknown is diluted so as to give a fluorescence slightly stronger to the naked eye than the 0·2 gamma/ml. solution. One cup is filled with 0·2 gamma/ml. fluorescein, the other one with the diluted unknown. The rod dipping in the 0·2 gamma/ml. solution is brought to, say, 50 mm. By moving the rod, both fields are matched and the concentration of the unknown read off from a reference diagram.

WALTER KOCH.

Department of Hygiene,
Hebrew University,
Jerusalem.
May 11.

[1] Weisberg, S. M., and Levin, J., *Ind. and Eng. Chem.*, Anal. Ed., **9** (1937).
[2] Schumacher, A. E., and Heuser, G. F., *Ind. and Eng. Chem.*, Anal. Ed., **12** (1940).
[3] Conner, R. T., and Straub, G. J., *Ind. and Eng. Chem.*, Anal. Ed., **13** (1941).
[4] Najjar, V. A., *J. Biol. Chem.*, **141** (1941).

Natural Selection in the Six-spot Burnet Moth

THE larva of the common six-spot burnet moth (*Zygaena filipendulæ* L.) forms an elongated cocoon on grass or other stems often at a height of six or more inches above the ground. Sometimes, however, the cocoon is spun on low vegetation or a twig in a hedge.

During last summer, when collecting large numbers of cocoons to breed the parasites, I noted that cocoons affixed to a coarse-meshed wire netting fence bordering a road had often been opened and the contents extracted. This was also the case with cocoons on a hawthorn hedge, but was not so with cocoons on tall upstanding grass stems in the open. The tentative conclusion that the enemy was a bird was confirmed when I saw a great-tit perch on the wire fence, open the end of a cocoon, pull out the larva inside, and thrust it into the mouth of a clamorous young one close by. The damage to this cocoon was similar to that previously noted. Thus it seems that a cocoon is liable to attack by a bird *which can reach it.*

This summer I have taken notes on the fate of all the cocoons on the wire fence for a length of about eighty yards. Each one, as soon as the imago emerged, or after it had been opened, was pulled off the fence and recorded. The results, during May 24–July 10, were as follows: opened and larva or pupa extracted, 22; opened, larva or pupa pecked and damaged but not extracted, 2; moth emerged, 8; moth formed but failed to emerge and died, 2; larva or pupa destroyed by Hymenopterous parasites, 3. Thus out of 37 on the fence, no less than 24 were destroyed by birds—a percentage of 64·8. I had hoped that it would be possible this year also to record the fate of cocoons out of reach of birds, but pressure of work has prevented this, and I have only the unrecorded experiences of last year.

Here is a promising and convenient subject for investigation by school scientific societies, for the