at 25·0° C. were fully grown. There followed a period of diapause when little or no development occurred. Next came a second active period, during which the small larvæ moulted and reached the fully grown stage, while the larvæ already fully grown pupated, forming the first-year group of pupæ. This alignment of alternating active and diapause periods, in individuals developing at different temperatures, and therefore at different stages in their development, indicates an internal rhythm of development common to all.

The length of the cycle in this rhythmic development under constant conditions is about forty-one weeks, compared with the annual rhythm of development under outdoor fluctuating conditions. It is considered that the shorter interval is the basic one, and that, when diapause is completed, activity (involving both moulting and pupation) will proceed as soon as the temperature is above the threshold for development. This also furnishes an explanation for the lack of variation in the time of pupation under outdoor conditions (29 individuals varied by less than 4 days at the end of two years development) compared with that under constant conditions (Fig. 1).

Some of the laboratory experiments commenced in August, some in November and some in December. Relative humidity was controlled, and any diurnal or seasonal changes in carbon dioxide concentration were masked. The experiments were carried out in complete darkness except for short exposures of the insects to daylight or to tungsten illumination when they were being examined. Some experiments were started with the progeny of adults from laboratory cultures, others with the progeny of adults from flowers, but these differences did not materially influence the results. All eggs were incubated at 20·0° C., 70 per cent relative humidity, and the experiments set up with newly emerged larvæ.

At 20° C. simple crossings were made between individuals taking two years to develop. The F_1 progeny fell into two groups (one-year and two-year) in approximately the same proportion as in the original population.

A more detailed account is in the press[1].

G. M. Blake
Pest Infestation Laboratory
(Department of Scientific and Industrial Research),
London Road, Slough.
Nov. 19.

[1] Blake, G. M., *Bull. Ent. Res.* (in the press).

A New Method for delaying Pupation in *Drosophila melanogaster*

Methods of delaying pupation in *D. melanogaster* are not only of interest in relation to the physiology of metamorphosis but also provide useful tools for developmental-genetical studies. Up to the present, the main method available has been that of Beadle, Tatum and Clancy[1]. They kept larvæ, aged less than 70 hr., on a low food-level provided by 1·5 per cent agar and 0·25 per cent brewer's yeast. According to Bodenstein[2] most of the larvæ starved in this way fail to survive, particularly, of course, those starved from early ages; moreover, the period for which pupation is delayed is highly variable, since in some cases larvæ pupate only one day late whereas in others the delay might be as much as five days.

A method has been found to delay pupation from six to seven days. The experiments were made with wild-type *Or.K.* stock. The eggs were laid over a 2-hr. period on agar medium and left for 20 hr., after which all the larvæ hatched during this period were removed. The remaining eggs were then raised on agar-molasses medium composed of 2 per cent agar, 17 per cent molasses and 1·5 per cent dry yeast. After 70 hr. from egg deposition (48 hr. after hatching), the larvæ were transferred to other vials containing the same food medium mixed with benzamide at concentrations of 2·0–6·0 per cent. In each vial, 12–15 larvæ were raised. Throughout the whole period of experiments, the temperature was kept at 25° C.

Nine days from egg-hatching, that is, five days after the control larvæ had pupated and one day after the flies had emerged from the pupæ, the test vials were examined. As shown in Table 1, at 2·0 per cent benzamide, about 88·8 per cent of the test larvæ have retained their larval characteristics, the remainder having become prepupal. The mortality-rate was insignificant.

Table 1. Effect of Benzamide on 48-hr.-old Larvæ of *D. melanogaster* (observed Nine Days after Hatching)

Conc. (per cent)	No. of test larvæ	Larvæ retarded		Pre-pupæ		Mortality-rate	
		No.	Per cent	No.	Per cent	No.	Per cent
2·0	54	48	88·8	5	9·2	1	2·0
4·0	69	66	95·6	—	—	3	4·4
6·0	69	67	97·1	—	—	2	2·9

At 4·0 and 6·0 per cent benzamide no pupation occurred, and nearly all the test larvæ remained in the food culture as mature third instar. These arrested larvæ were well able to withstand the experimental conditions, mortality-rate being small. When they were transferred to ordinary food, the percentage of recovery was very high. Pupation usually occurred 1–2 days after the transference. In other words, the whole period of delay was 6–7 days, 5 days with the treated food and 1–2 days with the ordinary one.

Table 2. Fate of Retarded Larvæ after Transference to Ordinary Food

Conc. of benzamide (per cent)	No. of transferred larvæ	Recovered larvæ	
		No.	Per cent
2·0	24	23	95·8
4·0	22	21	95·4
6·0	20	18	90·0

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A. Abd-el-Wahab*
Institute of Animal Genetics,
Edinburgh.
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* Present address: Zoology Department, University of Cairo.

[1] Beadle, C., Tatum, E., and Clancy, C., *Biol. Bull.*, **75**, 447 (1938).
[2] Bodenstein, D., *J. Exp. Zool.*, **82**, 329 (1939).

Collagen in the Perilemma of Insect Nerve

Insect nerves are enclosed in a sheath, the perilemma, made up of concentric layers of anisotropic protein micelles and resembling the connective tissue of vertebrates[1]. There is evidence that each lamina consists of rows of glycoprotein fibrillæ, oriented at right angles in successive layers, the whole embedded in a matrix of neutral mucopolysaccharide[2].