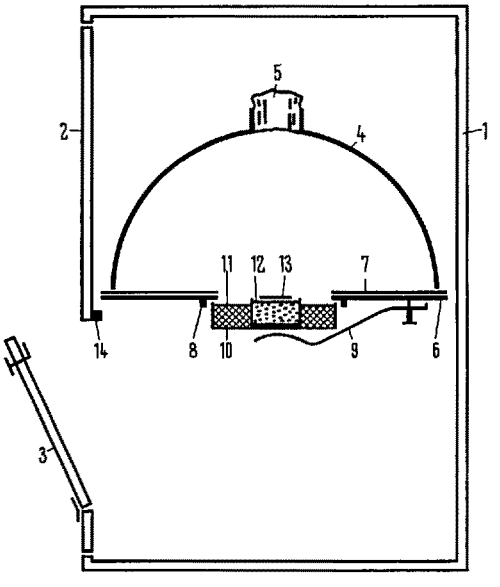
An Improved Method for the Collection of Large Numbers of Inseminated Eggs of *Drosophila melanogaster*

Drosophila eggs and embryos are convenient objects for radiobiological studies[1–4]. However, the extreme speed of development of the embryos brings up some experimental problems. RABINOWITZ[5] and SCHNEIDER-MINDER[6] have shown that at a temperature of 24–25 °C 1 mitotic cycle during cleavage lasts only about 8–9 min. It is evident that, using standard egg collection periods of 10, 30 or more min[4,7], the egg samples obtained from a population of actively laying females are not homogeneous with respect to developmental stages. Moreover it could be shown that different stages in the mitotic cycle during early cleavage exhibit different radiosensitivity[8]. Therefore in experiments on the mutagenic action of X-rays[9] or on chemical radioprotection[10], serious complications in the interpretation of the data can result from the inhomogeneity of the samples treated with respect to developmental stages. A convenient way to decrease these difficulties would be to shorten the egg collection period. The method to be described allows for the collection of ample numbers of eggs, deposited immediately after insemination[6], in periods as short as 3 min.

Stock cultures of *D. melanogaster* (Berlin wild) are reared at 25 °C in 200 ml bottles containing 40 ml standard medium (1000 ml water, 10 g agar, 75 g sugar, 150 g corn meal) and 2–3 drops of an aqueous suspension of living baker's yeast. New cultures are started with about 25 females and 25 males per bottle. After 24 h the flies are removed and about 5 g living yeast and some absorbant paper (200 cm^2) are added to each bottle. Every day 24 cultures are started. Adults from the 9- and 10-days-old cultures are collected daily, anaesthetized by CO_2 and pooled. With these flies about 6 'feeding bottles', each containing about 400 flies on well-yeasted medium, are set up. On each subsequent day the animals are transferred to fresh 'feeding bottles'. By the fourth day the flies are well fed and the females are actively laying eggs. The flies can now be used for new cultures or for experimental purposes.

If newly inseminated eggs are to be collected, well-fed flies are transferred to a special apparatus which is set up in a climatized room with humidity and temperature controlled to give a dew-point temperature of 24.4 ± 0.3 °C (ca. 96% relative humidity; 25 ± 0.3 °C). The apparatus (Figure), which is a modification of the one described by ULRICH[11], consists of 2 parts: a light-proof wooden box (35 × 30 × 45 cm), the front part (2) of which can be opened as a whole or a lid in the lower half (3) can be folded down, and the arrangement containing the flies, which can be fitted into the wooden box with the aid of 2 ledges fixed in the middle of the side walls. The latter part consists of a hemispheric bell-glass (4) with a diameter of 25 cm at its base and a hole at its upper end which is closed by a foam rubber stopper (5). The bell-glass stands on an aluminium plate (6) with a central hole of 9 cm in diameter. The plate is covered with a thick layer of blotting paper (7). A guiding ring (8) below the central hole and a spring (9) allow one to fix an egg-collection dish (10) to the plate. This dish consists of 2 petri-dishes (9.5 cm and 6 cm in diameter respectively). The smaller dish stands in the centre of the larger one and the space between the 2 dishes is filled with plaster (11). The inner dish is filled with the egg-laying medium (12). This medium is prepared by boiling a mixture of 500 ml water, 5 ml acetic acid, 100 ml malt extract, 85 g sugar and 135 g corn meal. Upon cooling of this medium 20 ml of a concentrated suspension of living yeast in water is added. After standing at room temperature for 1 day the fermenting medium is ready to be used. A piece of black paper with a relatively rough surface (5.5 × 2.5 cm) is soaked in 1% acetic acid and laid on the smoothed surface of the medium in the small dish (13). The piece of paper is the only wet place in the whole apparatus. As the females lay eggs only on a moist surface, eggs will be deposited exclusively on this strip of paper.

The egg collection procedure is as follows: non-anaesthetized flies (about 3000 females and 3000 males) are put into the apparatus through the opening at the top of the bell-glass. As soon as the females start to lay eggs in considerable numbers the egg-collection dish is changed 3–6 times at 10 min intervals. When the experiment starts, a first experimental dish is left in the apparatus for 3 min, the second for 7 min, the next again for 3 min, and so on. Only the eggs (50–100) which have been deposited during the 3 min periods are used for experiments, the eggs of the 7 min collections are discarded. If the piece of black paper is wet enough, all the eggs are laid on the paper and can be easily collected with a fine brush. The reason for the alternative 3 min/7 min change of the dishes is the following: (1) even the most careful change of the egg-collection dish disturbs some females which are laying eggs. These



The egg collection apparatus: 1, wooden box; 2, front part; 3, lid; 4, bell-glass; 5, foam rubber stopper; 6, aluminium plate; 7, blotting paper; 8, guiding ring; 9, spring; 10, egg-collection dish; 11, plaster; 12, egg-laying medium; 13, black paper; 14, switch.

[1] C. PACKARD, Radiology *25*, 223 (1935).
[2] H. LANGENDORFF und K. SOMMERMEYER, Strahlentherapie *82*, 316 (1950).
[3] H. FRITZ-NIGGLI, Naturwissenschaften *39*, 485 (1952).
[4] H. ULRICH, Verh. dt. zool. Ges., Hamburg 1956, 150 (1957).
[5] M. RABINOWITZ, J. Morph. *69*, 1 (1941).
[6] A. SCHNEIDER-MINDER, Arch. Julius Klaus-Stift. VererbForsch. *37*, 38 (1962).
[7] H. FRITZ-NIGGLI, Fortschr. Röntgenstr. *83*, 178 (1955).
[8] F. E. WÜRGLER, Int. Atomic Energy Agency, *STI/PUB/173*, 43 (1968).
[9] U. PETERMANN, Mut. Res. *5*, 397 (1968).
[10] B. MATTER, Radiol. clin. Biol. *36*, 299 (1968).
[11] H. ULRICH, Drosoph. Inf. Serv. *27*, 124 (1953).

females may keep the last inseminated egg in the uterus. Such an egg begins the embryonic development within the mother and is generally laid as an 'overaged' egg during the 7 min period. (2) Our experience has shown that females are too much disturbed by changing the dishes every 3 min and egg-laying activity decreases rapidly after about 1 h. With the alternative schedule egg collection can be extended for 6 h or more. If for a particular experiment the extreme short periods are not needed, regular changes every 5 min (or longer) are possible. Some tests showed that the collection period could not be reduced to less than 3 min without a drastic reduction in the number of eggs deposited.

To keep the disturbance of the flies to a minimum, the change of the dishes is done as follows: the egg-laying apparatus stands on a solid, vibration-free table. The lid of the wooden box is carefully lowered. An automatic switch (14) turns off the room light at the same time in order to prevent attraction of the flies by light. Now, in the dark, the spring is removed and the dish is gently rotated back and forth during about 10 sec. This causes the flies (which do not fly in the dark) to walk off the surface of the dish. Thereby they cross the ring of plaster which absorbs any fluid on the animals' legs. Thus neither the blotting paper nor the bell-glass ever get wet. A new dish is now fixed to the plate. Upon closing the lid of the box, the light in the room is turned on automatically.

The special temperature and humidity conditions are based on preliminary measurements of the dew-point temperature on the surface of a medium on which females have been laying eggs. Using these conditions in the whole experimental room has 2 advantages: (1) a dish brought into the apparatus has already the optimal temperature and (2) eggs collected from the apparatus remain under constant conditions[12].

Zusammenfassung. Es wird eine verbesserte Methode beschrieben, mit der innerhalb einer Sammelperiode von 3 min etwa 50–100 frisch besamte Eier von *Drosophila melanogaster* gewonnen werden können. Verglichen mit den bisher üblichen Sammelperioden von 10, 30 oder mehr min erhält man wesentlich stadienhomogenere Gelege. Eine weitere Verkürzung der Sammelperiode unter 3 min ist wegen der stark abnehmenden Anzahl Eier je Gelege nicht möglich. Vorausgesetzt, dass alle Störungen der Fliegen durch Erschütterungen, Licht, Temperaturschwankungen usw. ausgeschaltet werden, können z.B. für strahlenbiologische Experimente zahlreiche 3-min-Gelege im Laufe von 6 oder mehr Stunden gewonnen werden.

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STUDIORUM PROGRESSUS

Factors Influencing Rates of Tail Regeneration in the Lizard *Anolis carolinensis*

In an investigation of the somatotropic effects of certain hormones in the lizard *Anolis carolinensis*, tail regeneration was studied as one of a number of physiological variables related to growth[1]. In spite of a rigidly controlled experimental regime and use of only males of restricted age and size, considerable individual variation in tail regeneration was found. Although such variations have been reported[2,3] they have not been studied in detail. There are conflicting reports regarding certain possible regulatory factors in lacertilian tail regeneration, especially the role of the vertebral autotomy plane, and there have been speculations on largely uninvestigated factors such as epidermal involvement. We attempted to elucidate the basis for individual variation in the regenerative response in *A. carolinensis* by examining these and other factors, especially temperature.

Materials and methods. In early September, 60 adult male *A. carolinensis* (average snout-vent length 64.5 mm, body weight 5.0 g) were put at 32 ± 0.5 °C with 6 h light daily[4]. Some animals were injected with gonadotropins, or gonadotropins plus prolactin, but there were no significant differences in tail regeneration and the data were pooled for this analysis. Procedures for hand-feeding, assessing growth and autopsy are reported elsewhere[1].

The original tail (average length 124 mm, range 111–138) was amputated with a razor blade 18–21 mm behind the vent: amputated portions averaged 360 mg. None of the animals appeared to have had previously regenerated tails except at the very tip. The length of the regenerating tissue was measured weekly and after 6 weeks the newly regenerated portion was removed and weighed.

The epidermal condition at amputation and the position of amputation relative to the natural autotomy plane (Figure 1) was determined from histological preparations of the proximal 1.5 cm of the amputated portion: methods are described elsewhere[5].

In order to facilitate comparison between our results and those of previous workers who have used temperatures around 18–22 °C, a second experiment at 21 °C was conducted with 18 males in April. Ad libitum feeding maintained or increased the animals' weights. Severals were transferred to higher temperatures as described below.

Results. Tail regeneration at 32 °C (Figures 2 and 3). No detectable elongation occurred until 7–10 days after amputation, and then there was a period of rapid growth averaging 1.5 mm/day from the 14th to the 28th day. The average growth rate for the 10th to 42nd day after amputation was 0.98 mm/day. The mean length of the regenerated tail at the end of 6 weeks averaged 28.5 mm, representing 28% replacement of amputated tissue. Prominent

[1] P. Licht, Gen. comp. Endocr. *9*, 49 (1967).
[2] S. V. Bryant and A. d'A. Bellairs, J. Linn. Soc. (Zool.) *46*, 279 (1967).
[3] Y. L. Werner, Acta Zool. *48*, 103 (1967).
[4] P. Licht, Am. Midl. Nat. *79*, 149 (1968).
[5] P. F. A. Maderson and P. Licht, J. Morph. *123*, 157 (1967).