The figure shows embryonic development depending upon different laying conditions. Graphs A and B show the development of samples collected after 1 h of laying. Graphs C and D show samples after 1 h and 30 min laying, respectively, but with previous CO_2 treatment. Sample A was obtained after 24 h without rechanging the food and sample B after having discarded a former laying of 1 h. We can observe in graph A an overlapping between peaks of different stages. At 90 min after the start of the laying, when all the embryos should be in the syncytial stage, there are embryos at cellular blastoderm or gastrula stages, due to the retention effect. This effect is very variable; in some cases we observed up to 20% retention, mainly embryos older than gastrula. Sample B shows a lower retention effect indicating that the food conditions are an important factor.

The development graphs after CO_2 treatment (C and D) show sharper peaks which become free of embryos from later stages. The number of retained eggs in scores of 1000 embryos varies between 0 and 3 (the presence of these eggs may be fortuitous in part, because we have observed sometimes, just before the start of the laying period, flies bearing an egg adhering to the body). The treatment increases the amount of non-fertilized eggs about 5% over the control, thus the plateau of syncytium mortality seems to be higher. The different slopes and shifts of maxima are due to laying time, achieving good synchronization with 30-min laying. However, the number of eggs collected is less than half of the amount collected in a 60-min laying.

The reproducibility allows the harvesting of embryos of a defined stage merely by waiting long enough to reach the maximum of a stage after having removed the tray from the laying cage. The time for each stage in minutes after zero time for a 1-h laying is syncytium 90, syncytial blastoderm 145, cellular blastoderm 195, gastrula 255. For 30 min laying the times are syncytium 80, syncytial blastoderm 135, cellular blastoderm 180, gastrula 245.

The degree of synchronization achieved by this method is displayed in table 1, which shows the stage composition when a stage reaches the maximum. In the boxes there are the embryo percentages at their expected respective stages. It can be seen that the CO_2 treatment (C and D) eliminates the retention effect, therefore the fraction of more developed embryos becomes null. Non-fertilized eggs cause an increase of the fraction of syncytial embryos. The advantage of this method is clearly seen if we consider, for instance, the syncytial blastoderm (145 min) from B. Considering the mean number of nuclei per embryo of 120 for syncytium, 1440 for syncytial blastoderm, 6000 for cellular blastoderm and 12,000 for gastrula[3], the 145 min stage with 83.5% of syncytial blastoderm embryos, has in fact 38.2% of nuclei or cells from later stages.

Harvesting a large amount of eggs depends upon the number of flies per laying cage (more than 5000 flies is not useful) and the number of laying cages used. With this simple method of CO_2 treatment it is possible to use several laying cages simultaneously. In order to optimize the treatment, we have analyzed 4 consecutive layings after 1 anesthesia. The results are shown in table 2, which also shows the amount in g of eggs collected from each laying period. As should be expected, in the 2nd batch the retention effect begins to appear. The amount of eggs collected almost doubles in the 2nd batch but diminishes in subsequent batches. An alternative way is to repeat the CO_2 anesthesia before each laying period. Table 2 shows that the retention effect disappears absolutely in this way but the number of eggs decreases drastically. Therefore, to obtain a high yield, eggs may be collected twice after anesthesia but at the expense of homogeneity.

In conclusion, CO_2 anesthesia provides a simple method for harvesting large amounts of *D. melanogaster* embryos in the same developmental stage.

1 Sonnenblick, B.P., in: Biology of Drosophila, p.62. Ed. M. Demerec. Hafner Publishing Co., New York London 1950.
2 Elgin, S.C.R., and Miller, D.W., in: The Genetics and Biology of Drosophila, vol.2A, p.112. Eds M. Ashburner and T.R.F. Wright. Academic Press, London 1978.
3 Zalokar, M., and Erk, I., J. Microsc. Biol. cell. *25* (1976) 97.
4 Elgin, S.C.R., and Hood, L.E., Biochemistry *12* (1973) 4984.
5 Limbourg, B., and Zalokar, M., Dev. Biol. *35* (1973) 382.
6 Würgler, F.E., and Ulrich, H., in: The Genetics and Biology of Drosophila, vol.1C, p.1269. Eds M. Ashburner and E. Novitsky, Academic Press, London 1976.
7 Grossfield, J., and Sakri, B., J. Insect Physiol. *18* (1972) 237.
8 Hill, D.L., Drosoph. Inf. Serv. *19* (1945) 62.
9 Bownes, M., J. Embryol. exp. Morph. *33* (1975) 789.

0014-4754/84/030304-03$1.50 + 0.20/0

A simple, less stressful rat restrainer[1]

J.A. Owen, R.A.R. Tasker and K. Nakatsu

Department of Pharmacology and Toxicology, Queen's University, Kingston (Ontario, Canada K7L 3N6), 15 November 1982

Summary. The contruction of a simple, inexpensive animal restrainer is described. Data are given showing its utility in analgesia studies.

For many pharmacological experiments it is necessary to restrict the motion of small animals such as the rat. This was the case for our analgesia experiments in which the rat tail-flick method was employed. The most commonly available restrainers for small animals are plastic or Plexiglas® cylinders that fit firmly about the animal. These units, while effective for immobilization, cause animals to become somewhat agitated while they are being persuaded to enter the restrainers. The effect of restraint in inducing some degree of analgesia in rats has been documented[2]. Hence, the use of the plastic tube type restrainer may lead to variable results due to the presence of this additional parameter. In this communication we describe the construction of a simple, yet highly effective cloth rat restrainer. A comparison between the tail-flick latencies obtained with a commercial plastic restrainer and the cloth restrainer is presented.

Materials and methods. Construction. The construction of

the restrainer can be seen in figure 1. A 24×28 cm cloth (towel cloth is suitable) cover was held onto the 15×20 cm wooden base by 2 wooden strips (20×2 cm); each strip was secured by 2 bolts with wing nuts. The cloth was adjusted to snugly fit around the back of the rat while allowing sufficient space at the front for the animal to breathe normally.

Analgesia testing. Analgesia was assessed in 2 groups of 8 male Sprague-Dawley rats (200-250 g) by means of a tail-flick analgesia meter as described by Owen et al.[3]. The rats housed in the cloth restrainer were introduced into the restrainer when the analgesia testing commenced and remained there until the experiment was complete. On the other hand, rats were restrained in the plastic holders only for the period required for each analgesia measurement, although for a control comparison some rats were tested while being kept in plastic restrainers for the duration of the experiment.

Results and discussion. The tail-flick latency response times for rats housed in either plastic or cloth restrainers are shown in figures 2A and 2B. It is apparent from figure 2A that the mean tail-flick latencies obtained over the 1-h period were different for the 2 restrainers ($t=3.87$; $df=8$, $p \leqslant 0.01$). Use of the cloth restrainers gave far more consistent results than those obtained using plastic restrainers. The deviation of results about the mean at each time point is significantly less with cloth restrainers as shown in figure 2D ($t=4.19$; $df=8$, $p \leqslant 0.01$). This is further reflected by overall coefficients of variation over the assessment period for the cloth and plastic restrainers of 18.3% and 50.0% respectively. The decreased variability in results obtained with the cloth restrainer permits the detection of minor changes in nociceptive threshold which would be masked by the greater variability of results obtained with the plastic restrainers. In addition, the rats willingly enter and remain in the cloth holders for several hours at a time. Thus, the manipulation of large numbers of animals for the assessment of analgesia by tail-flick latency is simplified by having one restrainer for each animal, allowing the rat to be moved onto the tail-flick analgesia meter in its restrainer. By this method, tail-flick latencies can be assessed on 8 rats within a 2-min period.

These restrainers were also used with rats which had venous and cisternal cannulae. As the external ends to these cannulae were at the back of the neck and head, a hole was cut in the cloth cover of the restrainer to allow access to the cannulae.

The increased latencies observed using the plastic restrainer may reflect some degree of 'stress-induced' analgesia. Rats that were kept in plastic restrainers for the entire 1-h testing period were extremely agitated, showing gnawing and scratching behavior and often vocalizing. The coefficient of

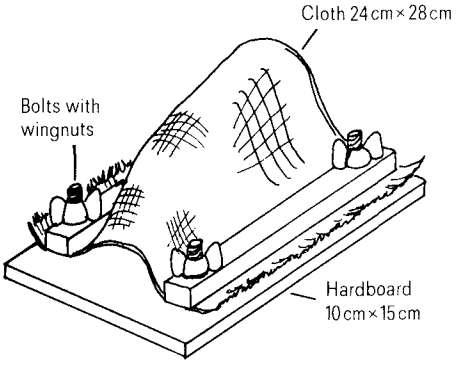

Figure 1. Restrainer. Rats voluntarily enter and remain in these restrainers for long periods.

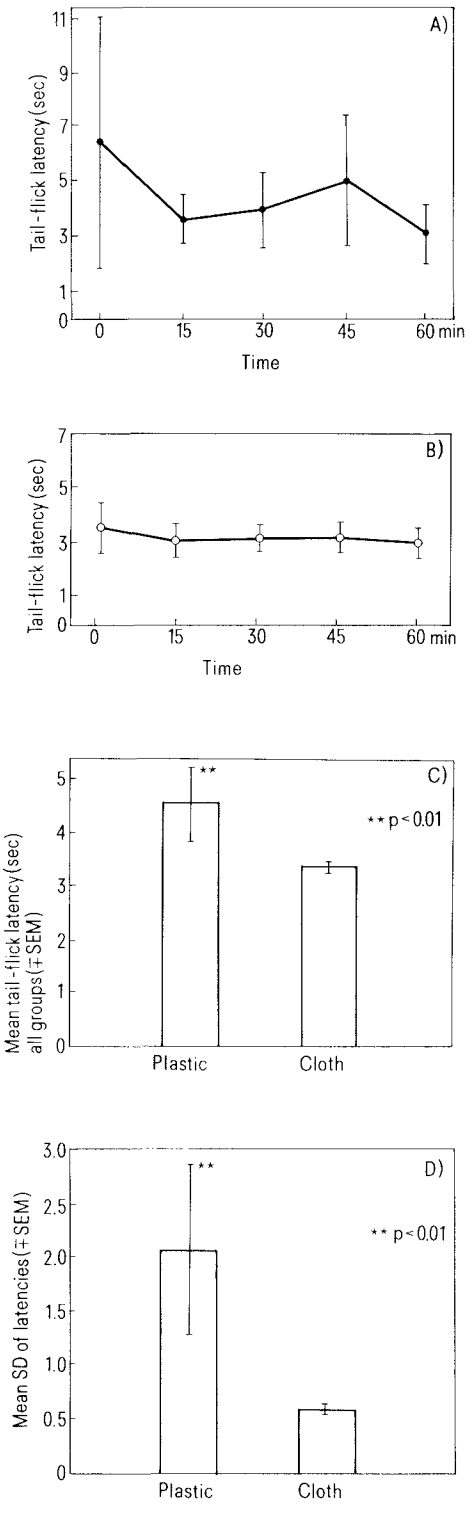

Figure 2. Comparison of reproducibility of rat tail-flick latencies using plastic and cloth restrainers. *A* and *B* Tail-flick latencies times were obtained as described in the text for plastic (A) and cloth (B) restrainers. Each point is the mean obtained from 8 animals; vertical bars represent SD. Note that the coefficients of variation are relatively large because no drugs were used which results in relatively short mean latencies. *C* Combined mean tail-flick latencies for all time points obtained using plastic (A) and cloth (B) restrainers. Vertical bars indicate SEM. *D* Comparison of the mean deviation of the tail-flick latency data obtained using plastic (A) and cloth (B) rat restrainers. Vertical bars indicate SEM.

variation for 4 rats under these conditions was 41.2%, considerably greater than that observed with the cloth restrainers.

In summary, the cloth rat restrainers represent an inexpensive and less stressful means of containing rats for assessment of analgesia by tail-flick latency. The results obtained through the use of this animal holder exhibit much less variability than those obtained in conjunction with the commercial plastic animal restrainers.

1 Supported by the Harry Botterell Foundation.

2 Madden, J., Akil, H., Patrick, R.L., and Barchas, J.D., Nature *265* (1977) 358.
3 Owen, J.A., Milne, B., Jhamandas, K., and Nakatsu, K., J. pharmac. Meth. *6* (1981) 33.

0014-4754/84/030306-03$1.50 + 0.20/0

Announcements

Italy

Courses

International School of Biophysics
14th course: Bioelectrochemistry II, membrane phenomena

Erice, Sicily, 5–15 November 1984

The course, sponsored by the BECS (Biological Energy Conversion Systems) Group, the Bioelectrochemical Society, the European Physical Society, the Italian National Research Council, the Sicilian Regional Government, and others, will have the following program:

Lectures. Structure and stability of membranes; their models; membrane lipids and proteins; breakdown and fusion – Transport of charged species through membranes; thermodynamics; mediated, nonmediated, active and passive transport; coupling mechanisms – Energy transduction; mitochondrial oxidative phosphorylation; chemiosmosis; photosynthesis – Effect of electric signals on membranes; basic electrophysiology; thermodynamics and conformational states; electric modulation of tissue growth and repair.

Seminars and round-table discussions. Interactions between external electric fields and membrane and membrane components.

Lecturers. H. Berg, Jena (GDR); M. Blank, New York (USA); R.S. Caplan, Rehovot (Israel); D. Chapman, London (UK); F. Conti, Camogli (Italy); J. de Gier, Utrecht (NL); D. Gräber, Berlin-West; E. Korenstein, Rehovot (Israel); P. Läuger, Konstanz (GFR); G. Lenaz, Bologna (Italy); B.A. Melandri, Bologna (Italy); I.R., Miller, Rehovot (Israel); E. Neumann, Bielefeld (GFR); A.A. Pilla, New York (USA); D.F. Wilson, Philadelphia (USA).

General information. Persons wishing to attend the course should write to Prof. G. Milazzo, Piazza G. Verdi 9, I-00198 Roma, Italy. They should specify: a) date and place of birth, together with their present nationality; b) degree and other academic qualifications; c) list of publications; d) present position and place of work; and include e) a letter of recommendation from their research group leader or from a professor of biophysics, physics, physiology or psychology.

Fees. Total fee including board and lodging is $ 500. Partial support can be given to some deserving students. The request for such aid must be specified and justified in the application letter.

Closing date for application is 20 August 1984.

Instructions to Authors

Experientia is a monthly journal of natural sciences devoted to publishing articles which are interdisciplinary in character and which are of general scientific interest. Considered for publication will be hitherto unpublished papers that fall within one of four categories:

Reviews (one-man and multi-author reviews)
Full Papers (in-depth reports not exceeding 4–6 printed pages)
Mini-reviews (1–2 printed pages)
Short Communications (1–2 printed pages)

Papers reporting on work that is preliminary in nature, or wherein animal experiments have been conducted without the appropriate anesthesia, will not be accepted.

Manuscript (including all tables and figures) must be submitted in *triplicate* and must be in *English*. *Title pages* should bear the author's name and address (placed directly below the title), a brief *abstract* (of approximately 50 words for short communications) mentioning new results only, and a listing of *key words*. *Footnotes* must be avoided. *Tables*, and then *figures*, are to follow the body of the text and should be marked with self-explanatory captions and be identified with the author's name. All *data* should be expressed in units conforming to the Système International (SI). *Drawings* are to be on heavy bond paper and marked clearly in black. *Photographs* should be supplied as glossy positive prints. Please note that we use two different systems for citing references. 1. For *Review Articles* and *Full Papers*, references should be arranged alphabetically and be numbered. Within the text, literature should be referred to by number and, where indicated, by author. The references should contain full journal article titles and the first as well as the last page of the article cited. 2. For *Short Communications*, an abbreviated bibliography is requested and references should be listed chronologically. Please consult a current issue of Experientia or inquire at the editorial office for details on form.

Authors are requested to specify under which section heading they would wish their communication to appear:

1. Chemistry, Physics, Biomathematics
2. Physiology, Pathophysiology (animals)
3. Physiology, Pathophysiology (plants)
4. Biochemistry
5. Anatomy, Histology, Histochemistry
6. Pharmacology, Toxicology
7. Molecular Biology
8. Immunology
9. Cellular Biology
10. Genetics, Developmental Biology, Aging
11. Oncology
12. Endocrinology
13. Neurobiology, Behavior
14. Environment, Ecology
15. Experimental Pathology and Clinical Research
16. Microbiology
17. New Methods and Apparatus

All incoming manuscripts are acknowledged immediately. Authors will be notified of the editorial board's publishing decision once their manuscripts have been evaluated by a minimum of two field experts. Fifty reprints of papers accepted for publication will be sent to authors free of charge; additional reprints may be ordered.

Manuscripts and all communications to the editors should be addressed to:

Experientia
Birkhäuser Verlag
P.O. Box 133
CH-4010 Basel/Switzerland
Tel. 061 23 18 10