An Anaesthetic for *Corophium volutator* (Pallas) and *Marinogammarus obtusatus* (Dahl), Crustacea, Amphipoda

During a study of respiratory ventilation in *C. volutator* and *M. obtusatus* it became necessary to anaesthetize to facilitate setting up the experiments. M.S. 222 Sandoz (Tricaine methanesulphonate) was chosen. It is apparent from BOVÉ's[1] review of M.S. 222 as an anaesthetic that it has been used extensively for fish and amphibia but infrequently, and only recently, for invertebrates.

In the crustacea STERBA[2] found M.S. 222 effective on *Daphnia pulex* and SCHWARTZ[3] used it in the transportation of the Brine Shrimp *Crangon septemspinosa*. However, workers at the Halifax Laboratory, Nova Scotia, found it unsatisfactory in the anaesthesia of *Homarus americanus*[4].

The following experiments were designed to ascertain the optimal effective concentration of M.S. 222 as an anaesthetic for the marine amphipods *C. volutator* and *M. obtusatus*. The optimal effective concentration is defined as the minimum concentration of M.S. 222 (in g/l of sea water) which will completely anaesthetize the above species within 30 min.

Two series of experiments were carried out. In the first, the times for complete anaesthesia and recovery were investigated. In the second, the effect of prolonged exposure to the optimal effective concentration of the anaesthetic on subsequent survival was examined. Both experiments were carried out in glass evaporating dishes of 12 cm diameter containing either 500 ml of sea water or freshly prepared solutions of M.S. 222 in 500 ml of sea water. All samples were aerated and maintained at 10 °C.

As this investigation was directed towards the use of M.S. 222 prior to experiments on respiratory ventilation only the largest individuals of the 2 species, *C. volutator*, average length 7 mm, and *M. obtusatus*, average length 9 mm, were selected. A total of 90 *C. volutator* and 54 *M. obtusatus* were used in the experiments in samples of 10 and 6 individuals respectively. The sexes were divided equally in each sample and most of the females carried eggs or larvae.

In the first series of experiments solutions of M.S. 222 of 0.4, 0.45, 0.5, 0.8 and 1.0 g/l concentration were used. A sample of amphipods was placed in each of the solutions and the time required to anaesthetize each individual was noted. The amphipods were removed from the anaesthetic, blotted and placed in recovery dishes containing aerated sea water. The time required for full recovery was observed. As the behaviour of each species preceding anaesthesia tended to follow a set pattern it was possible to describe a series of anaesthetic stages similar to that described by MCFARLAND[5] for fishes. This is illustrated in Table I. Total anaesthesia is defined as the condition in which the animals did not react to handling.

From the results of the first series of experiments the optimal effective concentration of M.S. 222 for both species was found to be 0.5 g/l. The effect of prolonged exposure to this concentration was studied by repeating the experiment; leaving the samples for 25 min to ensure anaesthesia. The animals were then kept in the anaesthetic for a further 15, 30, 60 or 120 min before being transferred to the recovery dishes.

Table I. Anaesthetic stages

Stage	Change in behaviour	
	C. volutator	*M. obtusatus*
I	Colour lightens, animals fall onto dorsal surface.	Swimming slows down, animals fall onto side.
II	Abdominal flexure followed by cessation of gross bodily movements (occasional twitch).	Pleopod beat becomes arrhythmic. Abdominal flexure followed by cessation of all movement (except twitches).
III	Total anaesthesia.	Total anaesthesia.

Table II. Survival of *C. volutator* and *M. obtusatus* in 0.5 g/l M.S. 222 solution

Time anaesthetized (min)	Average time of recovery (min)		% Recovery	
	C. volutator	*M. obtusatus*	*C. volutator*	*M. obtusatus*
15	24.2[a]	21.2 ± 0.9	100	100
30	23.2 ± 0.2	25.7 ± 0.3	100	100
60	20.8 ± 1.5	26.5 ± 1.2	100	100
120	21.5 ± 1.7	25.8 ± 1.2	100	100

Standard errors shown. [a] No standard error calculated.

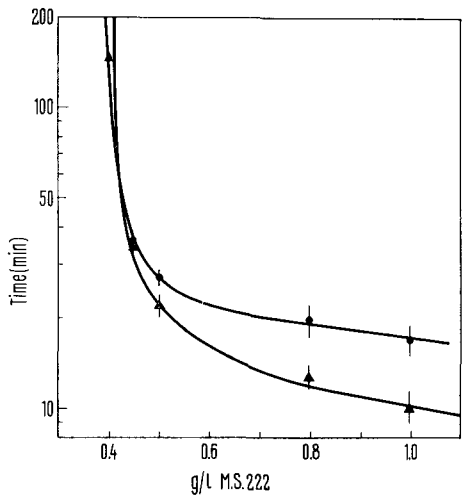

Fig. 1. The time taken to total anaesthesia (stage III) at different concentration levels of M.S. 222 in sea water in the marine amphipods. ●, *Corophium volutator*; ▲, *Marinogammarus obtusatus*. Vertical lines indicate the magnitude of the standard error at each point.

[1] F. J. BOVÉ, *MS-222 Sandoz, the Anaesthetic and Tranquilizer of Choice for Fish and other Cold-Blooded Organisms* (Sandoz Pharmaceutical Chemicals Division. Technical Bulletin).
[2] G. STERBA, Bull. Inst. oceanogr. Monaco. No. special 1D (1963). (1er Congrès International d'Aquariologie.)
[3] F. J. SCHWARTZ, Progve Fish Cult. *28*, 232 (1966).
[4] *Fisheries Research Board of Canada 1964*. Summary Report (Halifax Laboratory, Nova Scotia), p. 6.
[5] W. N. MCFARLAND, Publs. Inst. mar. Sci. Univ. Tex. *6*, 23 (1959).

The results are illustrated in the Figures 1 and 2 and in Table II. 0.4 g/l. M.S. 222 solution was barely effective with *M. obtusatus* and it was difficult to decide whether the animals had in fact reached stage III anaesthesia. *C. volutator* reached stage I only at this concentration level.

Survival of both species at the optimal effective concentration of 0.5 g/l for periods of up to 2 h was 100% and the time for recovery was unaffected by the length of anaesthesia. However, a side effect of M.S. 222 on gravid females was to induce the premature release of eggs or young from the brood pouch. In these premature young there was also 100% recovery from anaesthesia after each exposure time. The young also tended to recover more rapidly than the adults.

Preliminary investigations with a closely related amphipod species, *Corophium arenarium* (Crawford), showed the optimal effective concentration to be in the same range as the above 2 species.

These experiments have shown that M.S. 222 is an effective anaesthetic for the marine amphipods *Corophium volutator* and *Marinogammarus obtusatus*. It is interesting to note that the optimal effective concentration is relatively high (1:2000) when compared with the doses (1:25,000 to 1:12,000) recommended by BELL[6] for fishes. Doses of 1:2000 (0.5 g/l) which take 25 min to anaesthetize the amphipods will anaesthetize salmon and trout in under 2 min. EISLER and BACKIEL[7] found similar concentration effects of M.S. 222 on the times for anaesthesia and recovery of Chinook Salmon Fingerlings (*Onchorhynchus tshawyscha*). The fish, like the amphipods, are anaesthetized more rapidly and take longer for recovery after prolonged immersion in the anaesthetic. It must be emphasized, however, that the concentrations of M.S. 222 used by EISLER and BACKIEL[7] on the salmon fingerlings (1:33,000 to 1:2650) were much lower than those found to be effective with *C. volutator* and *M. obtusatus*. This higher concentration of M.S. 222 necessary to anaesthetize *C. volutator* and *M. obtusatus* compares well with STERBA's[2] work on *Daphnia pulex* where 1:4000 to 1:3000 is his recommended dose and also with SCHWARTZ's[3] value of 1:4000 which is the lowest concentration used on *Crangon septemspinosa*. A similar decreased sensitivity of crustacea, when compared with fish, has been observed when using quinaldine as an anaesthetic[8]. Crustacea such as *Hippolyte varians, Palaemon serratus* and *Carcinus maenas* were still unaffected while the shore fish *Blennius pholis, Cottus bubalis* and *Pholis gunnellus* were fully anaesthetized.

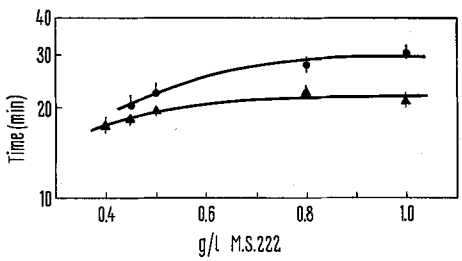

Fig. 2. The time taken for recovery from total anaesthesia (stage III) at different concentration levels of M.S. 222 in sea water in the marine amphipods. ●, *Corophium volutator*; ▲, *Marinogammarus obtusatus*.

Résumé. On a trouvé que l'anesthésique M.S. 222 Sandoz est effectif sur les amphipodes *Corophium volutator* et *Marinogammarus obtusatus*. Une concentration de 0.5 g/l peut anesthésier les animaux avec un délai de 30 min. Tous les animaux des deux espèces se remettent de l'anesthésie, même après 2 h dans cette concentration.

J. C. GAMBLE

Marine Science Laboratories,
Menai Bridge, Anglesey (U.K.), 10 January 1969.

[6] G. R. BELL, Bull. Fish. Res. Bd Can. No. 148 (1964).
[7] R. EISLER and T. BACKIEL, Trans. Am. Fish. Soc. *89*, 164 (1960).
[8] D. J. GROVE, personal communication.

Removal of Fertilization Membranes from Sea Urchin (*Lytechinus pictus*) Eggs

Many techniques have been used to remove fertilization membranes from sea urchin embryos[1–3]. Most of these techniques are either vigorous physical methods or chemical treatments designed to soften the membrane, digest it, or remove it by osmotic means. In this communication a technique is presented which has the advantages of being non-harmful to the eggs, of requiring no foreign substances in the medium, and of providing large quantities of membrane-free eggs quickly and easily.

The desired quantity of *Lytechinus pictus* eggs (obtained from the Pacific Bio-Marine Co., Venice, Calif.) was fertilized. After the fertilization membranes appeared (30–90 sec after insemination), the eggs were transferred to 15 cm³ centrifuge tubes and spun for 5–7 sec at about 1500 rpm (setting No. 5) in an International clinical centrifuge (Model CL). The supernatant sea water was decanted, the eggs were resuspended in sea water to a volume appropriate to the size of the homogenizer to be used, and the egg suspension was poured into the homogenizer. Dounce homogenizers with type B pestles (20-ml capacity, Kontes Glass Co., Vineland, N.J.) and Potter Elvehjem homogenizers with Teflon pestles (10-ml capacity, chamber clearance 0.004–0.006 in., A. H. Thomas Co., Philadelphia, Pa.) were satisfactory. The membranes were stripped from the eggs by allowing the pestle to settle by gravity through the egg suspension and then slowly withdrawing it. A sample was then examined microscopically for the presence of membranes. Normally,

[1] E. B. HARVEY, *The American Arbacia and Other Sea Urchins* (Princeton University Press, Princeton, N.J. 1956).
[2] D. MAZIA, J. M. MITCHISON, H. MEDINA and P. HARRIS, J. biophys. biochem. Cytol. *10*, 467 (1961).
[3] M. SPIEGEL and A. TYLER, Science *151*, 1233 (1966).