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.

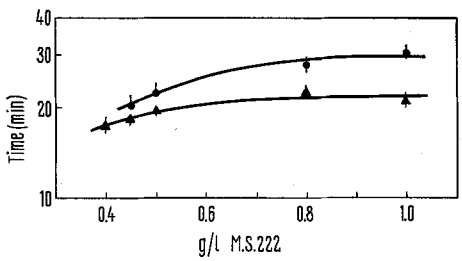

Fig. 2. The time taken for recovery from total anaesthesia (stage III) at different concentration levels of M.S. 222 in sea water in the marine amphipods. ●, *Corophium volutator*; ▲, *Marinogammarus obtusatus*.

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.

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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,

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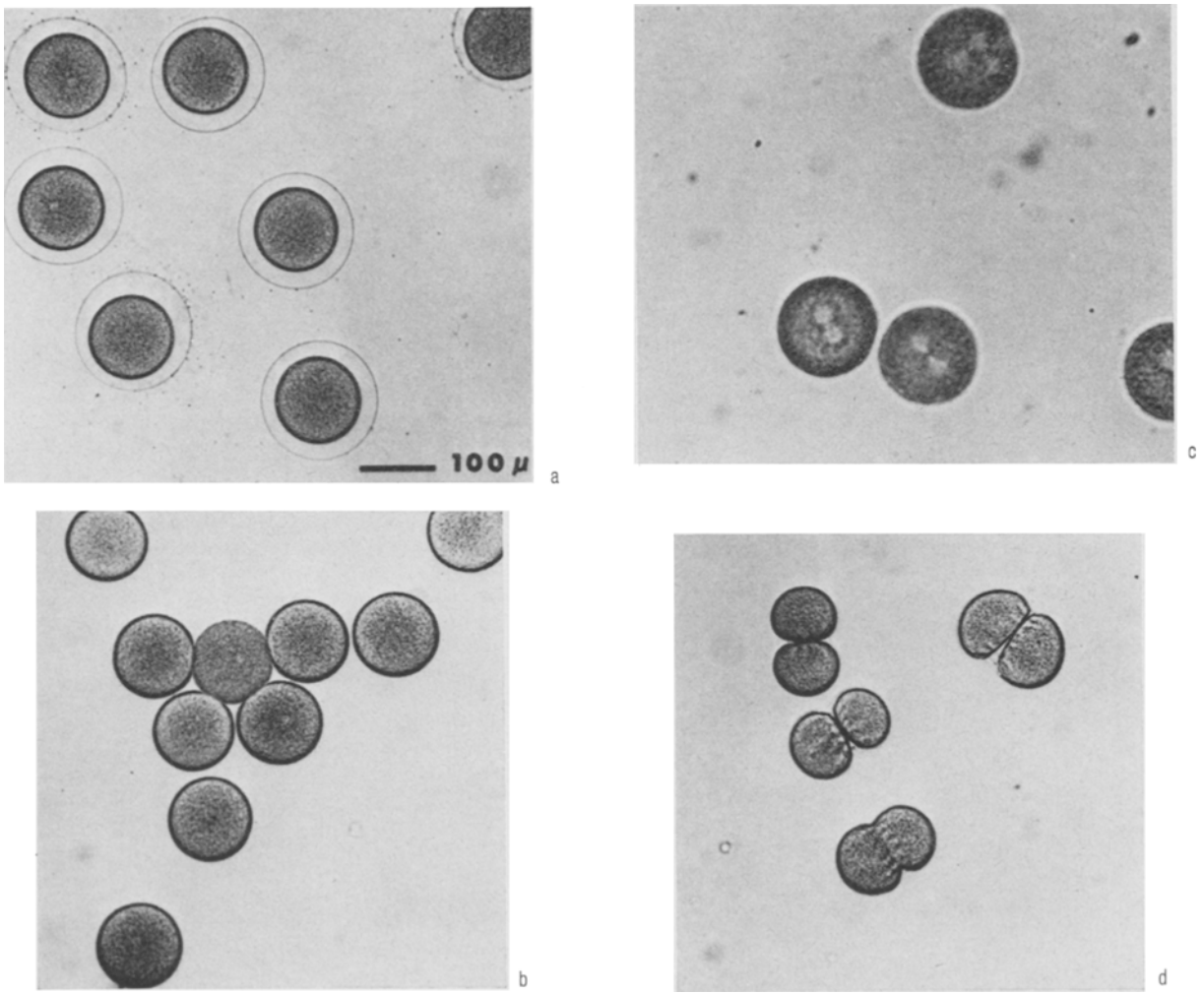
[3] M. SPIEGEL and A. TYLER, Science *151*, 1233 (1966).



(a) Fertilized eggs of the sea urchin *Lytechinus pictus* with fertilization membranes clearly present (100-μ scale included); (b) fertilized eggs after gentle homogenization to remove the fertilization membranes; (c) membrane-free eggs approaching first cleavage division (mitotic apparatuses apparent); (d) membrane-free eggs after first cleavage division.

all were removed. If some remained, the pestle was allowed to settle again through the egg suspension. All due speed, except for traverse of the pestle, was exercised throughout the procedure as the fertilization membranes were no longer easily removed after they had 'hardened' at about 5 min after fertilization.

Photomicrographs were taken using a Zeiss microscope with light field optics and a Leitz Aristophot camera. Using this technique, up to 100% of the fertilization membranes were removed from samples of fertilized eggs of the sea urchin *L. pictus*. The efficiency of membrane removal was independent of egg concentration. There was no noticeable damage to or disfiguration of the eggs. Figure 1a shows normal fertilized eggs with fertilization membranes. Figures 1b–d show homogenized eggs soon after membrane removal, in a late stage of first division, and in the two-cell stage, respectively. All figures are of the same magnification (100-μ scale included in Figure 1a).

When the homogenized eggs were gently centrifuged or permitted to settle under gravity, the supernatant sea water containing the fertilization membranes could be collected and/or the eggs could be resuspended in any medium desired (e.g., calcium-free sea water). The advantages of this technique are that it is rapid, no chemical additions to the medium are required, and large numbers of eggs can be simultaneously treated with no loss of cells. Whether or not the technique will work as efficiently with eggs of different diameter from other species is unknown[4,5].

Zusammenfassung. Eine einfache Methode für das schnelle Entfernen der Befruchtungsmembran von Seeigeleiern wird beschrieben.

B. R. BURCHILL[6] and C. H. BLOMQUIST

Biomedical Research Group,
Los Alamos Scientific Laboratory,
University of California,
Los Alamos (New Mexico, USA), 22 November 1968.

[4] This work was performed under the auspices of the U.S. Atomic Energy Commission.
[5] The authors acknowledge the assistance of Mrs. JULIAMARIE LANGHAM in taking the photomicrographs.
[6] Present address: Department of Zoology, University of Kansas, Lawrence, Kansas 66044 (USA).