FOREWORD

Marine contamination by petroleum, whether by natural seepage or by spills from ships at sea, by accidents in harbour or at offshore installations or by atmospheric or terrigenous input is by no means a new or rare phenomenon. In recent years however, the problems have been highlighted not only by the increased utilisation and marine transport of oil but also by a number of spectacular accidents which have raised questions about possible effects on the ecosystem. A number of detailed studies have been carried out in an attempt to answer these questions. The demands for such knowledge have been further increased by the various questions raised as a result of expansion of offshore exploration and exploitation for oil, particularly in environments hostile to these operations, in regions as far apart as the northern North Sea and the coast of Alaska.

Consequently, diverse aspects of the problem are being studied in several parts of the world by chemists and biologists who are often asking the same questions but using different approaches and sometimes producing conflicting views. Against this background, it seemed timely therefore to bring together a group of scientists from university, industry and government, actively engaged in such work, to examine and discuss common problems relevant to petroleum hydrocarbon contamination of the marine ecosystem and so a Workshop was sponsored by the International Council for the Exploration of the Sea, and held in Scotland at Aberdeen in September 1975.

The Workshop considered methodology, occurrence and fate in the environment, and effects on the ecosystem of petroleum hydrocarbons in the sea. Most of the papers presented and updated where necessary, are brought together in the present volume together with an edited version of the recorded discussion that followed each session. Of necessity, the reportage of the discussion is very brief although the proportion of time available for discussion compared favourably with that set aside for formal presentation of the papers. In preparing the discussion reports, the editors were assisted in particular by Dr R. Hardy, Dr R. Johnston, Mr P. R. Mackie and Dr I. C. White, and by comments from several contributors.

No attempt was made to produce specific recommendations but a study of the papers in this volume does give a clear indication of several lines of research which must be followed up before an adequate understanding can be reached of the effects of petroleum in the sea and it is evident that widespread monitoring operations will be fully effective only when the basis of our knowledge has been thus extended.

A list of participants to the workshop may be found in Appendix I.

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THE SEA URCHIN EGG AS A TEST OBJECT IN OIL POLLUTION STUDIES

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INTRODUCTION

Various kinds of organisms have been recommended as test objects in the study of marine pollution and a number of organisms have even reached a more or less official standing as material for routine testing of toxicological effects. The use of adult organisms imposes, however, certain limitations to the outcome of the toxicological testing, since the most sensitive period in the life span of an organism is the embryological period. As the knowledge of fertilization and differentiation was derived mainly from work on marine organisms and particularly on the gametes of sea urchins, it seems sensible to utilize this material also for toxicological testing (Hagström and Lönning, 1973).

A few examples will illustrate the use of sea urchin gametes and embryos as test material in pollution studies (cf. also Kobayashi, 1971; Lönning and Hagström, 1975a, b).

FERTILIZATION

The fertilization of the egg is a very sensitive process and a drop in the percentage or rate of fertilization or a tendency to polyspermy, reflect the action of an added test substance. Oil has a surprisingly small effect on the fertilization rate. If 1 ml/l of crude Ekofisk oil is added at insemination there usually is a weak delay in the fertilization rate. Oil dispersants added in the same concentration usually have more drastic effects, as exemplified by the fertilization rate experiment in Table 65, in which Ekofisk oil and various BP dispersants were tested. The substances were added at the moment of insemination. It appears that the new dispersant concentrate BP 1100 WD in 1/10 the concentration of BP 1100 and BP 1100 X (BP recommends dilution of the BP 1100 WD ten times before use) is less harmful to fertilization than the latter substances. However, if the concentrations are equal BP 1100 WD seems about as dangerous as the others. As can be seen from Table 65, BP 1100 WD in a concentration of 0·1ml/l slows down the fertilization rate, but the eggs are almost 100 percent fertilized. A comparison of BP 1100 WD with another dispersant concentrate, Esso Corexit 9527, shows that the latter interferes with fertilization already in a concentration of 1 µl/l and blocks fertilization completely in a concentration of 30—50 µl/l (Lönning and Hagström, 1976).

We also performed experiments in which the spermatozoa or eggs were only pretreated before they were used in fertilization experiments. By this method the effect of a test substance on the nucleus and on the cytoplasm and its components may be assessed. In the case of the sperm the amount of the cytoplasm is very restricted, and consequently the main effect of the pretreatment is likely to be confined to the haploid nucleus of the spermatozoon. Also the egg has a haploid nucleus but there the amount of the cytoplasm is much greater which may influence the outcome of a pretreatment. In the pretreatment experiments no test substance is present in the sea water medium at the moment of fertilization and during the ensuing development of the embryo.

The pretreatment of either of the gametes with crude oil many improve the fertilization rate somewhat. However, the resulting larvae often show disturbances in the differentiation, particularly of the skeleton. This is an indication that the pretreatment has interfered with the genome of the pretreated gamete. A striking

Table 65. *Strongylocentrotus pallidus*. Fertilization rate experiment (Hagström and Hagström, 1959). The substances were added at the moment of insemination

	% Fertilized eggs							
	Seconds after insemination							
	5	10	15	20	25	40	60	∞
Control	1	7	49	66	81	95	96	100
Ekofisk oil, 1 ml/l	0	10	28	68	73	88	93	100
BP 1100, 1 ml/l	0	1	2	2	5	7	10	29
BP 100 X, 1 ml/l	1	1	4	5	7	14	23	15
BP 1100 WD, 0·1 ml/l	1	6	21	46	57	79	90	99

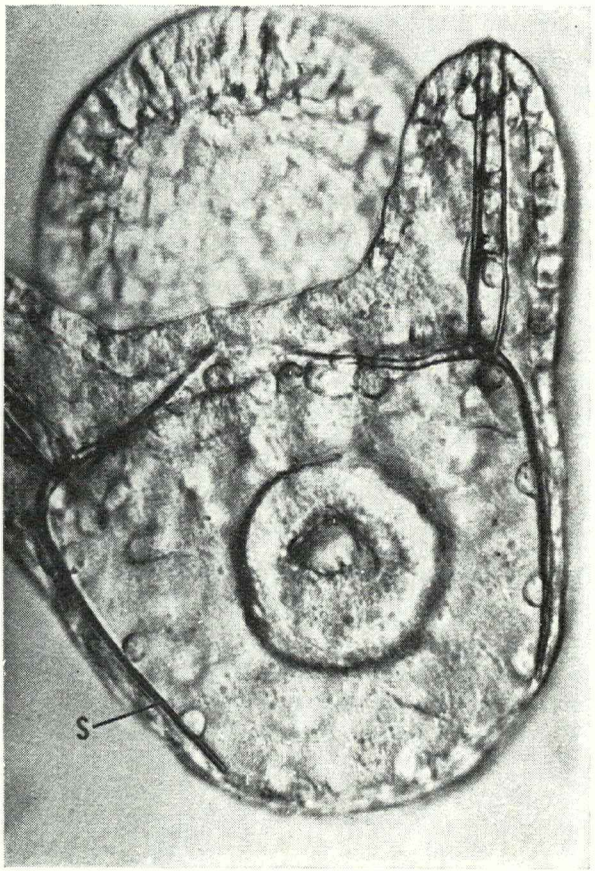

Figure 108. *Echinocyamus pusillus*. Control. A young pluteus seen in ventral view with a well developed skeleton (S). 1000 ×.

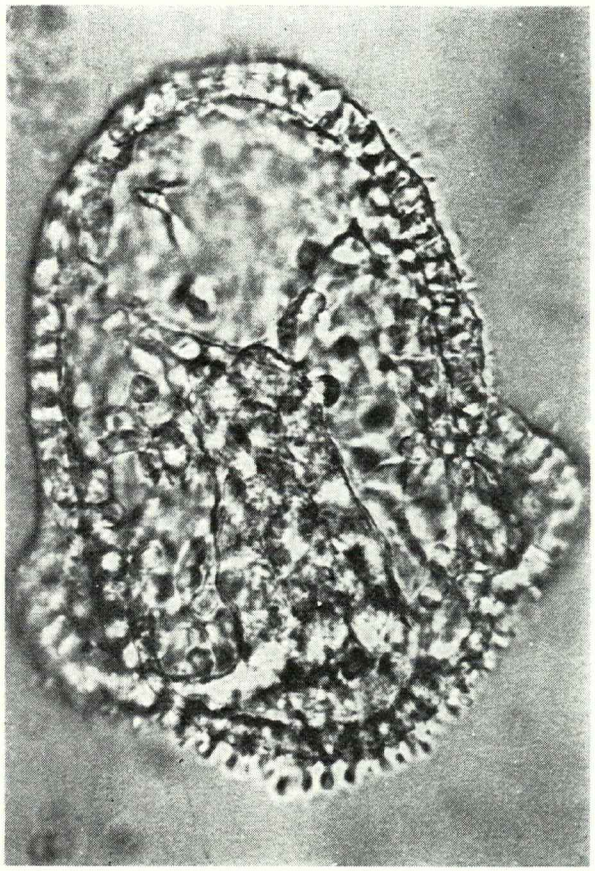

Figure 109. Larva from the same female as in Figure 108. Treatment from the 2-cell stage in BP 1100 WD, 10 µl/l. The formation of the skeleton is inhibited, and the ectoderm is affected, especially in the vegetal (lower) region. 1000 ×.

example of this was found in our earlier experiments on thalidomide which clearly indicated that the substance acts as a mutagen (Hagström and Lönning, 1973).

DEVELOPMENT

The early development is characterized by a rapid cleavage, whereas after hatching the cleavage rate slows down and there is a differentiation of organs. Since the eggs are rather transparent, changes in the cleavage rate or in the differentiation can be observed in the microscope directly. The addition of oil causes no serious changes up to hatching, whereas oil during later stages has severe influences on the embryo (Lönning and Hagström, 1975a). A delay or inhibition of the organization of the primary mesenchyme results more or less in an inhibition of the skeleton. Also the invagination of the intestine is often delayed and results in only a small intestine or exogastrulation.

Oil dispersants often have more far-reaching consequences for the larva; Figures 108 and 109 give an indication of this. The larva in Figure 109 had developed in BP 1100 WD, 10 µl/l. Not only is the formation of the skeleton inhibited, but also the ectodermal layer of cells is heavily affected, especially in the vegetal region of the larva.

The direct observations have been combined with some work on the ultrastructure of the larvae. Until now we have found very little effect after oil treatment whereas one of the least dangerous oil dispersants, Esso Corexit 8666, gives a marked accumulation of oil droplets in the cytoplasm (Lönning and Hagström, 1975a).

The experimental data which have accumulated during the last years suggest that crude oil has surprisingly modest effects on the development of the embryo whereas oil dispersants from various manufacturers have more serious influences on development. If crude oil and dispersant are mixed, as they usually are under

field conditions, the result may be even more deleterious to marine organisms than either substance alone (Lönning and Hagström, 1975a, 1976). We therefore think that the methods used in fighting oil spills should be carefully reconsidered.

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