Effects of hydraulic decompression and compression on deep sea amphipods

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See Table 1 for work site

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Introduction

Pressures in the range of several hundred atmospheres are known to affect the central nervous system of shallow water animals, causing a variety of motor disturbances which are both dose dependent and reversible (Macdonald et al., 1979; Menzies, 1974). At the cellular level comparable pressures affect both the steady state and the dynamic properties of axons and synapses (Wann & Macdonald, 1980). Deep sea animals might be expected to show a susceptibility to applied pressures which is proportional to their normal ambient pressure, and evidence consistent with this idea has been obtained from experiments with benthic amphipods collected from 1300m - 2500m depth (Macdonald and Gilchrist, 1978). In addition to measuring the tolerance of deep sea animals to high pressures, it is of equal interest to consider their tolerance to hydraulic decompression. In a previous report the first examples of hydraulic decompression symptoms were described in amphipods which had been collected in a pressure-retaining trap at 134 atm from 1300m depth. The symptoms consisted a mild flurry of uncoordinated activity as pressure approached one atmosphere, and the rapid inhibition of movement shortly thereafter. Similar animals collected from a depth of 2000m or more without being held at their normal ambient pressure were invariably immobile but they recovered activity when re-compressed to their normal ambient pressure (Macdonald and Gilchrist, 1978). Yayanos has also noted that decompression reversibly immobilizes deep sea amphipods (Yayanos, 1978).

Clearly the inhibition of motor activity associated with decompression from the animal's normal high ambient

pressure warrants further investigation, both as an interesting physiological index of adaptation to high pressure and as a potentially significant artefact in experiments using deep sea organisms which have been collected without pressure-retention (i.e. with decompression). This paper describes experiments in which live benthic amphipods were collected without pressure-retention in an immobilized condition from 4000-4325m depth and then promptly resuscitated by re-compression to 400 atm. The results of subsequent experiments in which the animals were subjected to pressures both higher and lower than normal are also described.

Methods

The experiments were part of the work carried out during the R.R.S. Challenger 12/78 cruise and the R.R.S. John Murray 11/79 cruise. Traps were baited with raw beef and either laid by an extended version of the deep mooring method previously described (Macdonald and Gilchrist, 1978) or by a "free-fall" method. In the latter case the trap was ballasted and equipped with an acoustic transmitter, a pyrotechnic release device, and nine Benthos buoyancy spheres. After several hours on the sea floor the ballast was released by acoustic command and the buoyant trap floated to the surface to be simultaneously tracked by the ship to within 1 km, and usually much less, of the surfacing point.

The normal ambient temperature of the trapped animals was between 2-3^{o}C but during collection the temperature within the traps increased significantly although it was not measured. The depth at which each trap was laid was determined by a precision echo sounder and occasionally verified by measuring the ambient pressure. Once on board the animals were

transferred from the traps to observational pressure vessels of a type previously described (Wann et al, 1979). Two independent apparatuses were used. One comprised a large cylindrical vessel (900 ml volume, 7.2 cm internal diameter, 1000 atm working pressure) and the other a pair of smaller vessels (210 ml volume, 5.7 cm internal diameter, 670 atm working pressure). Each apparatus incorporated a pneumatically powered pressure pump, a calibrated Bourdon tube gauge, and temperature-control equipment to regulate the temperature of the vessel at 5 ± 0.5°C. Bright light was required periodically to observe the animals in detail through the high pressure windows. Control experiments were carried out at atmospheric pressure at 5° ± 2°C, in the dark except for periods of observation.

Results

Trapping

Table 1 summarises the effectiveness of the traps. Amphipods were the only animals to be collected, comprising two species, Paralicella caparesca and Eurythenes grillus, with individuals ranging from 0.1 cm to 5 cm in length. Animals of less than 0.4 cm were not used in experiments. The number of animals collected on each occasion was highly variable. When removed from the trap all the animals were immobile and superficially appeared dead.

Three types of experiment were carried out. Animals selected for experiments were first subjected to resuscitation treatment, after which some were used in experiments in which their response to decompression was assessed whilst others were used in high pressure tolerance experiments.

Resuscitation experiments

Groups of mixed species were transferred from the traps as rapidly as possible, pressurised to 400 atm ± 3% in a matter of seconds and held at that pressure at 5°C for several hours. Control groups were simultaneously transferred to 5° water and held at atmospheric pressure. Animals were judged to be normal if their activity resembled that of healthy shallow water benthic amphipods, which display a characteristic posture if stationary and when active show a well defined repertoire of limb movements, additional to crawling and swimming. The deep sea amphipods were regarded as abnormal if they lay stationary in a straightened posture or, if active, their movements were unusual. Commonly observed abnormal movements include a slow pleopod beat and periodic ventrally-directed contractions of the whole body (ventral curling). Undoubtedly the bright light required to inspect the animals stimulated them, causing the moribund to stir and the active ones to move slightly, but the stimulus was common to both experimental and control animals.

The total period of resuscitation varied with the condition of the animals and the temptation to proceed with further experiments.

However, despite the variable resuscitation times it is clear from Table 2 that more than half the animals recovered some activity at 400 atm and that none recovered movement in the control experiments. Resuscitation was considerable in the experimental group, particularly in animals collected without delay at the surface (compare experiments 2, 3, 4, 6 and $6_{(78)}$ with 1, 5, 7 and 8).

It should be noted that subsequent experiments revealed or aided further recovery in some cases (see tolerance to high pressure) and the data in Table 2 are a minimal score of the capacity of the animals to resuscitate.

Response to decompression

Stepwise decompression experiments were carried out on a total of 53 animals, from experiments $4A_2$, 5A and 6B. Animals in experiment $4A_2$ were first held for 7 hours at 400 atm after experiencing a rapid decompression to atmospheric pressure of the type described below.

In all three step-decompression experiments pressure was reduced at a rate of approximately 70 atm/min, from 400 atm to 270 atm ± 3% and then held constant for 15 minutes, after which pressure was similarly reduced to 136 atm ± 3% and held constant for another 15 minutes. Finally the animals were decompressed to atmospheric pressure. The first decompression step caused a generalized increase in motor activity in most individuals, which faded in 15 minutes. The second decompression step caused a similar but lesser effect. The third step caused little movement and all individuals were lying stationary in a straightened posture within 4 minutes of experiencing atmospheric pressure. After 15 minute sojourn at atmospheric pressure the animals were restored to 400 atm over a period of one minute, after which the resumption of motor activity was variable. 5 of 9 animals in experiment $4A_2$ started to swim on recompression but in the other experiments recovery was much slower. All the individuals were fully recovered 6½ hours after recompression.

The second type of decompression experiment was necessitated by the use of pressure vessels with a maximum working pressure of only 670 atm for resuscitation. Animals which were decompressed after resuscitation in order to be transferred to the 1000 atm vessel for high pressure tolerance tests, yielded the following results. Decompression from 400 atm to atmospheric pressure in approximately one minute immobilized all individuals within 2 minutes of reaching atmospheric pressure (total of 47 individuals, experiments 2A, $4A_1$, $4A_2$, 3A and $6_{(78)}$). In the first three experiments the animals were kept at atmospheric pressure for 15 minutes and then rapidly recompressed to 400 atm, with the result that half the total recovered limb movements immediately and all resumed their pre-decompression level of activity within 60 minutes. In experiment $6_{(78)}$ recompression to 400 atm followed after 4 minutes sojourn at atmospheric pressure and all the individuals recovered promptly. In experiment 3A there was no recompression stage.

Tolerance to high pressure

60 individuals (54 P. caparesca, 6 E. grillus) in groups of approximately 10 were subjected to a pressure tolerance test in which pressure was increased in 50 atm steps at 5 minute intervals, starting at 400 atm. All the animals had been previously resuscitated; in experiments 3B and $6_{(78)}$ the tolerance test continued from resuscitation treatment whereas in others prior decompression and transfer to the 1000 atm vessel was necessary. In experiments 2A, 3A, $4A_1$ and $4A_2$ the decompression was rapid and in experiment 6B it was stepwise, but in all cases ample time for recovery

was allowed following recompression to 400 atm. The observations were difficult to quantify but the following summarises the results. Typically 2-5 of a group of 10 individuals would swim in response to compression steps, but this response failed to appear at pressures higher than 650-750 atm. Over the range 500-700 atm abnormal hyper-excitability culminated in an episode of ventral curling. Above 700 atm locomotor activity declined markedly and as the animals became immobilised they were seen to lie in a progressively straightened posture. Generally the vigour and prevalence of the hyperexcitable responses to compression were related to the level of locomotor activity prior to the test, but this had no effect on the inhibitory threshold. In experiment 2A for example, the pressure test was repeated after holding the animals for 6 hours at 400 atm. At the start of the second test the animals were more lively (8 of 10 swimming) than at the start of the first test (4 of 10 swimming). During the second test the hyperexcitable activity reached its peak at 650 atm with 9 animals showing ventral-curling, whereas in the first experiment peak hyperexcitability was also seen at 650 atm when only 2 animals exhibited ventral-curling. The maximum pressure applied in the tests was 700 atm and the dorsal body flexions, which are characteristic of most other pressurised amphipods, were not seen at any stage.

Discussion

The methods used to collect the benthic amphipods exposed the animals to a variety of stresses, the most significant of which were probably hydraulic decompression and an increase in temperature. The resuscitation experiments

demonstrate that 400 atm reverses many of the ill effects sustained during collection. In several favourable cases apparently complete recovery was achieved.

Animals in experiments 2, 3, 4, 6 and $6_{(78)}$ were not subjected to a delay at the surface during collection and showed a level of resuscitation superior to that in other animals. Experiments 2, 3, 4, 6 and $6_{(78)}$ score 62/76 animals normal and active as compared to 24/79 animals in experiments 1, 5, 7 and 8 which showed, at most, sub-normal movements (Table 2). The deep mooring method in which the trap is winched to the surface avoided the critical delay (Table 1) and yielded the healthy animals seen in the first group of experiments. The recovery of the traps laid by the free-fall method could hardly be carried out with less delay and clearly this method is not well suited for collecting live deep sea animals which are exposed to the surface pressure and temperature.

Hydraulic decompression, relatively high temperatures and other factors doubtless all contribute to the immobilizing effects sustained during collection, and their relative contributions remain to be assessed. High pressure is essential for resuscitation which could conceivably be merely the pressure reversal of thermal damage, because it is well known that pressure opposes the mild thermal denaturation of proteins (Johnson et al 1974). However the decompression experiments show it is likely that hydraulic decompression plays a significant part in the injury sustained during collection. The overall rate of decompression in the stepwise decompression experiments was approximately three times that experienced by the animals on their ascent to the surface,

suggesting that the immobilized condition observed on arrival could be caused by hydraulic decompression. Unfortunately shortage of time at sea prevented a thorough investigation of the relationship between decompression profile and the severity of immobilization. However cases were noted in which compression to 400 atm after a brief decompression test produced a prompt restoration of normal activity, whereas other cases occurred in which several hours at 400 atm were required for recovery. The latter would broadly match the resuscitation times.

The other main symptom seen during experimental decompression was a transient hyperexcitability which might be a generalized behavioural response to some unidentified stimulus. However the response, which lasted 5-10 minutes, was related to the pressure at which the decompression step was made and it seems more likely to be the result of some direct effect of decompression on the animals' neuromuscular system.

Both the excitatory and inhibitory effects of hydraulic decompression are new physiological phenomena, requiring more detailed quantitative description, but it is tempting to speculate on the mechanisms by which hydraulic decompression might exert reversible physiological effects. Perhaps the most obvious mechanism would be an effect on cell membranes, in which decompression would fluidise the bilayer structure. Thus the inhibition of movement caused by the hydraulic decompression of high pressure adapted amphipods might be the result of a perturbation of membrane structure broadly similar to that caused by other fluidising agents, such as anaesthetics

(Chin et al 1976; Macdonald and Wann, 1978). It will be interesting to see if the latter potentiates the former. The excitable effects of decompression are particularly interesting. Future experiments should try to eliminate unspecific behavioural responses and attempt to confirm a direct decompression effect on these animals, (a "decompression nervous syndrome"). (Bruner, 1975).

The slow recovery from the effects of a short exposure to atmospheric pressure implies a slow restoration process. In view of the likely perturbation of membrane structure caused by decompression, one attractive possibility is that ionic regulation is impaired by decompression, and the return of a normal ionic balance requires a relatively long time.

The measurements of the animals' tolerance to high pressure are interesting for two reasons, despite the obvious shortcoming that the animals may have been somewhat impaired by their prior treatment. First, compression caused hyper-excitability; and in particular the ventral curling movements were typical of the early stages of hyperexcitability seen in other, similar, pressurised amphipods from lesser depths. Second, hyperexcitability stopped short of dorsally directed flexions (convulsions) which are normally the climax to the hyperexcitable phase seen in pressurised amphipods. From a plot of the convulsion threshold pressure for amphipods obtained from 1 to 2500m depth (1-250 atm) the threshold pressure for convulsions in the present animals from 4000-4300$_m$ depths may be predicted to be approximately

850 atm (Macdonald et al, 1979). The prediction is not confirmed in the present results, perhaps because prior decompression irreversibly damaged that part of the nervous system on which pressure acts to elicit convulsions. It is worth noting that a deep pelagic amphipod, Lanceola sayana, fails to convulse at high pressure, but it also fails to exhibit any hyperexcitable responses to pressure, and its motor activity is abruptly inhibited at 350-400 atm. Many of the resuscitated amphipods exhibited a high level of apparently normal activity, which is inconsistent with the depressed metabolic rate reported in other deep sea animals (Childress, 1975; Smith, 1978; Torres et al, 1979; Somero and Siebenaller, 1979). Observations of the animals on the sea floor and in more elaborate high pressure aquaria are required to establish their normal level of activity.

Obviously many future experiments must be carried out on deep sea animals collected without change in pressure or temperature. Technically this is possible (Macdonald and Gilchrist, 1978; Yayanos, 1978) but necessarily our pressure-retaining traps are less effective at collecting amphipods than the large, simple traps we used in the present work. This factor, combined with logistic and practical problems of severely limited working time at sea have so far prevented us from carrying out more of the desired experiments.

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Table 1 Collection of live benthic amphipods from the Biscay abyssal plain ($47^{o}15'N$; $8^{o}33'-49'W$)

Trap No.	Depth, Metres	Time on bottom	Decompression (Ascent) time	Time from surface to in board	No. of amphipods	Ensuing Experiment No.
Deep Mooring Method						
1	4250	6h	6h*	30 min	30	1
2	4300	7h	1h 10m	5 min	200	2
3	4320	10h	1h 40m	" "	150	3
4	4300	10h	1h 20m	" "	30	4
5	4000	5h	2h	30 min	30	5
6	4200	5h	1h 20m	5 min	4	6
9 ≠ (78)	4300	14h	1h 55m	" "	400	6 (78)
Free Fall Method						
9	4275	16h	1h 15m	30 min	200	7
10	4320	5h	1h 15m	15 min	4	-
11	4300	10h	1h 35m	30 min	25	8
12	4325	5h 30m	1h 20m	40 min	40	-

* delayed at 6 depths between 3000m and 500m due to technical difficulties

≠ cruise Challenger 12/78. All other traps laid during John Murray 11/79.

Table 2. Resuscitation experiments on deep sea benthic amphipods from 4000-4300m depth. All individuals were immobile before compression to 400 atm at 5°C.

Expt No.	Time from in board to compression	No. of individuals		Peak activity (No.of animals, activity, time)	Duration of observations at 400 atm
		P.caparesca	E.grillus		
1A	25 min	10	0	3/10 slight limb movt. after 3hr	7hr 45min
1B	25 min	7	1	1/8 (E.grillus) weak pleopod movt. after 5 min sustained	7hr 45min
2A	10 min	9	1	3/10 swim and 4/10 slight limb movt. after 4hr 25 min	4hr 25min
2B	10 min	12	0	5/12 normal activity of which 1 swims, after 3 hr	3hr
3A	20 min	8	2	10/10 normal activity, with swimming, after 5 hr	5hr
[illegible]	15 min	13	0	13/13 normal of which 8 crawl or swim, after 4 hr	4hr
$1A_1$	15 min	10	0	10/10 normal after 8 hr	8hr 30min
$1A_2$	15 min	9	0	no observations made but 9/9 normal in subsequent experiments (see tolerance to decompression)	10hr 45min
5A	10 min	20	0	10/20 pleopod movt., subnormal, after 4 hr	4hr
5B	20 min	4	0	3/4 normal after 13 hr	13hr
7A	15 min	5	6	2/11 (E.grillus) limb movt. after 7 hr 30 min	22hr
7B	15 min	22	0	1/22 swim, 1/22 pleopod movt. after 8 hr	21hr 45min
[illegible]$_1$	5 min	0	2	2/2 weak limb movt. after 10min	10hr
$8A_2$	5 min	0	4	2/4 " " " " 10min	10hr
8B	20 min	0	2	2/2 " " " " 40min	6hr
$6_{(78)}$	20 min	5	3	5/8 " " " " 2 hr	3hr
Total		134	21	----------	---
Control Expt. at 1 atm					
1		9	0	no activity seen at any time	8hr
2		10	0	" " " " " "	4hr 30min
3		13	0	" " " " " "	5hr
5		11	0	" " " " " "	4hr
7		8	2	" " " " " "	22hr
8		0	16	" " " " " "	6hr
$6_{(78)}$		4	2	" " " " " "	4hr 30min
Total		55	20	----------	---

*2 pressure vessels connected together and used simultaneously.

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