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Gerard DREWA

Department of Biology, Academy of Medicine, Gdańsk

Effect of crude oil and detergent on the arylsulphatase (E.C. 3.1.6.1.) activity in *Euphausia superba* and *E. crystallorophias**)

ABSTRACT: Crude oil and light or heavy oils as well as the detergent ABS in concentrations of 5 and 50 ppm partially inactivate arylsulphatases in *Euphausia superba* and *E. crystallorophias*. After one hour exposure to detergent solutions the arylsulphatase activity in experimental animals transitorily increased. The arylsulphatase activity proved to be affected also in krill homogenates. The change of temperature, of the krill incubation in solution of crude oil or detergent, from $-1°C$ to $+1°C$ did not influence the enzyme activity. The damage of lysosomal membranes, by oils and the detergent, has been confirmed by the fact of enhanced penetration of Trypan blue into krill tissues.

Key words: alkylbenzene sulphonate (ABS), arylsulphatase (ASA), nitrocatechol sulphate (NCS), 4-nitrocatechol (4-NC)

1. Introduction

The development of transport and industry, consuming huge amounts of crude oil and of products of its processing, as well as the growing use of detergents are responsible for the pollution of many water bodies. Transportation of crude oil through seas, frequent shipwrecks, and cleaning are the main sources of pollution of the sea. For decontamination of the polluted area from crude oil various mixtures of surfactants are used. It has been found that in waters of Dead Vistula and Bay of Gdańsk during the summer season the concentration of detergents reached 2.64 ppm (Drewa, Zbytniewski and Pautsch 1975). Mann and Schmid (1961) reported that the concentration of dodecylbenzene sulphonate in the rivers of the German Federal Republic ranged from 1.0 to 16.2 ppm. On the sea routes of Pacific Ocean the concentrations of aromatic hydrocarbons ranged from 0.04 to 0.82 ppb (Lee and Nicol 1978).

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Even the relatively clean waters of the Antarctic Ocean are in danger to be polluted with crude oil and detergents. Rather small amounts of crude oil and detergents in the antarctic waters are coming from the fishing boats and scientific stations existing there. Many countries are looking for the natural gas and crude oil in the antarctic region. This is the potential danger of the pollution of this region in the near future.

Pollutants contaminating the aquatic biocoenoses are damaging the plants and animals. Metabolic disorders, acute and chronic toxicity, disturbances of embryonic development are responsible for the ecological disequilibrium and disturbances in the food chains. There are many reports on the effect of hydrocarbons from crude oil on the sea animals and sea biocoenoses (Nelson-Smith 1970). These works concern the influence of detergents and hydrocarbons on the animals living in the seas of temperate climate. All these papers may be divided into several groups: the first group investigated the viability and behaviour of animals exposed under laboratory conditions in variable concentrations of pollutants (Mann 1962a, Lüdemann and Kayser 1963, Mann and Schmid 1965, Riger 1975, Bigford 1977, Pautsch 1978). The second group observed the embryonal development and larval development of eggs and larvae living in variable concentrations of toxicants (Mann and Schmid 1961, Byrne and Calder 1977, Cucci and Epifiano 1979, Laughlin and Neff 1979). The third group investigated the differences in the chemical composition of the body, the influence of pollutants on the chemical composition of the animal body, and on the physiological processes after short or long term exposure of experimental animals to the action of various pollutants (Lang 1967, Donaldson 1976, Drewa, Zbytniewski and Pautsch 1977, Kurelec et al. 1977, Edwards 1978, Drewa et al. 1979, Walczak 1979).

The last group observed the fate of pollutants in the aquatic biocoenoses, the rate of their biodegradation by microorganisms, their penetration into various animals tissues and their metabolism in living organisms (Mann and Schöberl 1976, Neft et al. 1976, Walker et al. 1976. Rossi and Anderson 1977).

We found no publications concerning the effects of detergents and crude oil on the animals living in antarctic water bodies. It may be supposed that the pollutants may be more toxic for water animals living in temperate regions than in the antarctic regions. It has been found in may papers that the effect of pollutants is stronger in higher temperatures (Mann 1962b, Laughlin and Neff 1979).

Physiological processes as well as biochemical reactions in living organism depend on the normal structure and functions of biological membranes (Tappel 1969). To the biological membranes belong all structures surrounding the cell organelles and cytoplasm. Biological membranes are composed of protein and lipid components. Hydrocarbons present in crude oil as well as detergents influence the permeability of biological membranes and in higher concentration they destroy the protein-lipid structure of membranes. The release of the lysosomal enzymes that follows the labilization of the membranes leads to cell autolysis. Detergents and crude oil penetrate the deeper tissues of living organisms through integumenta, digestive system

and respiratory epithelium (Lee et al. 1976, Drewa, Zbytniewski and Pautsch 1979). Lysosomes are surrounded by elementary biological membranes. These organnelles are involved in intracellular digestive processes and take part in the destruction of microorganisms. Lysosomes also remove defective cell tructures. In lysosomes are also cumulated exogenous substances, which could not be metabolized because of lacking proper enzymes. It has been found that pesticides belong to such substances (Tappel 1969).

Arylsulphatases (E. C. 3.1.6.1.) are lysosomal enzymes taking part in the cell digestive processes as well as in the moulting of *Crustacea* (Shimony and Nigrelli 1972). ASA hydrolyse sulphuric esters of phenols. Taking into consideration the importance of ASA in *Crustacea* it has been decided to assay the activity of these enzymes in *Euphausia* exposed to solutions of crude oil, fuel oils and a detergent.

2. Material and methods

Euphausia superba Dana and *E. crystallorophias* Holt et Tattersall were collected in the Ezcurra Inlet during the austral summer 1979/1980. Animals were kept in cooled 10 liter tanks. Experiments were carried out at temp. $-1°$ and $+1°C \pm 0.5°$. The experiments were performed with animals adapted for 24 hours to laboratory conditions. No significant difference has been found in the enzyme activity between females and males and therefore animals to he experiments were caught at random, regardless of the sex (Drewa and Jackowska 1980). The mean body weight of the krill was 750 ± 100 mg and the length of the animals ranged from 39 to 51 mm.

The influence of pollutants on the ASA activity was examined in four groups. First group: animals were incubated in sea water with addition of crude oil. Second group: light fuel oil was added to the sea water. Third group: heavy fuel oil was added to the sea water. In the fourth group krill was incubated in solutions of the detergent. In each experimental group two concentrations of pollutant were used, i.e. 5 ppm and 50 ppm. The water in containers was aerated in order to assure the mixing up of the pollutant with the water. Because of biodegradation, evaporation, and accumulation of pollutants in the animals tissues the solutions of pollutants were renewed every 24 hours. The Ramashkin crude oil (from Iraq) as well as the fuel oils were obtained from the Gdańsk Oil Refinery. The detergent used was ABS (technical grade) belonging to anionic detergent. Parallelly with each experimental group a control group was examined.

After 1, 6, 12, 24, 48 and 96 hours of exposure animals were caught at random, rinsed with distilled water, dried on lignin and then homogenized in ten volumes of redistilled water. The whole animals together with the chitin carapax were homogenized. The obtained homogenates were centrifuged at $1500 \times g$ for 10 min. The ASA activity was estimated in supernatant using the Roy's method (Roy 1958) modified by Błeszyński (Błeszyński and Działoszyński 1965). NCS is hydrolyzed by ASA to 4-NC and

sulphate ion. The enzyme activity was expressed in nM of 4-NC per mg of protein. The protein concentration in the homogenates was assayed according to Lowry et al. (1951). In one series of experiments the enzyme activity was assayed after adding the pollutants to krill homogenates in a way to obtain concentrations of 5 and 50 ppm.

The trypan blue penetrates into lysosomes *in vivo*. The content of this dye may serve as a measure of the lysosomal membrane permeability (Holtzman 1976). The Trypan blue accumulation in the hepatopancreas and in the abdomen muscles was assayed after exposing the krill for 24 hours to the pollutants in the concentration of 50 ppm in 0.0001% Trypan blue. The absorbance was read in 1 cm cells against control homogenates. LC_{50} has been determined for *E. superba* in a 48-hour test. Results were treated statisticaly by the "t"-test of Student (Documenta Geigy 1977).

3. Results

The ASA activity in the homogenates of *Euphausia superba* and *E. crystallorophias* is shown in Tables I-VI. The enzyme activity was expressed in nM of 4-NC per mg of protein. There is no difference in the enzyme activity in *E. superba* incubated at the temp. −1 or +1 C. The animals were not incubated in other temperatures because of their inadequacy for *Euphausia*. The mean temperature (summer) of the Ezcurra Inlet water is 0.07—0.51 C.

Table I.

ASA activity in the homogenates of *Euphausia superba* after the animals have been exposed to 5 ppm of some pollutants, temp. −1 C. Enzyme activity expressed in nM of 4-NC/mg protein

Pollutant used	Hours of animals incubation					
	1	6	12	24	48	96
Control	1.79	1.87	1.87	1.86	1.79	1.86
± S. D.	0.08	0.07	0.07	0.06	0.08	0.07
Light fuel oil	1.69	1.64	1.48	1.56	1.67	1.78
± S. D.	0.05	0.10 b	0.06 a	0.08 a	0.06	0.03
Heavy fuel oil	1.87	1.73	1.64	1.68	1.72	1.72
± S. D.	0.07	0.05 b	0.05 a	0.04 b	0.08	0.04 b
Crude oil	1.89	1.86	1.78	1.78	1.78	1.79
± S. D.	0.05	0.02	0.03	0.03	0.01	0.01
Detergent ABS	2.86	1.76	1.46	1.17	1.13	1.02
± S. D.	0.06 a	0.03	0.04 a	0.03 a	0.03 a	0.02 a

Difference significant at the level of: a — p 0.005 b — 0.025 p 0.01

Table II.

ASA activity in the homogenates of *Euphausia superba* after the animals have been exposed to 50 ppm of some pollutants, temp. −1°C. Enzyme activity expressed in nM of 4-NC/mg protein

Pollutant used	Hours of animals incubation					
	1	6	12	24	48	96
Control	1.79	1.87	1.87	1.86	1.79	1.86
± S. D.	0.08	0.07	0.07	0.06	0.08	0.07
Light fuel oil	0.87	1.25	1.22	1.19	1.29	1.29
± S. D.	0.04 a	0.08 a	0.06 a	0.08 a	0.04 a	0.02 a
Heavy fuel oil	1.34	1.37	1.46	1.33	1.44	1.42
± S. D.	0.03 a	0.07 a	0.05 a	0.04 a	0.04 a	0.04 a
Crude oil	1.65	1.62	1.57	1.49	1.50	1.48
± S. D.	0.03 b	0.02 a	0.01 a	0.01 a	0.01 a	0.01 a
Detergent ABS	2.59	1.64	1.28	1.10	0.93	0.75
± S. D.	0.06 a	0.03 a	0.02 a	0.03 a	0.02 a	0.02 a

Difference significant at the level of: a — $p < 0.005$ b — $0.025 < p < 0.01$

Table III.

ASA activity in the homogenates of *Euphausia superba* after the animals have been exposed to 5 ppm of some pollutants, temp. +1°C. Enzyme activity expressed in nM of 4-NC/mg protein

Pollutant used	Hours of animals incubation					
	1	6	12	24	48	96
Control	1.79	1.80	1.76	1.81	1.81	1.79
± S. D.	0.06	0.06	0.06	0.07	0.06	0.06
Light fuel oil	1.71	1.63	1.36	1.38	1.58	1.66
± S. D.	0.05	0.06 b	0.05 a	0.05 a	0.07 a	0.07 a
Detergent ABS	2.64	1.67	1.40	1.18	1.13	1.03
± S. D.	0.08 a	0.05 b	0.05 a	0.04 a	0.04 a	0.03 a

Difference significant at the level of: a — $p < 0.005$ b — $0.025 < p < 0.01$

After one hour of incubation of *E. superba* the light fuel oil decreased the enzyme activity by 52% in both temperatures (Table II and IV). After longer periods of incubation in this pollutant the enzyme activity increase again but even after 96 hours of incubation this increase was not very high and at both temperatures the activity was by about 30% lower than in control animals.

Exposure to the detergent gave inverse effects: after one hour of incubation an increase of the enzyme activity was observed in both concentrations of the pollutant, but higher ASA activity has been found in animals exposed to 5 ppm of detergent. In both temperatures the ASA activity was higher by about 50% at 5 ppm and by 40% at 50 ppm of the detergent (Ta-

Table IV.

ASA activity in the homogenates of *Euphausia superba* after the animals have been exposed to 50 ppm of some pollutants, temp. +1°C. Enzyme activity expressed in nM of 4-NC/mg protein

Pollutant used	Hours of animals incubation					
	1	6	12	24	48	96
Control	1.79	1.80	1.76	1.81	1.81	1.79
± S. D.	0.06	0.06	0.06	0.07	0.06	0.06
Light fuel oil	0.89	1.07	1.08	1.14	1.20	1.20
± S. D.	0.04 a	0.06 a	0.05 a	0.06 a	0.05 a	0.05 a
Detergent ABS	2.50	1.55	1.25	1.13	0.98	0.80
± S. D.	0.08 a	0.06 a	0.05 a	0.05 a	0.04 a	0.04 a

Difference significant at the level of: a — $p < 0.005$

Table V.

ASA activity in the homogenates of *Euphausia crystallorophias* after the animals have been exposed to 5 ppm of some pollutants, temp. −1°C. Enzymy activity expressed in nM of 4-NC/mg protein

Pollutant used	Hours of animals incubation					
	1	6	12	24	48	96
Control	2.39	2.34	2.29	2.36	2.41	2.28
±S.D.	0.05	0.06	0.07	0.06	0.09	0.08
Light fuel oil	2.34	2.08	1.91	1.61	1.47	1.52
±S.D.	0.09	0.10 a	0.04 a	0.09 a	0.06 a	0.05 a
Heavy fuel oil	2.39	2.11	2.02	2.12	2.17	2.10
±S.D.	0.09	0.06 a	0.06 a	0.07 b	0.07 a	0.09 a
Crude oil	2.41	2.39	2.18	2.30	2.34	2.21
±S.D.	0.06	0.07	0.05	0.06	0.06	0.07
Detergent ABS	3.46	2.45	1.83	1.53	1.57	1.30
±S.D.	0.08 a	0.08	0.04 a	0.05 a	0.05 a	0.04 a

Difference significant at the level of: a — $p < 0.005$, b — $0.025 < p < 0.01$

ble I—IV). After 6-hour or longer incubation a decrease of ASA activity was noted. After 96 hours of incubation the decrease of activity in both temperatures was 50% at 5 ppm and 60% at 50 ppm of the detergent. The ASA activity in the control animals was significantly higher in the *E. crystallorophias* than in *E. superba* (Table V and I). The interspecies differences of the arylsulphatase activity of the hepatopancreas in the genus *Euphausia* have been found by Drewa and Jackowska (1980). All pollutants used in our experiments gave similar effects in both species of *Euphausia* (Table I—VI). The ASA activity in homogenates of *E. crystallorophias* incubated in solutions of the detergent was initially higher in both

Table VI.

ASA activity in the homogenates of *Euphausia crystallorophias* after the animals have been exposed to 50 ppm of some pollutants, temp. −1°C. Enzyme activity expressed in nM of 4-NC/mg protein

Pollutant used	Hours of animals incubation					
	1	6	12	24	48	96
Control	2.39	2.34	2.29	2.36	2.41	2.28
± S. D.	0.05	0.06	0.07	0.06	0.09	0.08
Light fuel oil	1.25	1.91	1.61	1.31	1.19	1.07
± S. D.	0.08 a	0.07 a	0.03 a	0.03 a	0.03 a	0.03 a
Heavy fuel oil	1.77	1.71	1.70	1.77	1.81	1.71
± S. D.	0.05 a	0.06 a	0.06 a	0.08 a	0.07 a	0.07 a
Crude oil	2.10	2.05	1.92	1.93	1.94	1.83
± S. D.	0.07 b	0.06 b	0.05 a	0.07 a	0.08 a	0.06 a
Detergent ABS	3.13	2.04	1.63	1.37	1.24	1.03
± S. D.	0.06 a	0.07 b	0.05 a	0.05 a	0.06 a	0.05 a

Difference significant at the level of: a $p < 0.005$ b $0.025 < p < 0.01$

Table VII.

Increase of absorbance (750nm) in krill tissues after 24 hours exposure to pollutants of 50 ppm concentration with 0.0001% trypan blue added

Pollutants	Hepatopancreas	Abdomen muscles
Control	100.0%	100.0%
Light fuel oil	131.2%	103.8%
Heavy fuel oil	120.1%	104.3%
Crude oil	114.0%	102.8%
Detergent ABS	116.7%	108.6%

concentrations but later a decrease of the enzyme activity has been observed. The decrease in the enzyme activity was stronger after the incubation of animals in the light fuel oil than in the heavy fuel oil and crude oil.

Table VII shows an increase of the enzyme activity in krill tissues after animals exposed to pollutants at 50 ppm for 24 hours in the presence of 0.0001% Trypan blue. It was found that most of the stain cumulated in hepatopancreas and lesser amounts in the muscles of the abdomen. Highly significant differences occurred between animals exposed to detergent and oils. The results of experiments on the detergent action *in vitro* confirmed both the high sensitivity of lysosomal membrane system to this pollutant and a different mode its action as compared to that of crude oil and fuel oils (Table VIII).

Table VIII.

ASA activity in vitro in homogenates incubated 30 min. with pollutants

Pollutants	5 ppm	50 ppm
Control	100.0%	100.0%
Light fuel oil	112.4%	177.2%
Heavy fuel oil	108.4%	124.9%
Crude oil	108.4%	140.2%
Detergent ABS	85.2%	62.9%

The following LC_{50} values for detergent, crude oil and light and heavy fuel oils were obtained: 200 ppm, 700 ppm, 500 ppm and 550 ppm.

4. Discussion

According to many papers the water soluble fraction of crude oil or fuel oil was usually added to experimental tanks (Bigford 1977, Cucci and Epifiano 1979). These water soluble components belong to volatile and nonvolatile compounds. Volatile compounds evaporate more quickly and are more toxic and theirs solubility in water is lesser. Nonvolatile compounds are better soluble in water, their effect on the water organisms is longer and appears later. Under static conditions, even if the tank content is changed every 24 hours, some oil components were lost due to adsorption and evaporation (25 to 50%) Cucci and Epifiano 1979, Van Der Linden (1978).

In the present work it seemed more reasonable to add the crude oil or fuel oils directly to experimental tanks. During the whole period of experiments the water was stirred in order to assure the mixing up of water with the pollutants.

In many reports different results have been obtained even after using the same crude oil at the same concentration. Therefore Mann (1976) suggested to use the same general conditions for testing of toxic substances to unify the experiments and obtain comparable results. These data should comprise the dimensions and age of animals, the duration of the experiment and the concentration of pollutants. Salinity and temperature should meet the optimal conditions in which the animals live. Taking into consideration all mentioned above terms, reservations, factors is not easy to compare our results those of other authors.

The pollutants used cause inactivation of the enzyme during the first hours of experiments. It may be supposed that in such a short time the labilization of biological membranes takes place under the influence of oil hydrocarbons as well as under the influence of the detergent. Rossi and Anderson (1977) have observed that *Neanthos arenaceo-*

dentata (Polychaeta) incorporate aromatic hydroconbons after 1 hour of exposure to these pollutants. After one hour of exposure the saturation of tissue with these compounds has been maximal. No significant differences has been found in the ASA activity between the control and experimental krill exposed to 5 ppm of crude and fuel oils. But exposure of the krill to 50 ppm, particularly of the light fuel oil resulted in significant differences in enzyme activity. The aromatic hydrocarbons penetrate into the organism through the whole integumentum, through the digestive system and through the respiratory epithelium. The results of Lee, Ryan and Neuhauser (1976) support this assumption. The crab *Callinectes sapidus* assimilated 2.5% of the labelled aromatic hydrocarbons (benz/a/pyrene and methylcholanthrene) and the rest was excreted. Some 50% of the absorbed hydrocarbons have been found in the hepatopancreas, the rest in other tissues. Light and heavy fuel oils are more toxic crude oil because of higher concentration of di- and tricyclic aromatic hydrocarbons as compared to crude oil.

Crude oil and fuel oils are mixtures of different compounds. Beside hydrocarbons in the crude oil there are many compounds containing sulfur and nitrogen. The crude oil toxicity for water animals results from the presence of aromatic hydrocarbons. The low molecular weight hydrocarbons are aromatic hydrocarbons. The low molecular weight hydrocarbons are responsible for the acute toxicity whereas the high molecular weight ones are responsible for the chronic toxicity (Lee and Nicol 1978). The more rings are present in the molecule of the hydrocarbon, the more toxic is the compound for animals (Laughlin and Neff 1979).

Under normal conditions the animals are exposed to a very broad range of salinity and temperature. The increase of temperature is followed by higher toxic effects of pollutants (Laughlin and Neff 1979). Similar effects exerted by temperature on the toxicity of pollutants have been reported in Mann's paper (Mann 1962b).

In our experiment no difference has been found in the ASA activity in animals incubated in solutions of pollutants at different temperatures. It may be supposed that the experimental temperatures did not exceed the normal temperatures typical of the environment of krill.

The changes in ASA activity under the influence of crude oil or detergent have probably been the result of the membrane labilization. The influence of pollutants on the enzyme activity varied. According to Kurelec et al. (1977) the exposure of *Clupea pilchardus* and *Salmo trutta* to the fuel oil has enhanced benz/a/pyrene monoxidase activity in *Clupea pilchardus* and *Salmo trutta* living in the region of disaster increased by about 450% and 900% respectively (Kurelec et al. 1977).

The aromatic hydrocarbons of crude oil and detergents may inhibit or enhance the enzyme activities. After one hour of exposure an increase of ASA activity has been observed but after a long period of incubation the enzyme activity decreased. It is difficult to verity the hypothesis that the initial increase of the ASA activity is the result of the enzyme induction. Perhaps the rise in the enzyme activity is the effect of destroying of the lysosomal membranes.

The hydrophobic groups of detergents attach to the lipid components of biological membranes. The hydrophilic groups of detergent turn to the water phase. This disorganization in membranes structure leads to protein denaturation membrane labilization. In consequence the lysosomal hydrolases leak to the cytoplasm (Lang 1967). The longer is the exposure to the solutions of detergent, the greater is the described effect: after 96 hours of exposure of animals to 50 ppm of the detergent the ASA activity decreased down to 40% of the control value (Table II, IV and VI).

The enzyme inactivation by hydrocarbons has not been so high as that by the detergent. The highest enzyme inactivation was observed in the solutions of light fuel oil. The longer as the period of incubation of animals in the solutions of crude oil, the higher was the ASA activity in the homogenates but even after 96 hours of incubation the enzyme activity was lower in experimental animals as compared to the control krill.

It may be supposed that the incubation of krill in the environment containing crude oil fraction mobilizes the defence mechanisms. The increase of resistance of the crab *Eurypanopeus depressus* larvae exposed to water soluble fraction of Kuwait crude oil for a long period has been found by Cucci and Epifiano (1979). It has been found that the rate of mortality of *zoea* stage III and IV decreased if the larvae of the stage I and II were exposed to water soluble crude oil fraction prior to the action of this pollutant.

The experiments with the detergent or the fuel oils added to the krill homogenates support the supposition that pollutants labilize lysosomal membranes. The addition of the detergent to the homogenates in concentrations of 5 or 50 ppm has inhibited the enzyme activity by 85% and 63% respectively. Both detergent concentrations increased the enzyme activity *in vivo* as well *in vitro* after one hour of incubation and decreased its activity after the longer period of incubation.

The crude oil hydrocarbons increase the ASA activity *in vitro* by 8% and 40% respectively. The results are different from those obtained *in vivo*. The decrease of the ASA activity *in vivo* resulted probably from the labilization of lysosomal membranes and leaking of the enzymes from lysosomes with simultaneous inhibition of the enzyme synthesis *de novo*.

The experiments with Trypan blue support the view that pollutants damage the lysosomal membranes. Trypan blue was added to the incubation milieu of animals. After 24 hours of incubation there has been found 3% to 8% of this dye in the experimental animals. The dye penetration into the organisms has been the highest after exposure to solution of detergent and light fuel oil and the lowest in animals incubated in solutions of crude oil. It has been found that the first two pollutants i.e. detergent and light fuel oil react easily with the lipid components of biological membranes and increase their permeability (Drewa, Zbytniewski and Pautsch 1977). LC_{50} of the tested pollutants supplied evidence that detergent and light fuel oil are more toxic than crude oil or heavy fuel oil. The aim of the present work is to call attention to the possibility of toxic effects of pollutants in antarctic waters. It is well known (Neff et al. 1976, Rossi and Anderson 1977) that aromatic

hydrocarbons as well as detergents are responsible for disturbances of embryonal development and physiological processes of some marine animal species.

5. Conclusions

1. The changes of the ASA activity and the LC_{50} values indicate that in our experiments the detergent ABS and light fuel oil are the strongest toxicants.

2. Over the temperature range from -1 °C to $+1$ °C there was no difference of the ASA activity in krill exposed to pollutants in the concentrations of 5 and 50 ppm.

3. The detergent and crude oil hydrocarbons decrease the ASA activity, except that after one hour exposure of krill to the detergent the enzyme activity increases.

4. *In vitro*, the crude oil and fuel oils increase the enzyme activity, but the detergent is an inactivator of ASA.

5. The changes of ASA activity are the result of labilization of lysosomal membranes. This has been confirmed by the fact that the dye Trypan blue is cumulated to a higher degree — as compared to control experiment — in the hepatopancreas of the krill after it has been exposed to pollutants wit addition of the dye.

6. Summary

Euphausia superba and *E. crystallorophias* have been incubated in water with crude oil, light and heavy fuel oils as well as with the detergent ABS in concentrations of 5 and 50 ppm. After 1, 6, 12, 24, 48 and 96 hours of exposure the ASA activity has been estimated in krill homogenates.

The detergent ABS increases the ASA activity after the first hour of krill exposure in both concentrations (Table I—VI). After this time an evident decrease of activity occurs. On the other hand crude oil and its fractions cause ASA inactivation alerady after the first hours of experiment. The experiments were carried out at two different temperatures: -1 C and $+1$ C. Similar results have been achieved for *E. superba* and *E. crystallorophias* in both temperatures.

In Table VII the increase of the Trypan blue accumulation has been shown in hepatopancreas and muscles of the krill exposed 24 hours to the pollutants in concentration of 50 ppm with 0.0001% of Trypan blue. Most of the dye was cumulated in hepatopancreas, less in the abdomen muscles. The highest difference was found after incubation with the detergent and light fuel oil.

The results of the experiments *in vitro* with the detergent are similar (Table VIII).

LC_{50} (48 hour test) has been estimated for the detergent, crude oil, light and heavy fuel oil and following values have been obtained respectively: 200 ppm, 700 ppm, 500 ppm and 550 ppm for *E. superba*.

Crude oil hydrocarbons and the detergent labilize the biological membranes, among them the lysosomal membranes. As a result of the destruction of the lipid structure of membranes the acid hydrolases are released to the cytoplasm. The ASA inactivation under the influence of the used pollutants is probably the result of the enzyme release and the inhibition of the enzyme synthesis *de novo*.

7. Резюме

Вставлено крыля *E. superba* и *E. crystallorophius* в воду с примесью нефти, лёгкого и тяжелого приводного масла и детергента ABS в сгущениях 5 и 50 ppm. После 1, 6, 12, 24 и 96 часов экспозиции обозначено активность асылосульфата (ASA) в гомогентах из крыля.

Детергент ABS вызывает увеличение активности ASA после первого часа экспозиции крыля в обоих сгущениях а после этого периода выступает понижение активности энзима (таблица I—VI). Нефть и её две фракции вызывают инактивацию ASA уже в первых часах опыта. Опыт проведено в двух вариантах температур: в температуре $-1°C$ и $+1°C$. Такой же эффект после применения потулянтов получено для *E. superba* и *E. crystallorophias* в обоих вариантах температур.

В таблице VII представлено рост активности ASA в процентах в мышцах крыля после сутичной инкубации этих животных в полютантах в сгущении 50 ppm с примесью лазури, трипана в 0,0001%. Наиболее красителя было нагромождено в печёнко-желудочных частях и немного в брюшковых мылцах. Самые большие разницы констатировано после детергента и легкого приводного масла. *In vitro* детергент понижает активность энзима (таблица VIII).

После 48 часов обозначено LC_{50} для *E. superba* инкубованных в детергенте, нефти, лётком и тяжелом приводном масле. Эти величины достигают соответственно: 200 ppm, 700 ppm, 500 ppm и 550 ppm. Углеводороды выступающие в нефти а также детергент лабилизуют биологические плёнки. Из-за ветшания лицидовой структуры дизосомальных плёнок наступает освобождение кислых гидроляз от цитоплязмы. Понижение активности ASA у крыля под влиянием применяемых полютантов является вероятно итогом онактивации энзима полютантами, освобождением энзима от цитоплязмы или задержанием его синтеза *de novo*.

8. Streszczenie

Eksponowano kryla *E. superba* i *E. crystallorophias* w wodzie z dodatkiem ropy naftowej, lekkiego i ciężkiego oleju napędowego i detergentu ABS w stężeniach 5 i 50 ppm. Po 1, 6, 12, 24, 48 i 96 godzinach ekspozycji oznaczono aktywność arylosulfatazy (ASA) w homogenatach z kryla.

Detergent ABS powoduje zwiększenie aktywności ASA po pierwszej godzinie ekspozycji kryla w obu stężeniach, a po tym okresie następuje spadek aktywności enzymu (tabela I—VI) Ropa naftowa i jej dwie frakcje powodują inaktywację ASA już po pierwszych godzinach doświadczenia. Doświadczenie przeprowadzono w dwóch wariantach temperatur: w temperaturze $-1°C$ i $+1°C$. Podobny efekt po stosowaniu polutantów uzyskano dla *E. superba* i *E. crystallorophias* w obu wariantach temperatur.

W tabeli VII przedstawiono przyrost aktywności ASA w procentach, w tkankach kryla po 24 godzinnej inkubacji tych zwierząt w polutantach w stężeniu 50 ppm z dodatkiem błękitu trypanu w ilości 0.0001%. Najwięcej barwnika kumulowało w wątrobotrzustkach, mniej w mięśniach odwłokowych. Największe różnice stwierdzono po detergencie i lekkim oleju napędowym. *In vitro* detergent obniża aktywność enzymu (tabela VIII).

Po 48 godzinach wyznaczono LC_{50} dla *E. superba* inkubowanych w detergencie, ropie naftowej, lekkim i ciężkim oleju napędowym. Wartości te wynoszą odpowiednio: 200 ppm, 700 ppm, 500 ppm i 550 ppm.

Węglowodory zawarte w ropie naftowej oraz detergent labilizują błony biologiczne, w tym również błony lizosomalne. Wskutek niszczenia struktury lipidowej błon lizosomalnych, następuje uwalnianie kwaśnych hydrolaz do cytoplazmy. Spadek aktywności ASA u kryla pod wpływem stosowanych polutantów jest prawdopodobnie wynikiem inaktywacji enzymu przez polutanty, uwalnianiem enzymu do cytoplazmy lub zahamowaniem jego syntezy *de novo*.

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AUTHOR'S ADRESS:
Dr Gerard Drewa
Zakład Biologii Instytutu
Biologii Medycznej
Akademia Medyczna
Dębniki 1a
80-210 Gdańsk, Poland