radioactivity eluted as parent compound and 10.6% eluted as the metabolite. Shark urinary metabolite when analyzed by HVPE or by paper chromatography showed similarity to the urinary metabolites of dog, monkey, rat and mouse. It was ninhydrin negative, unlike alanosine and had retained the UV absorption characteristics of the parent compound. Since the urinary metabolite and brain metabolite behaved similarly on anion exchange column, we presume that the brain metabolite shares these characteristics as well.

The observation that the majority of the radioactivity present in the brain is in the form of the parent compound diminished the possibility that a metabolite less soluble than the urinary metabolite or the parent compound was responsible for accumulation of radioactivity in the brain. Hence, a few simple experiments were conducted to find out if the bulk of the radioactivity (present as the parent compound) is retained by any preferential binding of the same to the macromolecules. Twelve thousand g supernatant of the brain carried 67% of the total radioactivity and the rest was with the pellet. Six percent of the radioactivity associated with the pellet could further be released if the homogenate was made 5% with respect to PCA prior to centrifugation. An additional 15% of this associated radioactivity could be removed after repeated washing of the pellet with methanol. These figures indicate that only a low percentage of the parent compound and/or metabolite is actually associated with and presumably incorporated into proteins or nucleic acids. Exhaustive dialysis of the brain homogenate indicated that about 70% could be dialyzed out. The amount of non-dialyzable radioactivity is once again the same as that associated with the pellet. It was therefore necessary to find out whether the residual radioactivity associated with the pellet was due to any preferential binding of the drug to macromolecules present in the brain. This was achieved by doing an equilibrium dialysis experiment. Control brain extract was dialyzed against buffer containing DL-1-[^{14}D]alanosine. Analysis of the contents of the bag and the dialysate showed that there was no preferential binding of the drug to the macromolecules of the shark brain. Non-dialyzable radioactivity is therefore associated with macromolecules by interactions which are not ionic and different from other non-covalent bindings.

Though more lipophilic forms have a better chance of being retained in the brain, small molecules similar to amino acids have good chances of entering the brain. In fact, the behavior of L-alanosine in neonatal mouse brain causing hypothalamic lesions similar to the ones caused by high doses of L-aspartic and L-glutamic acids (Proc. Amer. Assoc. Cancer Res. 18:219, 1977) indicates how similar this drug is to these amino acids. Though the present analysis does not say the final word on the mechanism of retention of L-alanosine, however, it has opened up possibilities for water soluble drugs for effective treatment versus brain tumor. Nonetheless, on the strength of the efficacy of this drug against mouse brain tumor and these results reported here, it has been recommended by the Drug Development Program of NCI for early clinical trials against human brain tumors.

EFFECTS OF WATER SOLUBLE FRACTIONS FROM CRUDE OILS ON DEVELOPMENT OF SAND DOLLAR EMBRYOS

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Until the supplies of petroleum are exhausted, marine life will be threatened periodically by accidental spillage of crude oil. Therefore it is pertinent that effects of water-soluble components from these oils be observed in a variety of marine organisms. The developing echinoderm embryo is a potentially useful one for determining effects of pollutants, since the eggs and sperm can be readily obtained and the embryo can be reared under controlled conditions.

For the studies reported here gametes were obtained from the sand dollar, *Echinarachnius parma*. after injection of about 1 ml of 0.5 M KCl into the mouth area of the animal. Eggs from a single female were washed in a 250 ml beaker and then fertilized by adding 10 drops of a 1% suspension of sperm. Incubation embryo suspensions was carried out in filtered sea water at 16°. Data from an experiment were accepted on

if the developmental success of the controls was 80% or better. Observations of the developing embryos were made at times appropriate to detect effects on first cleavage, regularity of early cleavages, cell shapes and sizes during early cleavage, rate of early development, completion of the blastula, hatching of the blastula, and developmental anomalies of the gastrula, prism and pluteus.

Water soluble fractions (WSF) were obtained from samples of Kuwait crude oil (KC), South Louisiana crude oil (SLC), and a Venezuelan bunker C residual oil (BC). These fractions were prepared by stirring vigorously in a magnetic stirrer 1 part of oil with 9 parts of filtered sea water for 20 hours. Upon separation of the phases, the aqueous layer was obtained and used.

In order to observe effects generally, eggs were fertilized in the WSF and remained in it throughout the duration of the experiment. The results of this treatment can be seen in Table 1. Kuwait crude WSF at full strength did not allow development beyond the blastula. Lower levels had no effect on development. On the other hand, SLC and BC at full and half strength of the WSF did not allow first cleavage. Less effects were noted from exposure to 10% WSF of SLC and BC. Clearly, these aqueous extracts of oils produced serious effects on development and the effects due to KC were different from those of SLC and BC.

TABLE 1

Effects of water soluble fractions from crude oils on sand dollar development

Concentration of WSF[a]	Effects on Development[b]		
	Kuwait Crude	S. Louisiana Crude	Bunker C
100%	Blastular arrest	No cleavage	No cleavage
50%	None	No cleavage	No cleavage
10%	None	70% cleavage 20% survive to pluteus	80% cleavage All survive to pluteus
1%	None	None	None

[a]Eggs were fertilized in WSF and remained in that medium for the duration of the experiment.

[b]Development was observed until the controls reached the late pluteus stage (72 hrs.)

Experiments were performed to determine the relationship between effect of WSF and amount of embryogenesis which preceded its introduction. Embryos were placed in WSF from SLC and BC 9 minutes post fertilization. The results (Table 2) were identical to those obtained when the eggs were fertilized in the WSF solutions. These experiments were repeated, delaying the WSF exposure until 60 minutes post fertilization, until immediately after first cleavage (105 min), and until the blastula was formed (20 hrs). As seen in Table 2, no cleavage or further development occurred after embryos were placed in 50% and 100% WSF from SLC and BC. Embryos placed in 100% WSF from BC and SLC at the blastula stage exhibited arrest of development and death. Thus it appears that the SLC and BC extracts arrest sand dollar development at whatever stage they are introduced.

This type of effect on echinoderm development is similar to effects observed from inhibitors of aerobic ATP synthesis. As seen in Table 3, the uncoupling agent 2,4-dinitrophenol (DNP) prevents cleavage and development at levels of 10^{-3} M and 10^{-4} M. At lower concentrations, more development is allowed but ultimately none of the embryos survived. If embryos at the blastula stage are exposed to 10^{-3} M DNP, development is arrested as had been shown with WSF from SLC and BC.

Finally, the reversibility or extent of recovery from these effects was noted. Embryos were placed in the toxic medium 1 hour post fertilization and then removed to normal sea water medium 1 hour later. In

TABLE 2

Effects on WSF of sand dollar development using various dose initiation times

WSF		Time after fertilization embryos placed in WSF			
		9 min	60 min	105 min[a]	20 hrs[b]
SLC	100%	No cleavage	No cleavage	Arrested-2 cells	Blastular arrest
	50%	No cleavage	No cleavage	Arrested-2 cells	--
	10%	20% develop to pluteus	--[c]	--	--
	1%	No effect	--	--	--
BC	100%	No cleavage	No cleavage	Arrested-2 cells	Blastular arrest
	50%	No cleavage	No cleavage	Arrested-2 cells	--
	10%	80% develop to pluteus	--	--	--
	1%	No effect	--	--	--

[a]At 105 min, first cleavage had just occurred.

[b]At 20 hrs, the blastula was formed.

[c]Dashed lines indicate experiments were not run at that concentration.

TABLE 3

Effect of dinitrophenol on sand dollar development

DNP[a]	Developmental effects[b]
10 M	No cleavage
10 M	No cleavage
10 M	85% arrested blastulae, all dead at 2 days
10 M	30% arrested blastulae, all dead at 2 days
10 M	30% arrested blastulae, all dead at 2 days

[a]Embryos were placed in DNP solutions 9 min post-fertilization.

[b]Development was observed through pluteus stage in the controls (55 hrs)

In Table 4, it can be seen that with 50% WSF from SLC and BC and with DNP some reversibility occurred. Again it should be noted that the effects from DNP are similar to those caused by the oil samples. It is reasonable to speculate that the WSF from SLC and BC may be acting on the sand dollar embryo to inhibit aerobic energetics metabolism.

Samples of crude oils were kindly provided by the American Petroleum Institute.

TABLE 4

Reversibility of effects of oil WSF and DNP on sand dollar development

Medium[a]		Developmental Effects
SLC	100% WSF	No cleavage
	50% WSF	75% formed blastulae, then died
BC	100% WSF	No cleavage
	50% WSF	75% formed blastulae, then died
DNP	10^{-3} M	Slowed development to abnormal gastrula, then died
	10^{-4} M	Slowed development to abnormal late gastrula, then died

[a]Embryos were placed in toxic medium 1 hour post-fertilization and removed to sea water 1 hour later.

EFFECT OF DILUTION ON BETA-ALANINE FLUXES IN SKELETAL MUSCLE OF *Raja erinacea*

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When marine elasmobranchs enter an environment more dilute than sea water, the solute concentrations in both the extracellular and intracellular fluids are markedly reduced (Forster and Goldstein, Am. J. Physiol. 230:925-931, 1976). Several amino acids are among the intracellular solutes whose concentration is most markedly reduced. This finding raises the question of whether a change in the transport mechanism for amino acids is involved in the cellular response to dilution.

In this communication I describe an initial series of experiments in which the effects of changing extracellular osmolarity on the fluxes of beta-alanine across the muscle membrane were determined. These fluxes were selected for study because it has been found in skate muscle (Boyd, Cha, Forster and Goldstein, J. Exp. Zool. 199:435-442, 1977) that this is one of the amino acids whose concentration is more drastically reduced after dilution, moreover it appears that this amino acid is not metabolized by muscle tissue (Goldstein, Personal Communication).

Bundles of muscle fibres were obtained from the pelvic fin of *Raja erinacea*. These muscles run between two tendons that can be readily dissected. To obtain pieces of tissue suitable for flux experiments bundles of fibres weighing less than 100 mg were isolated under the dissecting microscope. Care was taken to remove all the fibres that appeared damaged. The electrical excitability of the bundles was checked after dissection and at the end of the experiment.

For the flux determinations the bundles were first incubated in 4 ml of a labeled amino acid solution for one hour. Immediately after loading the muscles were soaked in a series of tubes containing 3 ml of nonradioactive solution for carefully timed intervals. The solutions were then counted to determine the radioactivity leaving the muscle during each period. At the end of the experiment the muscles were weighed and prepared for counting by dissolving them in 0.3 ml of Nuclear Chicago Solvent. By adding up in reverse order the corrected counts of the effluents to the radioactivity remaining in the bundle at the end of the experiment it was possible to determine the radioactivity present in the muscle at the midtime of each collecting period. Graphical analysis of these results (see Figure 1) showed, that the efflux process is made up of the sum of two exponentially decaying components. This analysis taken together with previous measurements of isotope washout from skeletal muscle (Harris, J. Physiol. 166:87-98, 1963) indicates that the fast component corresponds to washout of the labeled amino acid from the extracellular space while the slow component represents efflux from the intracellular compartment. According to this analysis the extrapolated intercept of the slow component corresponds to the amount of intracellular amino acids at the end of the incubation in the loading solution.