Figure 1 illustrates an experiment in which we added adenosine to the perfusion fluid to reach a concentration of 10^{-4} M. The addition of adenosine rapidly increases the volume of rectal gland secretion, in this case reaching about 20 times the resting level. As the data summarized in Table 1 show, the increase in the rate of secretion caused by adenosine was already discernible when 1 x 10^{-6} M adenosine was employed. Maximum stimulation was observed with 10^{-4} M adenosine.

TABLE 1

Dose dependence of the effects of adenosine on the volume of fluid secretion (ml/hr/g wet weight)

Control	10^{-6} M	5 x 10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M
0.032	0.062	-	2.17	3.95	3.78
0.040	0.073	0.56	-	2.38	2.46
0.041	-	0.81	1.43	4.66	-
0.035	0.069	-	0.85	1.72	-

Analysis of the fluid emerging from the secretory duct indicates that during the action of adenosine, the composition of the secreted fluid is barely modified. The Cl- content during the control period was 498.7 ± 62.1 mEq/liter (n=18), while during stimulation it was 501 ± 98.1 mEq/liter.

Figure 2 illustrates one of the experiments performed to test whether other adenosine derivatives would also increase the rate of secretion. Two points are clearly shown: First, while inosine had a stimulatory effect; the stimulation was minor when compared with the effects of adenosine. Indeed, 10^{-3} M inosine was necessary to produce effects similar to those obtained with 5 x 10^{-6} M adenosine. Second, ATP had large stimulatory effects; in the illustration, 10^{-3} M was used, but in other experiments we found that as little as 10^{-4} M produced a clear-cut but smaller stimulation. However, when 10^{-5} M were used, no effects were observed. Analysis of the secreted fluids collected in these experiments showed that, as in the case of adenosine, the Cl- and Na concentration during the action of the chemicals was nearly the same as those found during the resting condition.

Our results show that the rate of transport by the salt gland is markedly enhanced by both adenosine and ATP. The fact that the former is more effective than the latter, and that the effective concentrations of both agents are small compared with the levels of ATP in most cells, suggest that they do not constitute a direct source of energy for the transport process. One of several attractive possibilities is to suppose that as in other tissues (Ann. Rev. Biochem. 47:655-686, 1978) purine derivatives combine with specific receptors.

Such a possibility ought to be explored further since adenosine is an ubiquitous substance that could have an important role as a mediator in the regulation of salt and water metabolism in various epithelia. Supported by the New York Heart Association.

DISTRIBUTION OF ^{14}C-BENZO(a)PYRENE IN THE LOBSTER, *Homarus americanus*, AT VARIOUS TIMES AFTER A SINGLE INJECTION INTO THE PERICARDIAL SINUS

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Certain polycyclic aromatic hydrocarbons (PAH) that occur in high boiling fractions of crude and refined petroleum have been implicated as carcinogens, mutagens, cytotoxins, and immune suppressants in various marine vertebrate and invertebrate species (Effects of Petroleum on Arctic and Subarctic Marine Environments and Organisms, Vol. II, 1977, Academic Press). Previous studies (for example, Dunn and Stich, Bull. Environ. Cont. Toxicol. 15:398, 1976) have shown that marine invertebrates accumulate benzo(a)pyrene, a PAH which occurs as a widespread pollutant, from the water column. Consequently, we have investigated the disposition of ^{14}C-benzo(a)pyrene in the lobster, a marine crustacean that is an important food source. We were particularly interested in the tissues that were the major storage sites for this PAH, and whether or not the hydrocarbon residues were unchanged benzo(a)pyrene or some of its oxidative metabolites.

Male and female hard-shelled lobsters, 310-600 g, were purchased locally. [7,10-^{14}C]Benzo(a)pyrene (^{14}C-BP) (60.7 mCi/mmole) was supplied by Amersham-Searle and purified by elution through an alumina column to 99.5% radiochemical purity as demonstrated by high pressure liquid chromatographic analysis (HPLC). The radiolabeled benzo(a)pyrene was diluted with unlabeled benzo(a)pyrene (Aldrich, Gold Label) prior to use, dissolved in Emulphor:dimethyl sulfoxide:water (1:1:8 by volume), and administered by injection into the pericardial sinus. Each lobster received 1 mg ^{14}C-BP (3.7 μCi)/kg. After treatment the dosed animals were placed in lobster pots immersed in a 1000 gallon holding tank equipped with flowing seawater (12-15°C).

TABLE 1

Specific activity of various lobster tissues or fluid following the pericardial injection of ^{14}C-benzo(a)pyrene (1 mg/kg)

dpm/mg tissue or/μl plasma
Time after administration

Tissue	1 Day	3 Day	7 Day	2 Week	4 Week	6.5 Week
Hepatopancreas	133.7 ± 12.2*	130.1 ± 28.1	137.6 ± 15.9	113.8 ± 48.0	107.7 ± 3.6	72.9 ± 16.6
Green gland	13.2 ± 2.0	9.5 ± 2.4	9.3 ± 1.3	13.8 ± 3.5	8.4 ± 2.1	7.5 ± 1.9
Intestine	8.8 ± 0.6	9.3 ± 2.0	7.2 ± 4.1	11.8 ± 2.9	8.7 ± 2.8	8.5 ± 2.5
Heart	9.6 ± 4.5	4.5 ± 1.5	5.2 ± 1.9	6.9 ± 2.2	3.9 ± 1.3	4.3 ± 1.0
Egg masses	13.2 - 16.6**	5.4 - 21.7	7.4 - 18.1	5.9 - 13.1	8.2 - 23.9	14.7 - 15.9
Male gonad	11.0 - 17.4	6.9 - 8.9	5.7 - 6.4	6.5 - 9.5	6.5 - 5.6	3.9 - 6.7
Gill	4.0 ± 1.2	2.5 ± 0.5	2.6 ± 0.9	3.5 ± 1.1	2.6 ± 0.4	2.8 ± 2.1
Stomach	6.6 ± 2.6	5.7 ± 0.6	3.6 ± 0.8	4.6 ± 1.3	2.8 ± 0.3	3.9 ± 1.3
Tail muscle	6.5 ± 0.8	3.9 ± 0.5	3.7 ± 0.6	5.5 ± 2.0	3.1 ± 0.2	2.6 ± 0.5
Claw muscle	5.2 ± 1.3	3.1 ± 0.3	3.6 - 4.4	4.2 ± 0.9	2.9 ± 0.2	2.9 ± 1.0
Fecal contents	16.4 ± 7.1	40.9 ± 12.7	14.9 ± 6.6	33.3 ± 16.1	20.6 ± 6.1	21.1 ± 14.0
Plasma	1.7 ± 0.9	0.9 ± 0.1	1.1 ± 0.2	1.9 ± 0.7	1.3 ± 0.5	1.6 ± 0.8
N	4	4	4	4	4	5
Recovery of dosed radioactivity (%)	95.4 ± 10.0	101.1 ± 10.0	107.7 ± 9.4	86.5 ± 19.2	65.7 ± 4.9	63.9 ± 11.4

*Mean ± S.D.

**Range, N = 2

Plasma was withdrawn from the pericardial sinus just before sacrifice. Subsequently, the tissues were dissected from the carcass and weighed. Triplicate aliquots of plasma and duplicate samples of tissue (60-230 mg) were digested in NCS. Radioactivity was determined by liquid scintillation counting using the external standard method.

The specific radioactive content of plasma, several tissues, and fecal matter, at various time points after ^{14}C-BP administration, is shown in Table 1. The hepatopancreas (HP) contained the highest concentration of radioactivity of any organ sampled, accounting for 80-92% of the total benzo(a)pyrene-derived radioactivity present at all time points (1 day to 6.5 weeks).

Intestine, gonads, heart, and green gland were intermediate in specific radioactive content between the HP and muscle, stomach, or gill. The high specific activities present in feces may indicate this to be a major pathway of elimination for the compound. However, the ^{14}C-BP-derived radioactivity was persistent in all tissues sampled. Essentially all of the dosed radioactivity was recovered in lobsters 7 days after treatment, and about 65% of the injected radioactivity remained in the animals even after 6.5 weeks.

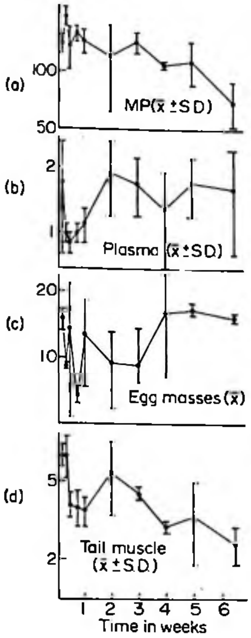

Figure 1. Semi-log plots of specific activity (dpm/mg or dpm/ul) of several lobster tissues and plasma at various time points after a single pericardial injection of ^{14}C-benzo(a)pyrene (1 mg/kg).

Depuration of the BP-derived radioactivity from 1 to 6.5 weeks was seen in almost all tissues examined as is exemplified by HP and tail muscle (Figures 1a and 1d). The scattering encountered among individual values made meaningful half-lives difficult to estimate. However, a semi-log plot of the total radioactivity remaining in the lobster did indicate a half-life of about 2 months. Egg masses, however, showed no such loss of radioactivity (Figure 1c), their specific activities being, in fact, higher at 6.5 than at 2 or 3 weeks. This may be of toxicological significance since some PAH have teratogenic properties. The specific activity versus time plots for the various tissues also demonstrated some interesting redistribution effects. Radioactivity content of the hepatopancreas increased between 1 and 2 days (Figure 1a) which coincided with a loss in radioactivity in most of the other tissues, especially plasma (Figure 1b). Tail muscle (Figure 1d) was typical of all other tissues sampled; there was a rapid initial decrease in specific activity during the first week, followed by a prolonged decline in BP-derived radioactivity.

These fluctuations in radioactivity distribution are consistent with an initial uptake of ^{14}C-BP from the hemolymph by a single, predominant compartment (HP), followed by release from this compartment back into the circulating hemolymph and subsequent uptake into secondary compartments. Finally, there is a gradual release of radioactivity from all compartments to the hemolymph and, in turn, to the external environment. Such a redistribution pattern has been repeatedly observed in mammalian species with lipophilic polychlorinated biphenyl (PCB) and polybrominated biphenyl (PBB) isomers (Tuey and Matthews, Drug Metab. Disp. 5:444, 1977).

Benzo(a)pyrene is known to be converted to many different metabolites by the microsomal cytochrome P-450-dependent monooxygenase system, and certain of these metabolites are much more toxic than the parent hydrocarbon itself. In fact, benzo(a)pyrene 7,8-dihydrodiol-9,10-epoxide is a potent carcinogen and mutagen in mammalian systems. Thus, the presence of benzo(a)pyrene derivatives, such as benzo(a)pyrene 7,8-dihydrodiol, that are proximate metabolic precursors for carcinogenic and mutagenic compounds in seafood could constitute a risk to human health. Consequently, we were also interested in the chemical form of the ^{14}C-BP-derived radioactivity that was present in the major storage sites of the lobster (i.e., hepatopancreas and tail muscle).

Aliquots (0.25 g) of frozen hepatopancreas or tail muscle from lobsters treated with ^{14}C-BP (1 mg/kg) 3 or 6.5 weeks before sacrifice were thawed, homogenized in water and extracted with hexane (De Pierre et al., Anal. Biochem. 63:470, 1975). The metabolites in the aqueous fraction (0.5-1.5% of the radioactivity) have still not been identified. The hexane extracts (containing 98.5-99.5% of the radioactivity) were and analyzed by HPLC, essentially as described by Holder et al. (Proc. Natl. Acad. Sci. USA 71:4356, 1974). The elution of ^{14}C-BP and its metabolites from the HPLC column was compared to that of authentic benzo(a)pyrene metabolites (kindly supplied by the National Cancer Institute-NIH Carcinogenesis Research Program).

Most of the radioactivity stored in lobster hepatopancreas after ^{14}C-BP administration was unchanged hydrocarbon (97% of the radioactivity in the hexane extract of hepatopancreas from lobsters 3 weeks after treatment, and 83% of the radioactivity 6.5 weeks after treatment). However, there was a slight increase in the amount of benzo(a)pyrene metabolites in the hepatopancreas over time. Three weeks after the administration of ^{14}C-BP, 3.3% of the hexane-extracted radioactivity from hepatopancreas occurred as phenolic metabolites of benzo(a)pyrene (primarily 3-hydroxybenzo(a)pyrene) whereas 17% of the hexane-extracted radioactivity was identified as phenolic metabolites of benzo(a)pyrene 6.5 weeks after treatment with ^{14}C-BP.

In contrast, benzo(a)pyrene metabolites comprised a greater proportion of the radioactivity stored in tail muscle. At 48 hr after dosing with ^{14}C-BP phenolic metabolites accounted for 38% of the radioactivity in the hexane extracts of muscle homogenate, and 6.5 weeks after treatment approximately 50% of the hexane-extracted radioactivity occurred as phenolic metabolites of benzo(a)pyrene. Moreover, there were slight changes in the amount of radioactivity that remained in the aqueous phase after homogenization and hexane extraction of tail muscle, which were not observed with the hepatopancreas: Two days post-treatment about 91% of the total tail radioactivity was hexane extractable, but only 88% after 6.5 weeks. To date, the metabolites in the aqueous fraction have not been identified but dihydrodiols, polar conjugates of phenols and dihydrodiols, and glutathione conjugates are all likely candidates.

In summary, benzo(a)pyrene-derived radioactivity is very persistent in the lobster ($t_{0.5}$ approximately 2 months), is slowly eliminated via the feces, and may accumulate in egg masses. The radioactivity present in hepatopancreas, the major storage compartment in the lobster, occurs predominantly as benzo(a)pyrene although minor amounts of metabolites are also found.

IONIC CONTRIBUTIONS TO THE SHORT-CIRCUIT CURRENT ACROSS THE OPERCULAR EPITHELIUM

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During previous studies with isolated opercular epithelia, a number of observations on the influence of inorganic ions on the short-circuit current (Isc) were made but not pursued. This year it was decided to return to these observations and investigate further the influences of the major biological ions on the generation of the Isc across this epithelium. This was accomplished by ion substitution experiments by one of the following methods: (1) continual perfusion with ion-substituted Ringer after the establishment of steady-state conditions with normal Ringer, (2) aspiration and rinsing of the chamber several times with ion-substituted Ringer, (3) gradual substitutions by small volume titrations. Epithelia from seawater (SW)-adapted killifish, *Fundulus heteroclitus*, were dissected, mounted in lucite chambers, and short-circuited by methods previously described (Degnan et al., J. Physiol. 271:155-191, 1977).

The Isc across the operculum is a Cl^- current (Karnaky et al., Science 195:203-205, 1977), while the unidirectional Na^+ movements can be described as passive under both short- and open-circuited conditions (Degnan et al., J. Physiol. 271:155-191, 1977; Bull. MDIBL 17:69-71, 1977). Other studies have shown that the transepithelial potential difference (p.d.) across the operculum under open-circuit conditions was primarily Na^+ sensitive and probably a Na^+ diffusion potential on top of an electrogenic Cl^- transport potential (Degnan and Zadunaisky, Amer. J. Physiol., in press).

The effect of Na^+ substitution on the electrical properties across the operculum is summarized in Table 1. Bilateral substitution reduced the Isc 97.9% while unilateral mucosal and serosal substitutions reduced the Isc 85.0% and 17.3%, respectively. The relationship between the bilateral Na^+ concentration and the Isc was a typical Michaelis-Menten curve (Figure 1a). Serosal Na^+ substitution produced an initial increase in the Isc, which peaked near 130% of the control level around 80-100 mM. This was followed by a decline in the Isc to a steady-state level, usually between 50-70% of the control level at zero serosal Na^+ (Figure 1b). Mucosal Na^+ substitution resulted in a linear decrease in the Isc which approached zero as the concentration approached zero (Figure 1c). This response was observed whether there was normal (151 mM) or zero serosal Na^+. The effect of Cl^- substitution on the electrical