Reports

Petroleum Hydrocarbons: Uptake and Discharge by the Marine Mussel Mytilus edulis

Abstract. *The common marine mussel* Mytilus edulis *has been observed to rapidly take up mineral oil, [^{14}C]heptadecane, 1,2,3,4-tetrahydronaphthalene, [^{14}C]toluene, [^{14}C]naphthalene, and [^{3}H]3,4-benzopyrene from seawater solution. This species of mussel did not metabolize any of these compounds, and transfer of the mussel to fresh seawater, after exposure to the hydrocarbon in solution, resulted in the discharge of most of the hydrocarbon, although significant amounts remained (between 1 and 400 micrograms per mussel). The nontoxic paraffinic hydrocarbons mineral oil and heptadecane were taken up (10 milligrams per mussel) to a much greater extent than the aromatic hydrocarbons (2 to 20 micrograms per mussel).*

Much has been written about the effects of petroleum on marine life [see the review of Nelson-Smith (*1*)], but few experiments have dealt with the uptake and discharge of specific petroleum hydrocarbons by members of the marine food web. An examination by Blumer *et al.* (*2*) of oysters and other shellfish exposed to an oil spill revealed the presence of large quantities of petroleum compounds in the body tissues. In initial investigations we studied the uptake and discharge of mineral oil by the mussel *Mytilus edulis*, a common intertidal filter feeder along the Pacific coast. Success with these experiments led us to believe it would be useful to study the uptake of a wide range of petroleum hydrocarbons by mussels. Several aromatic and polycyclic aromatic compounds [3,4-benzopyrene, naphthalene, 1,2,3,4-tetrahydronaphthalene (tetralin), and toluene] were selected because of their toxic or carcinogenic properties. Mineral oil and heptadecane were chosen as examples of nontoxic paraffinic hydrocarbons. Heptadecane is found in petroleum and is also a naturally occurring hydrocarbon in many marine organisms (*3*), including mussels.

Solutions consisting of the following amounts of hydrocarbons, each dissolved in 1 liter of seawater, were used for the uptake experiments: 32 to 100 μg of naphthalene, 100 to 500 μg of toluene, 2 to 200 μg of benzopyrene, 10 to 100 μg of tetralin, 1 g of mineral oil, and 1 to 8 mg of heptadecane. In an attempt to aid the dissolution of the mineral oil, which consisted of only purified paraffins (J. T. Baker Company), and tetralin, we sonicated these substances in seawater which contained Celite. The concentrations of Celite in the final media were maintained well below 50 mg per liter to avoid adverse effects on the filtration rate (*4*). The other hydrocarbons were shaken in seawater and formed true solutions.

Young mussels (average dry weight not including the shell, 0.30 g) were collected off the pier at the Scripps Institution of Oceanography; each mussel was placed in a 2-liter beaker containing 1 liter of seawater and the dissolved hydrocarbon. Air was continuously bubbled into the beakers during the experiments. In some experiments mussels were transferred to fresh seawater after a period of exposure to the hydrocarbon, so that we could observe the discharge of the hydrocarbon.

After exposure whole mussels and dissected organs (Tables 1 and 2) were weighed, rinsed with methanol, and extracted with a mixture of chloroform and methanol (2 : 1 by volume). For mussels exposed to mineral oil, benzopyrene, naphthalene, and tetralin, the mussel lipid extracts were dried over nitrogen, weighed, and applied to a silicic acid column; the hydrocarbon fraction was eluted with redistilled petroleum ether [for details of the method see (*5*)]. We analyzed the hydrocarbon fraction by gas-liquid chromatography (Varian Aerograph series 1800), using a 3 percent OV-1 column. The hydrocarbon fractions from the experiments with benzopyrene and naphthalene were applied to silicic acid thin-layer plates and separated with a mixture of petroleum ether and benzene (1 : 1 by volume). Spots were made visible with iodine, and we identified the components by comparing their R_F values with those of authentic standards. The aromatic hydrocarbons were eluted with ethanol, and their concentrations were determined spectrophotometrically at their absorption maxima (between 250 and 360 nm).

After each time interval (see Tables 1 and 2) for the experiments with radioactive hydrocarbons ([^{3}H]3,4-benzopyrene, [^{14}C]toluene, [^{14}C]naphthalene, and [^{14}C]heptadecane), three mussels were removed, the tissues of each mussel were dissected and rinsed three times with methanol, and lipids were extracted from the separated tissues. A 200-μl fraction of the extract was added to Aquasol (New England Nuclear), and the radioactivity was counted in a liquid scintillation counter (Beckman DPM 100). The hydrocarbon remaining in the tissue extracts after the methanol rinses was considered to be hydrocarbon that had been taken up by the mussel. The radioactivity values given in Tables 1 and 2 for each sample time are the averages for three mussels. Lipid extracts were also applied to silicic acid thin-layer plates and developed in a mixture of petroleum ether and benzene (1 : 1 by volume) and in chloroform. The positions of the radioactive compounds on the plates were determined by autoradiography with single-coated, blue-sensitive, x-ray film (Eastman Kodak).

The naturally occurring hydrocarbon content of marine mussels is approximately 1 mg per mussel. Gas-liquid chromatography of these hydrocarbons revealed a series of straight-chain and branched-chain hydrocarbons from C_{16} to C_{26}, the major components being pristane, a branched C_{19}, eicosene (C_{20} with one double bond), and heneicosahexaene (C_{21} with six double bonds). Blumer *et al.* (*2*) have reported a similar pattern for oysters from the Atlantic, and we had previously identified the polyunsaturated C_{21} hydrocarbon (with

Table 1. Uptake and discharge of [^{14}C]heptadecane by *M. edulis*. Each mussel was placed in a 2-liter beaker containing 6.2 mg (7×10^6 count/min) of [^{14}C]heptadecane in 1 liter of seawater. The amounts of heptadecane taken up are expressed in milligrams of heptadecane per gram (dry weight) of tissue. The total activity taken up in each tissue is expressed in counts per minute. After 24 hours of exposure to the hydrocarbon the mussels were transferred to seawater free of hydrocarbon.

Time (hours)	Whole mussel		Tissue							
			Gill		Mantle		Adductor muscle		Gut	
	Amount	Activity	Amount	Activity	Amount	Activity	Amount	Activity	Amount	Activity
2	0.14	6,000	0.57	400	0.08	2,000	0.01	200	0.16	3,300
3	0.19	10,000	0.48	2,900	0.21	3,500	0.11	1,900	0.06	2,100
24	6.0	1,000,000	13.0	20,000	7.8	320,000	1.4	16,000	20.0	600,000
48	1.0	150,000								
120	2.8	190,000								
360	3.2	260,000	2.8	98,000	1.3	37,000	0.4	2,800	4.2	130,000

six double bonds) in algae (*6*). We were unable to detect any aromatic hydrocarbons in these mussels by spectrophotometric or by mass spectrometric techniques.

Although high concentrations of mineral oil were rapidly taken up by the mussels, these hydrocarbons appeared to have no effect on the mussels. Between 10 and 15 mg of mineral oil was taken up by each mussel within 2 days, and longer exposures did not result in further uptake. Gas-liquid chromatography of the hydrocarbon fraction from these mussels demonstrated that no significant alteration of the mineral oil had taken place after 6 days of exposure. After the mussels, which had been exposed for 3 days to mineral oil, were transferred to seawater free of hydrocarbons, they discharged over 90 percent of the mineral oil, but gas-liquid chromatography showed that some mineral oil (between 200 and 400 μg) was still present, with losses of the short-chain hydrocarbons and consequent enrichment of the longer-chain hydrocarbons (longer than C_{22}).

The pattern of uptake and discharge for [^{14}C]heptadecane was similar to that for mineral oil (Table 1). Within 20 minutes [^{14}C]heptadecane could be detected in *M. edulis*, which indicates how efficiently hydrocarbons can be filtered from the surrounding seawater. Although the concentrations of mineral oil used were much higher than those of heptadecane, the maximum uptake of mineral oil was the same as that of heptadecane (approximately 10 mg). Autoradiographs indicated that no breakdown of [^{14}C]heptadecane had occurred at the end of a 24-hour period.

Tetralin was found to be highly toxic at a concentration of 100 parts per million. At lower concentrations mussels exhibited paralysis, identified by loss of the ability to close an open shell after someone tapped on it, and by the recovery of this response when they were transferred to fresh seawater. Approximately 20 μg of this hydrocarbon were taken up by each mussel; 80 percent of this amount had been discharged after the mussels had been kept in fresh seawater for 24 hours.

Only 3 to 10 μg each of [^{14}C]naphthalene (Table 2), [^{14}C]toluene, and [^{3}H]3,4-benzopyrene were taken up by the mussels. Toluene, naphthalene, and benzopyrene did not have a lethal effect at the concentrations used (up to 1 mg per liter). Some of these aromatic compounds may inhibit the filtering ability of mussels. It has been suggested that microscopic chemoreceptors in bivalves detect the presence of toxic particles, and that impulses from these organs cause changes in the muscle fibers of the gills, bringing about changes in the filtration rate (*4*).

By autoradiography we demonstrated that the radioactive aromatic compounds tested were not metabolized by the mussels. Copepods also lack the ability to metabolize aromatic hydrocarbons (*7*). In contrast, vertebrate systems have enzymes in the microsomes of the liver that hydroxylate polycyclic aromatic compounds, and the hydroxylation of 3,4-benzopyrene is considered an important aspect of its carcinogenic action in mammals (*8*). The hepatopancreas of the mussel may lack the hydroxylating enzymes present in vertebrate livers. Apparently the bacteria present in mussels also lack the ability to metabolize aromatic hydrocarbons, although other workers have isolated marine bacteria that are able to metabolize aromatic hydrocarbons (*9*).

In all the uptake experiments reported here hydrocarbons were rapidly taken up by gill tissues (Tables 1 and 2). When excised gill tissue was exposed to radioactive hydrocarbons, it quickly absorbed radioactivity, whereas excised mantle and adductor muscle did not absorb significant amounts of radioactive hydrocarbons. We hypothesize that the gill tissue of mussels has a micellar layer which absorbs the hydrocarbons and then passes the hydrocarbons to other tissues. An important function of the gill during the filter feeding of bivalves is to separate the food particles, presumably by size, and to transport them to the labial palp before ingestion.

After long periods of exposure to hydrocarbons high concentrations of hydrocarbons were found in the gut (Tables

Table 2. Uptake and discharge of [^{14}C]naphthalene by *M. edulis*. Each mussel was placed in a 2-liter beaker containing 32 μg (850×10^6 count/min) of [^{14}C]naphthalene in 1 liter of seawater. The amounts of naphthalene taken up are expressed in micrograms of naphthalene per gram (dry weight) of tissue. The total activity taken up in each tissue is expressed in counts per minute. After 4 hours of exposure to the hydrocarbon the mussels were transferred to seawater free of hydrocarbon.

Time (hours)	Whole mussel		Tissue							
			Gill		Mantle		Adductor muscle		Gut	
	Amount	Activity	Amount	Activity	Amount	Activity	Amount	Activity	Amount	Activity
4	7	36,000	9	2,600	2	7,400	6	5,800	7	20,000
6	4	25,000	22	4,400	2	5,800	4	2,700	3	12,000
76	3	7,500	4	500	1	2,300	1	700	2	4,000

1 and 2) (the gut refers to all tissues other than the mantle, gill, and adductor muscle). The hepatopancreas may be the site of hydrocarbon storage in mussels; recent investigations have shown that hydrocarbons are stored in the gallbladder and liver of marine fish (*10*).

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18.6-Year Earth Tide Regulates Geyser Activity

Abstract. *Over 40 years of records from Yellowstone National Park, Wyoming, show that the 18.6-year tidal component strongly regulates the frequencies of eruption of Grand and Steamboat geysers. The frequency of Grand Geyser increases with increasing tidal force and that of Steamboat Geyser decreases, which suggests that tidal dilatation is one factor affecting heat flow to a geyser.*

It has been established that earth tidal forces have a regulatory influence on geothermal activity (*1*). The interval between eruptions of Riverside Geyster in Yellowstone National Park was found to be especially responsive to the fortnightly and semiannual components of the tidal forces. It was also found that, over a 6-year period in which careful records were kept, the interval between eruptions of Old Faithful Geyser in California decreased essentially linearly with increasing tidal force. The variations in geyser activity are attributed to mechanical straining by the tidal forces of the geothermal area in which the geyser is located. Such strains could be expected to influence the rate of heat flow to the geyser. Further examination of the Yellowstone records shows that at least two geysers, Grand and Steamboat, respond dramatically to the 18.6-year tidal component. This appears to be the first time that the influence of the 18.6-year component has been observed in a solid earth geophysical phenomenon.

The earth tidal forces, arising from the gravitational attraction between the earth, the moon, and the sun, cause the ebb and flow of the ocean tides and, in addition, deform the solid part of the earth, producing strains of the order of 10^{-7} which vary in synchronization with variations in the tidal forces. The maximum variation in gravity due to lunar and solar attraction is about 1 part in 5×10^6 times the acceleration of gravity. Since the earth rotates on its own axis, the moon revolves around the earth, and the earth around the sun, the tidal force is constantly changing, and its magnitude at any one place on the earth depends on the relative positions of the earth, moon, and sun. In general, the variations are periodic, having the following approximate frequency components: semidiurnal, diurnal, fortnightly, semiannual, 8.8-year, 18.6-year, and 20,900-year. The overall tidal force, although it is made up of numerous periodic components, does not itself display a periodically regular pattern. The values of the variation in gravity due to tidal forces are readily calculable by using Longman's method (*2*).

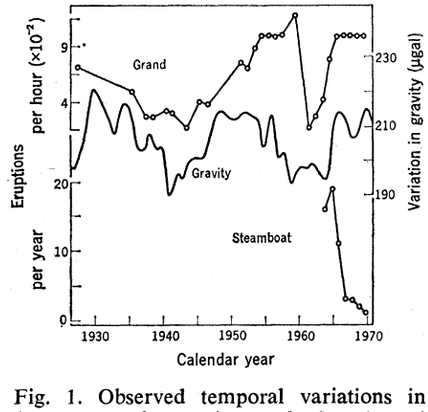

Fig. 1. Observed temporal variations in frequency of eruptions of Grand and Steamboat geysers and computed average annual spread between maximum and minimum gravity due to earth tidal forces.

The 18.6-year component arises from the inclination of the orbital planes of the earth and the moon, about 5° with respect to one another. The inclination causes the average tidal force to vary about 10 percent during an 18.6-year period.

Many records have been kept by the park naturalists on geyser activity in Yellowstone National Park. These records, usually in the form of periodic informal reports and personal notebooks, while often fragmentary, can be pieced together to give fairly reliable histories of the activities of some of the geysers. The activities of two of the major geysers, Grand and Steamboat, are especially noteworthy when compared with moderately long-term changes in earth tidal forces.

Grand Geyser is situated in the Upper Geyser Basin. During an eruption it will play to a height of 30 to 60 m, for anywhere from 15 minutes to 1½ hours. According to Marler (*3*), the interval between its eruptions varies from 8 hours some seasons to over 40 hours other seasons, with two periods of dormancy over the four decades of observations. Steamboat Geyser lies in the Norris Basin, some 30 km from Grand Geyser. It is the most stupendous geyser in the park, erupting to a height of 100 to 130 m and playing several hours. The characters of the two basins are quite different (*4*). Upper Geyser Basin is the older of the two, containing many perfect geysers such as Old Faithful, Grand, and Giantess. The younger Norris Basin exhibits a much wider range of phenomena, from very new geysers to well-established ones.

The historical records of the activities of Grand and Steamboat geysers are presented in Fig. 1, where average frequency of eruption has been plotted as a function of year. The data for Grand cover the relatively long period from 1927 through 1969; those for Steamboat are less extensive, being available only for the 6-year period from 1963 through 1969. The annual average earth tidal force is also plotted in the same figure. The ordinate in this