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Bacterial Oxidation of Hydrocarbons in Marine Sediments.*

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It has been demonstrated[1] that certain bacteria, widely distributed in nature, are capable of utilizing petroleum hydrocarbons as a source of energy. Recently Grant and ZoBell[2] have demonstrated the presence of such bacteria in marine environments.

If such bacteria are active in marine sediments, *in situ,* they may account for the dearth of hydrocarbons[3] in this material; thereby influencing the present theories of petroleum genesis. Therefore studies were undertaken to obtain more information on factors which may influence the activity of these bacteria in potential source-sediments.

Experimental. The sediments used in this investigation were samples of bottom mud from Mission Bay and from the Channel Island region. To demonstrate the presence of hydrocarbon-oxidizing bacteria in these materials, small amounts of the sediment were added to tubes containing 75% sea water, 0.1% NH_4Cl and a large drop of sterile paraffin oil. Uniform turbidity in the tubes or a pellicle of growth at the oil-water interface after 7 to 9 days incubation indicated the presence of these organisms. These cultures were purified by repeated transfers and by plating procedures. Using this technic, cultures able to oxidize a variety of hydrocarbons have been isolated from most of the sediments tested.

To test hydrocarbon-oxidation in the sediments, the muds were mixed with sterile paraffin oil, or in some cases a lubricating oil, and were added to sterile bottles. Oxidation of the hydrocarbon was determined by the extent of oxygen consumption which was measured manometrically.

Table I shows the results of a typical experiment in which 50 g of sediment from Mission Bay were used. The test cultures contained 2 ml of the particular oil. The controls represent biological oxidation of the organic matter in the muds. It is evident that considerable O_2 was consumed in the presence of the hydrocarbon. The quantity of hydrocarbon oxidized was calculated on the assumption that 3.5 mg of O_2 are necessary to completely oxidize 1 mg of a hydrocarbon. The O_2 consumed in 9 days was approximately ¾ the total capacity of the bottles, indicating that hydrocarbons may be oxidized in sediments stored in containers if sufficient oxygen is present. Even if suitable examination of marine sediments do not reveal the presence of hydrocarbons it does not preclude the possibility of their

TABLE I.
Milliliters of Oxygen Consumed by Bacteria in Mud After Different Periods of Time at 25°C. The Amount of Hydrocarbon Oxidized Is Also Given.

	Incubation period in days 3	5	7	9	Hydrocarbon oxidized after 9 days
	ml	ml	ml	ml	mg
1. Mud only	0.5	1.5	2.7	4.5	
2. " "	1.3	3.3	3.9	5.4	
3. " + paraffin oil	3.4	8.9	12.6	16.3	4.82
4. " + " "	1.3	6.5	11.7	15.3	4.17
5. " + lubricating oil	3.5	11.1	18.4	22.1	6.88
6. " + " "	2.2	7.4	14.3	20.4	6.20

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1 Bushnell, L. D., and Haas, H. F., *J. Bact.,* 1941, **41**, 653.

2 Grant, C. W., and ZoBell, C. E., Proc. Soc. Exp. Biol. and Med., 1942, **51**, 266.

3 Trask, P. D., *Problems of Petroleum Geology,* Am. Assn. Petrol. Geologists, Tulsa, Okla., 1934.

having been formed, but subsequently oxidized by bacteria, *in situ.*

It was noticed in the course of these experiments that the amount of water present in the sediments seemed to affect the oxygen consumption. To test this observation an experiment similar to that outlined in Table I was set up. Controls and tests to which 25 ml of sterile 75% sea water was added were included. The addition of water did not seem to affect the oxidation of the hydrocarbon since the relative amounts oxidized in the tests with and without added water were the same. The controls without water consumed much more oxygen than did those with added water. Thus it would seem that hydrocarbons in marine sediments are oxidized preferentially to organic matter.

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Hormonal Requirements for Pregnancy and Mammary Development in Hypophysectomized Rats.*

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Studies on the hormonal requirements for the production of traumatic deciduomata in hypophysectomized rats indicated an essential role of the hypophyseal lactogenic hormone presumably due to its luteotrophic properties.[1] Cutuly has recently reported[2-4] the maintenance of pregnancy in hypophysectomized rats with pituitary lactogenic preparations evidently impure and not of the highest potency. The investigation reported below revealed that neither a crude lactogenic preparation equal in potency to those used by Cutuly nor purified lactogenic hormone constituted adequate therapy for establishing pregnancy in the hypophysectomized rat. However, when estrone† was injected with lactogenic preparations successful implantation occurred.

The rats used were 60 to 70 days of age. They were bred and then hypophysectomized within 24 hours. Daily injections were made beginning on the day of operation. Glucose was given intraperitoneally in a dose of 200 mg 4 times a week. In some cases thyroxin was injected daily subcutaneously. Estrone was administered subcutaneously in sesame oil and the pituitary preparations were injected subcutaneously in aqueous solution. Animals were sacrificed at different stages of pregnancy or at term and the state of development of the implants or fetuses, and of the mammary glands noted.

In a preliminary experiment it was not possible to establish pregnancy in 6 rats injected with 1 μg of estrone daily; nor was it possible in 7 rats injected with 2 to 5 mg (16 to 62 I.U.) of impure lactogenic hormone, nor in 5 rats injected with 2 mg (60 I.U.) of purified lactogenic hormone. The lactogenic preparations were found to be free of the other gonadotrophins. When 60 μg of an FSH-ICSH combination capable of causing estrin formation in the hypophysectomized rat were injected daily with 5 mg (62 I.U.) of the crude lactogenic extract into 7 rats, all became pregnant and 4 were able to maintain or deliver some normal young. Cutuly's results were also duplicated when the same dose of crude lactogenic preparation was

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1 Evans, H. M., Simpson, M. E., Lyons, W. R., and Turpeinen, K., *Endocrinology,* 1941, **28,** 933.

2 Cutuly, E., PROC. SOC. EXP. BIOL. AND MED., 1941, **47,** 126.

3 Cutuly, E., PROC. SOC. EXP. BIOL. AND MED., 1941, **48,** 315.

4 Cutuly, E., *Endocrinology,* 1942, **31,** 13.

† The estrone used in these experiments was kindly supplied by Parke Davis & Co.