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.

TABLE I.
Condition of Implants and Mammary Glands in Pregnant Rats Hypophysectomized and Maintained by Injection of Lactogenic Hormone and Estrone.

Daily hormone dose (Subcutaneous)							
Lactogenic mg	Estrone μg	Thyroxin μg	Days injected	Necropsy day	Body wt change g	Condition of implants	Mammary gland
2	1	10	13	14	+27	7 normal-sized, dead	LA++
2	1	10	13	14	—36	no sites	DAB
2	1	10	13	14	— 5	12 living	LA++
2	1	10	16	16	+ 9	10 "	LA+
2	1	10	16	16	+ 2	3 " , 7 resorbing	LA+
2	1	10	16	16	—14	no sites	DAB
2	1	10	11	11	—11	1 normal	LA+
2	1	10	11	11	—10	3 "	LA+
2	1	10	11	11	—11	9 "	DAB
2	1	10	11	11	+ 6	10 "	LA+
2	1	10	11	11	— 1	8 resorbing	DAB
2	1		11	11	—10	5 "	LA+
2	1		11	11	— 6	5 "	LA+
2	1		11	11	— 8	3 " , 1 normal	LA+
2	1		11	11	— 6	no sites	DAB
2	1		11	11	—12	" "	DAB
2	1		11	11	—13	" "	DAB
4	1		11	11	+ 7	11 resorbing	LA+
4	1		11	11	— 8	8 "	LA+
4	1		11	11	+ 8	2 " , 8 normal	LA+
4	1		11	11	— 5	5 " , 1 "	DAB
4	1		11	11	+ 2	5 " , 2 "	DAB
4	1		11	11	+ 9	5 " , 3 "	LA+
4	1		11	11	0	10 "	LA+
4	1		11	11	—11	1 "	DAB
4	1		11	11	—14	6 "	DAB
4	1		11	11	— 5	7 "	LA+
4	1		11	11	— 6	8 normal	LA++
4	1		11	11	—22	9 "	DAB

Note: All rats received 200 mg of glucose intraperitoneally 4 times weekly. The estrone was given in 0.1 cc sesame oil. The lactogenic hormone assayed approximately 30 I.U. per mg. The daily dosage was given in 0.5 cc of 2% aqueous solution of butanol. LA+ and ++ refer to beginning lobulo-alveolar development characteristic of the first half of pregnancy. DAB indicates presence of ducts and alveolar buds only. Hypophysectomized animals, uninjected or injected with either estrone or lactogenic hormone, always failed to implant following copulation.

given with 1 μg of estrone daily. In this experiment only 2 of 34 rats failed to implant. Of special interest were 3 rats injected with the lactogenic preparation from day 1 to 12 only, and given estrone every second day over the same period. Two of these rats had living young at term and the third showed signs of recent fetal death on day 23. This result was foreshadowed in the experiments of Pencharz and Long[5] in which pregnancy was maintained without treatment in rats hypophysectomized after day 11.

An attempt was next made to establish and maintain pregnancy with estrone and pure lactogenic hormone (*i.e.*, hormone which had been proven to be electrophoretically homogeneous, or preparations of equal potency, approximately 30 I.U. per mg). The dosages used were chosen from our earlier work[6] in which it had been possible by treating hypophysectomized rats with 60 I.U. of pure lactogenic hormone and 10 I.U. of estrone, to induce lobulo-alveolar mammary development equal to that usually observed in the first half of normal pregnancy. As shown in Table I, 29 rats received 1 μg of estrone daily, subcutane-

5 Pencharz, R. I., and Long, J. A., *Science*, 1931, **74**, 206.

6 Lyons, W. R., Simpson, M. E., and Evans, H. M., *Anat. Record* (Suppl.), 1942, **82**, 38.

ously and of these 17 were injected with 2 mg and 12 with 4 mg of lactogenic hormone. Since the lower dose seemed to be just as efficacious as the 4 mg level, the data are not treated separately. Nor are the rats which received thyroxin considered separately since the results did not differ from those obtained in the other animals. Of the 29 rats, only 5 showed no sign at necropsy of having been pregnant. The other 24 animals succeeded in implanting a total of 175 embryos as judged by the number of normal or resorbing implantation sites. Eighty of these (present in 14 rats) appeared to have been maintained in a healthy condition through day 11. As shown in the table, living fetuses were also found on days 14 and 16. No attempt was made in this experiment to maintain pregnancy until term. The majority of the rats showed lobulo-alveolar mammary growth equaling that of normal mid-pregnancy. Most of the rats lost weight even in cases where fetal and placental growth were found to have continued normally.

Summary. The same daily doses of purified lactogenic hormone (60 I.U.) and estrone (10 I.U.) capable of inducing beginning lobulo-alveolar mammary growth in the hypophysectomized rat, also ensured successful implantation in rats hypophysectomized and injected from the day of sperm. Since only about one-half of the estimated number of implantations were found to have developed normally through mid-pregnancy it follows that these 2 pure substances in the doses used did not adequately substitute for the intact pituitary of a pregnant rat. When injected alone neither lactogenic hormone nor estrone permitted implantation to occur.

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Antiluminescent Activity of Antibiotic Substances.

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As has been pointed out previously,[1,2] certain substances prepared from fungi or actinomycetes are capable, in high dilution, of interfering with the bio-luminescence of luminescent bacteria. This observation has been applied to a larger series of antibiotic substances isolated from fungi, actinomycetes, and bacteria, or of simple chemical nature.

The antiluminescent test was carried out as previously described.[1,2] Although a standard of aspergillic acid with 256 R. units was included in every test, the results were read in gamma required to produce blacking out per 1.0 ml of a 24-hour culture of *Photobacterium fischeri* without any reference to the standard. This also was true of the antibacterial test which was read in gamma required to inhibit for 18 hours the growth of 1,500,000 organisms of a culture of *Streptococcus pyogenes* per 1 ml of beef-heart broth. With all substances at least 2 tests were carried out, and with several of them many tests were made. If 2 tests did not give close agreement in the absolute values obtained, enough were made to obtain an average value. The results are shown in Table I, which gives the substances in decreasing order of (1) antiluminescent activity, and (2) antibacterial activity. Also shown is the antiluminescent-antibacterial (AL/AB) ratio.

Certain points of interest arise. Thus, many new antibiotic substances have been shown to have antiluminescent activity. One of these is the chemical compound, tolu-p-quinone;[3] 2 of them—pyocyanase and pyocyanin—are bacterial in origin; 2—streptothricin[4] and actinomycin[5]—are from actinomycetes; and the remainder are from fungi.

1 Rake, G., McKee, C. M., and Jones, H., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 273.

2 Jones, H., Rake, G., and Hamre, D. M., *J. Bact.*, in press.