luteum preparations in amounts equal to, or greater than, the quantity necessary to sustain pregnancy in the castrate rabbit.[3] It is equally clear that this inhibition of ovulation is not effected by altering the sensitivity of the ovarian follicle as measured by the response to P.U. If we may accept our injections of crystalline progesterone as a satisfactory hormonal substitution for the corpus luteum of pregnancy or pseudopregnancy, we might then conclude that the corpus luteum hormone suppresses ovulation not by any direct action on the ovarian follicles, but by some interference at a site more central in the chain of the ovulation-provoking mechanism.

8935 C

Periphytic Habits of Some Marine Bacteria.

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In studying the factors which influence the increased bacterial activity[1] during the storage of samples of sea water collected for bacteriological analysis, ZoBell and Anderson[2] noted that multiplication occurs more rapidly in small volumes than in large volumes of the water. Similar observations were made on fresh water by Whipple[3] who reported that after 24 hours' storage there were 300 bacteria per cc. in a gallon, 7,020 per cc. in a pint and 41,400 per cc. in 2 ounces of water which initially contained 77 bacteria per cc. Whipple attributed the difference to the oxygen content of the water, but in sealed receptacles ZoBell and Anderson[2] found that oxygen was not the controlling factor. They noted a direct relationship between the area of solid surface exposed to the stored water and the bacterial activity in it.

In continuing these studies, freshly collected sea water was filtered by gravity through a Buchner 4G sintered-glass filter into a 50-liter bottle. After shaking the bottle to mix the water and to insure its complete oxygenation, the water was siphoned into glass-stoppered bottles varying in capacity from 10 cc. to 10,000 cc., which were stored in a waterbath at 16°C. The bacterial population

[3] Allen, W. M., and Corner, G. W., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 403.

[1] Waksman, S. A., and Carey, C. L., *J. Bact.*, 1935, **29**, 531.

[2] ZoBell, C. E., and Anderson, D. Q., *Biol. Bull.*, 1936, **71**, 324.

[3] Whipple, G. C., *Tech. Quart.*, 1901, **14**, 21.

was determined immediately and at 24-hour intervals thereafter by plating procedures. The oxygen-content was estimated by the Winkler method on duplicate bottles of water. Representative findings are summarized in Table I which gives the maximal bacterial population found in each volume of stored water and the oxygen-content after 20 days' storage.

TABLE I.

The oxygen-content of sea water stored at 16° C. in glass-stoppered bottles of different capacities after 20 days and the maximal bacterial population (3 to 5 days) in similar bottles. The water initially contained 5.46 cc. of oxygen per liter and 231 bacteria per cc.

Volume of sea water	10 cc.	100 cc.	1000 cc.	10,000 cc.
Oxygen per liter	2.59 cc.	2.90 cc.	3.68 cc.	4.17 cc.
Bacteria per cc.	1,475,000	1,080,000	673,000	382,000

The data in Table I show that the greatest consumption of oxygen occurs in the smallest volumes of stored sea water in which are also found the most bacteria. However, the maximal bacterial populations appear within 3 to 5 days, whereas the daily determination of oxygen reveals that the most rapid consumption occurs after the 5th day or, at a time when the bacterial population of the water is rapidly receding. Although the bacterial population of the water may drop to only a few thousand per cc. after 10 days, the rate of oxygen-consumption continues unabated until after the 12th to the 20th day. This indicates that there are many more bacteria respiring than the number which are demonstrated by plating procedures, or else there is something in the water besides the bacteria which causes the consumption of oxygen. Several different kinds of experimentation prove that the consumption in stored sea water after the plate-count decreases is caused by the respiration of periphytic bacteria which have attached themselves so firmly to the walls of the glass receptacle that they are not dislodged by vigorously shaking the bottle prior to sampling.

First, as reported above, the most rapid consumption of oxygen occurs in sea water stored in the smallest receptacles which present the largest solid-surface area per unit volume of water. Further increasing the solid surface by the addition of glass beads, glass rods or silicious sand accelerates the rate of oxygen-consumption although available organic matter limits the total amount of oxygen consumed. The larger surface permits the attachment of more periphytic bacteria and shortens the distance between the solid surface and each unit of water containing the nutrients and oxygen.

Second, when the sea water is carefully siphoned after the 10th day of storage into another receptacle filled with nitrogen to prevent absorption of oxygen from the air, oxygen-consumption is greatly retarded and becomes proportional to the number of bacteria in the water. However, when the original container is refilled with sterile sea water, oxygen-consumption begins almost immediately and at approximately the same rate as when it was interrupted by the removal of the initial water. This is interpreted as showing that the majority of the respiring bacteria are tenaciously attached to the walls of the container. Fresh water bacteria have a similar tendency to adhere to submerged glass slides.[4] Henrici[5] recommends the term "periphytic bacteria" to describe those which grow attached to submerged surfaces rather than the term "attachment bacteria" which ZoBell and Allen[6, 7] applied to marine bacteria which they found growing on glass slides submerged in the sea.

Third, the direct microscopic observation of slides submerged in different volumes of stored sea water reveals a relationship between the number of periphytic bacteria attached to the glass and the oxygen consumption—assuming the bacteria are evenly distributed over the entire solid surface of the receptacle as on the glass slides. This is illustrated by the data in Table II. While there are more bacteria

TABLE II.

Bacteria per cc. of sea water, oxygen-content per liter and bacteria per sq. cm. of glass slide submerged in different volumes of sea water after 14 days at 16° C. The solid surface offered by each receptacle including the slide and the ratio of the total volume to the solid surface are also given.

Volume of sea water	120 cc.	1225 cc.	13,220 cc.
Area of solid surface	168 sq. cm.	660 sq. cm.	3194 sq. cm.
Ratio of cc. : sq. cm.	1 : 1.14	1 : 0.54	1 : 0.24
Bacteria per cc.	216,000	34,000	62,000
Oxygen per liter	3.53 cc.	4.38 cc.	4.71 cc.
Bacteria per sq. cm.	12,900,000	30,500,000	43,600,000

per unit area of solid surface in the larger receptacles, there are not as many periphytes per unit volume of water as in the smaller rereceptacle. This is attributed to the closer proximity of the water to the solid surface in the small receptacles. More periphytes per unit area are supported by the greater amount of nutrients in the larger volumes of sea water but the distance of these nutrients from the periphytes renders them less available.

The concentration of nutrients in sea water limits the activity of

4 Henrici, A. T., *J. Bact.*, 1933, **25**, 277.

5 Henrici, A. T., *J. Bact.*, 1936, **32**, 265.

6 ZoBell, C. E., and Allen, E. C., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1409.

7 ZoBell, C. E., and Allen, E. C., *J. Bact.*, 1935, **29**, 239.

bacteria therein.* When the solid surface in stored sea water is increased tremendously by the addition of silicious sand or inert colloidal substances, the total bacterial population reaches only 10 to 100 million per cc. including estimated periphytes. Upon the addition of a little organic matter it greatly exceeds this number regardless of the volume of the receptacle in which the water is stored or the area of solid surface. However, when more than 10 mg. of nutrient material such as peptone is added per liter of sea water, the proportion of periphytes which develop is decreased. In sea water containing more than 100 mg. of peptone per liter the beneficial effect of solid surfaces is masked by the great abundance of bacteria which appear in the water. Under these conditions bacterial activity in large volumes of water is just as great as in small volumes. ZoBell and Anderson[2] have advanced a theory to account for these phenomena and the studies are being continued.

8936 C

Quantitative Determination of Vibratory Sensibility.

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The determination of the threshold of vibratory acuity is a valuable part of the neurological examination, particularly in the diagnosis of lesions affecting the posterior columns of the spinal cord. There have been numerous studies in which an attempt has been made to obtain a quantitative measure of acuity. The tests have all been made with a tuning fork of some description, usually with some device designed to measure the intensity of the vibration, an end which, in the opinion of the workers themselves, was not satisfactorily attained.

We have, therefore, constructed an instrument which produces a vibration of constant frequency, the intensity of which can be varied over a considerable range, and measured accurately. It consists of an iron pole-piece which constitutes the vibrating member, and to which is attached a round, metal button 12.5 mm. in diameter which is placed over the bony prominence to be tested. This pole-piece

* According to Krogh and Keys (*Biol. Bull.*, 1934, **67**, 132), natural sea water contains less than 10 mg. organic matter per liter, much of which is not utilizable by bacteria.