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11. This study was aided by grants (MOR-27) from the American Cancer Society. I am indebted to H. A. Bern and K. B. DeOme for their guidance, to C. H. Li for the purified pituitary hormones, to G. K. Hawkins of Schering Corporation for the estrone and progesterone, and to K. Pfister of Merck, Sharp and Dohme for the cortisol. Mammotropin was from Li preparation No. L2738E and growth hormone from Li preparation No. L2732D. Other preparations of these hormones are currently being employed, and their final activity in the media are being determined by standard bioassay methods.

7 August 1957

Controlling Growth of Test Bacteria for Antibiotic Assays through Anaerobiosis

Sarcina lutea and *Micrococcus pyogenes* are standard Food and Drug Administration test organisms used in assay methods for antibiotics, including penicillin, aureomycin, and chloromycetin. Both bacterial species, if not obligate aerobes, display strong aerobic characteristics.

The inability of these antibiotic-sensitive bacteria to grow well in an oxygen-deficient environment prompted a study of the effects of strict anaerobiosis on (i) growth retardation when strains of these bacteria were imbedded in thin layers of agar, (ii) survival, under this condition, during storage at warm temperatures, and (iii) retention of sensitivity to antibiotics following resumption of growth.

Anaerobiosis was induced either by removal of air from the environment through high vacuum or by saturating the environment with nitrogen. Three milliliters of melted, sterile nutrient agar, seeded with a test organism, were layered into a disposable, plastic petri dish (60 mm in diameter). After solidification of the agar, the seeded plate was inserted into an aluminum-polyethylene pouch with low oxygen-transmission characteristics (*1*). Evacuation of the pouch to 29.0 in. (76 cm) of vacuum and automatic heat-sealing of the open lip was carried out with a high-speed, Flex-Vac controlled-atmospheric packaging machine (*2*). Other pouches were evacuated similarly and then gassed with nitrogen and sealed by heat. All sealed pouches (Fig. 1) were held 1 day at approximately 20°C during transit to the analytical laboratory, and then were stored at 34°C for a period of days. At specific intervals, 1, 3 and 13 days, some pouches were removed for examination of bacterial survival and of sensitivity to antibiotics.

The pouches were torn open to permit re-entry of oxygen. Then paper disks saturated with standard penicillin solutions of various concentrations were applied to the seeded nutrient agar surfaces; the plates were then incubated at 33°C for 6 hours or more to develop zoning.

The results are partly presented in Table 1. Strict anaerobiosis induced by automatic vacuum packing of aluminum-polyethylene pouches completely prevented growth of these bacteria at exposure temperatures of 20°C and 34°C. *Sarcina lutea* and *Micrococcus pyogenes* (*3*), under these conditions, survived for at least 12 days at 34°C, and removal of anaerobiosis by tearing open the pouch reinitiated growth in the agar with unimpaired sensitivity to penicillin. Generally, however, growth of bacteria after they had been kept dormant in the warm, airless environment was not as dense or as rapid as in the case of those grown on

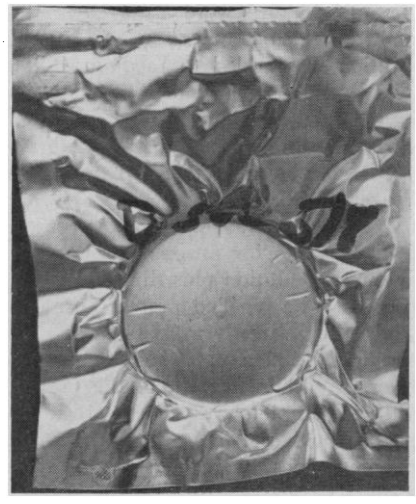

Fig. 1. Aluminum-polyethylene pouch with seeded agar plate under 29-in. (76 cm) vacuum. The concavity of plate is an indicator of vacuum level.

control plates (*4*), but with only a few exceptions, this did not seriously impair disk assay analysis. A few plates, three in this lot of 53 that were tested, failed to show growth after incubation for reasons not readily apparent.

The application of nitrogen to induce anaerobiosis produced results comparable to those obtained with high vacuum. Growth of bacteria held under nitrogen pack after 12 days' storage at 34°C was not as dense and rapid as that on control plates (*4*) that had been kept in cold storage under atmospheric conditions, but no significant loss of sensitivity against penicillin was observed. Carbon dioxide has not yet been tested, but in a well buffered medium, it conceivably would act in the same manner as nitrogen.

It is interesting that *Sarcina lutea* and *Micrococcus pyogenes* in most, but not all, instances survived and also retained sensitivity to penicillin when exposed in the absence of oxygen to long and extremely warm-temperature storage. More research in this area may show an application for these results. If growth control can be standardized with anaerobiosis by using an inexpensive, lightweight, gas-impervious package, a simple field test for the detection of antibiotics in milk and other biological materials might possibly ensue. An urgent need exists for a simple field test that would indicate effectively when milk has become adulterated with antibiotics at the farm. Such adulteration occurs after therapy of mastitis-afflicted dairy cattle with antibiotic infusions of the udder (*5*).

Perhaps, with proper selection of test organisms, a pocket disk or cup assay test for on-the-spot screening of antibiotics from natural sources might be designed. Furthermore, continued study with high-speed, vacuum and gas packaging of bac-

Table 1. Effect of anaerobiosis on growth of antibiotic-sensitive bacteria and on their retention of sensitivity to penicillin. Control plates were seeded and kept under atmospheric conditions for 13 days at 5°C. Vacuum plates were seeded and kept under vacuum for 1 day at 20°C and then 12 days at 34°C. Nitrogen plates were seeded and kept under a nitrogen atmosphere for 1 day at 20°C and then 12 days at 34°C. Measurements of zone diameter and observations of growth were made after 6 to 10 hours' incubation at 33°C.

Penicillin standard* (unit/ml of fluid)	Control plates		Vacuum plates		Nitrogen plates	
	Zone diameter (cm)	Growth†	Zone diameter (cm)	Growth†	Zone diameter (cm)	Growth†
			Sarcina lutea			
0.25	2.1	Excellent	2.2	Good	2.3	Good
0.50	2.4	Excellent	2.4	Good	2.6	Good
			Micrococcus pyogenes			
0.25	2.2	Excellent	2.3	Fair	2.4	Fair
0.50	2.5	Excellent	2.5	Fair	2.5	Fair

* Applied on agar with 1.3 cm. disk.
† No initial growth was apparent when the sealed container was opened, whether the plates were control plates or vacuum or nitrogen packed after storage for 13 days at respective temperatures.

terial plates may result in an easier and more effective means of growing and counting anaerobes in the laboratory than is employed presently.

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2. Standard Packaging Co., Clifton, N.J. The rate of machine used in this study is 1800 units per hour.
3. American Type Culture Collection, *Catalogue*, ed. 5 (1949); *M. pyogenes* var. *aureus*, No. 9801 and *S. lutea*, No. 10054.
4. Control plates were those containing nutrient agar seeded with test bacteria and held under atmospheric conditions for 13 days at 5°C prior to assay for penicillin.
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28 June 1957

Growth Chamber with Light of Solar Intensity

Conventional plant growth chambers utilize primarily fluorescent lamps, which produce a maximum illumination of only 2000 to 2500 ft-ca (*1*). No descriptions have been found of chambers in which the intensities are comparable to those of full sunlight. Summer sunlight intensities in the United States at noon on relatively clear days range from around 9000 to 14,000 ft-ca, depending upon the exact time of year, altitude, latitude, and sky conditions. Therefore, experimental studies with plants having these high light requirements cannot be carried out in conventional chambers. This would include plants whose natural occurrence is restricted to open areas receiving full sunlight.

The performance of the chamber described in this report (Fig. 1) has been satisfactory, not only in its mechanically successful operation since November 1956, but also in that several herbaceous species requiring full sun for normal development have been grown from seedling to maturity without evidence of shade effects.

This chamber is a modification of an existing one, in which all parts except those required for illumination were present. It is 1.4 m wide, 1 m deep, and 1.1 m high. After a survey of commercially available lamps had been made, a battery of 36 G-E R-40 300-w reflector spots was chosen. The lamps were installed in porcelain bases having ball-and-socket joints (*2*), which were in turn clamped to a frame forming a square grid with 15-cm centers. Thus the bulbs were only 2.5 cm apart. Heat-resistant asbestos wiring was used throughout.

These lamps have an emissivity of 3500 total lumens and a nominal beam spread of 30 deg. The 10-deg center cone varies from 13,500 to 14,500 candlepower, with a drop to 6000 at the 20-deg circumference, and 2000 at the 30-deg circumference. Total lumens for a 10-deg cone are 330; for a 20-deg cone, 970; and a 30-deg cone, 1450. The approximate average initial intensity at 1.5 m is 560 ft-ca over a circle 23 cm in diameter, 440 ft-ca over a circle 45 cm in diameter, and 276 ft-ca over a circle 76 cm in diameter (*3*). The accumulated foot-candles from the overlapping intensity patterns of each of the 36 lamps varies from about 12,000 in the center of the grid to 8500 in the outer portion. Although intensities increase as the distance is reduced, the inverse square law does not hold because of the multiple light sources and the cone-shaped intensity pattern of each source. These calculated intensities have been verified by direct measurement. By slightly angling the lamps, the grid area at 1.3 m (on the floor of the chamber) can be made to receive almost uniform illumination.

The only other suitable sources located were mercury-type lamps such as the G-E B-H6 1000-w lamp of 65,000 lu, which is used in search-lights, and the G-E A-H9 3000-w lamp of 120,000 lu, which is used for high bay lighting. For some installations these lamps may be preferable to incandescent lamps. Drawbacks include the absence of built-in reflectors, the elaborate cooling system needed by the former and the size of the ballast for the latter. Uniform illumination would be difficult to achieve, and several lamps would be required for a chamber of the size described here. None of the street-lighting lamps investigated were satisfactory. Despite a range in size from 1000 to 25,000 lu these lamps were not small enough to permit the close spacing required to build up high intensities.

Approximately 70 percent of the energy used by a 300-w incandescent lamp is dissipated as heat energy. A water filter consisting of a box with 6-in. metal sides and a glass bottom was designed to remove most of this heat. Water inflow is through a perforated pipe across one side, and the outflow is through a series of holes drilled on the opposite side. Water depth is controlled by vertical spacing of the outlet holes.

In the range of wavelengths from 0.6 to 3.0 μ, the amount of radiation absorbed from sunlight by a layer of water 1 cm thick is 26.97 percent, while only 0.01 percent in the range from 0.2 to 0.6 μ is absorbed. For a layer 10 cm thick, 45 percent of the radiation from 0.6 to 3.0 μ is absorbed, while for a 100-cm layer, the amount of absorption is increased to only 63.34 percent. However, the 0.08-percent radiation absorbed in the range from 0.2 to 0.6 μ by a 10-cm layer is increased to 7.5 percent for a 100-cm layer (*4*). When a 10-cm layer is used in this chamber, heat radiation is reduced to a level comparable to that received from the sun. At 1.3 m from the source, radiation received is 1.1 g cal/cm² min, whereas at 0.9 m it is increased to 1.65 g cal/cm² min.

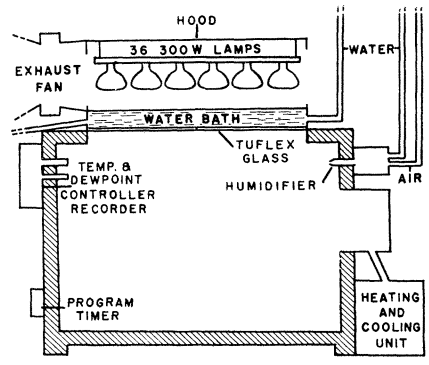

Fig. 1. Cross-sectional diagram of growth chamber 1.4 m wide, 1 m deep, and 1.1 m high.

The floor is composed of a ¼-in. sheet of tempered plate glass (*5*) which has four times the resistance to thermo shock and three to five times the resistance to impact shock of ordinary plate glass. This glass permits 90 to 93 percent transmission in the visible spectrum, with a cut off between 0.31 and 0.36 μ and a slow fall beyond 1 μ.

A Foxboro dewpoint temperature and humidity recording and controlling system is used, in connection with a 750-w heater and a 1 hp refrigeration unit, each operated with a fan for forced circulation. Moisture is supplied by an atomizer utilizing compressed air at a pressure of 30 lb/in.² For most uses humidity control may not be necessary because of transevaporation, which occurs naturally within the chamber. A more satisfactory system, especially for low or high temperatures and humidities, would employ recirculating air, with the heating and cooling units located outside the box (*6*). It should be emphasized that Fig. 1 is diagrammatic and is thus suggestive of a variety of modifications.

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