NOTES

APPARATUS FOR LARGE SCALE ANAEROBIC CULTURES IN AN ATMOSPHERE OF HELIUM

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Studies of obligate anaerobes are hampered in most laboratories by the paucity of adequate equipment for these special procedures. One of us (A. E. B.) has adapted containers for large scale anaerobic cultures which maintain a high degree of anaerobiosis for several weeks at incubator temperatures.

Steel milk cans[1] were selected for their strength of construction and ease of handling and transporting in the laboratory. A ten gallon milk can accommodates 116 standard petri dishes or 150 tubes (25 by 150 mm) and is very useful if floor level incubator areas are available. A five gallon can (approximately 20 inches in height and 11 inches in diameter) accommodates 56 petri dishes and can be used in standard laboratory incubators. A high pressure stopcock was soldered into an opening through the lid of the container. A layer of a soft sealing clay,[2] approximately one-half inch thick, was molded under the rim of the lid and extended downward on the plug to ensure perfect closure. It has not been found necessary to replace or to remold the clay after 6 months' operation. The lids may be secured to the cans with metal bolts. We have never obtained an increase in pressure, during the incubation interval, sufficient to dislodge the lid or to penetrate the seal.

Anaerobiosis is obtained by vacuum and replacement with gas to atmospheric pressure. We employ helium because it is inert, inexpensive, and apparently does not affect the metabolism of the organisms we have studied. By means of a Y connection, the gas valve on the lid of the can is connected with the helium tank and the vacuum pump. A mercury manometer is placed in the line between the Y connection and the pump, with a 3-way glass T-shaped stopcock provided to permit a closed system measurement of vacuum or pressure in the can. With a Megavac pump and a one-third hp electric motor, we have consistently obtained a pressure of 20 mm of mercury in a can filled with culture plates. When maximum vacuum is obtained, the lead to the pump is closed and the motor is cut off. The gas is admitted slowly to the can until atmospheric pressure is attained. We have found that five consecutive flushings and final replacement with helium to atmospheric pressure provide optimum conditions for mass cultivation of obligate anaerobes on solid and in liquid media.

The method described has yielded luxuriant surface and broth cultures of

[1] Obtained from Sears, Roebuck and Company, no. 32KO8852.

[2] "Plasticine"—obtained from Baltimore Biological Laboratories, Inc., Baltimore, Md.

Clostridium histolyticum, *C. novyi*, *C. sporogenes*, *C. perfringens*, *C. tetani*, *Corynebacterium acnes*, and *Streptococcus anaerobius* after five days' incubation at 34 C. In one experiment a duplicate series of plates inoculated with *C. tetani* failed to produce growth in the same interval and temperature when incubated in a jar designed to provide anaerobiosis by use of illuminating gas and a heat activated catalyst. Excellent sporulation of *C. tetani* and *C. novyi* was observed on plates of brain heart infusion agar after 14 days' incubation; whether mass sporulation occurs on solid media in a shorter interval has not been determined. In a beef heart infusion broth, *C. tetani* produced large numbers of spores in four days.

A design of the container and system employed will be furnished on request.

CHANGES INDUCED IN THE H ANTIGENS OF SALMONELLA BLEGDAM[1]

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Edwards and Moran (Proc. Soc. Exptl. Biol. Med., **61**, 242, 1946) showed that changes could be induced in the H antigens of *Salmonella in vitro* by growth in agglutinating sera. Bruner and Edwards (J. Bact., **53**, 359, 1947) reported alterations of the nonspecific antigens of the *Salmonella*. Bruner (J. Bact., **57**, 387, 1949) demonstrated that it was possible to transform *Salmonella oranienburg* to *S. montevideo*. The H antigens of these two microorganisms fall in the g-complex of the antigenic mosaic of the *Salmonella*, and the fact that one type can be induced from another indicates that the numerous serological types now listed within this group possibly originated from a single ancestral strain, or from a few primitive strains. *Salmonella blegdam* also possesses the factor g, and this note deals with the changes induced in its H antigens.

S. blegdam antiserum absorbed by *Salmonella enteritidis*, and *S. blegdam* absorbed by *Salmonella moscow* were employed in the study. The diagnostic formula of *S. blegdam* is IX,XII: gmq-. Absorption by *S. enteritidis* (IX,XII: gm-) removed all the O agglutinins from the *S. blegdam* antiserum as well as the H agglutinins gm, but permitted those for factor q to remain. By absorbing *S. blegdam* antiserum with *S. moscow* (IX,XII: gq-) agglutinins only for factor m were retained in the serum. Single factor m and q antisera were also obtained by cross absorbing *S. enteritidis* and *S. moscow* antisera, but they were not as satisfactory as the absorbed *S. blegdam* antiserum. In all cases the m and q antisera were treated with chloroform and tested for sterility before use.

Five strains of *S. blegdam* were used in the study and a total of 30 single colonies was examined. In each case growth from a single colony was employed

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