slow-moving growth factors may include not only vit. B_{12}, B_{12a}(8) and B_{12b}(9); but also nucleic acids, nucleotides(10), and other substances relatively insoluble in the *n*-butanol, that may serve as vit. B_{12} substitute growth factors for the test microorganism. Some resolution of these factors has been accomplished by altering the conditions of chromatography, such as using different solvents(4), treating the paper strips with salts (9), and extending the period of solvent flow.

That the comparative bioautograph technic using *E. gracilis* and *L. leichmannii* as described above gives further insight into the complex nature of the slow-moving group of factors is evident. *E. gracilis* showed a growth response to the vit. B_{12} of Cobione, but did not respond to the one or more unidentified slow-moving factors found in the DNA preparations which were highly growth-promoting for *L. leichmannii*. Additional work is needed, however, on the response of *E. gracilis* to vit. B_{12a} and B_{12b} before further information on the identity of the slower-moving factors can be ascertained by this procedure. Additional evidence that *E. gracilis* does not respond to many of the growth factors that are known to be active for the *L. leichmannii* strains, is apparent in all of the chromatograms presented in Fig. 1. Its lack of response to any of the rapidly moving factors substantiates and supplements the work and suggestion of Hutner *et al.*(1). The comparative bioautograph method may thus serve as a useful tool in the study of the slow-moving factors, as a qualitative method of indicating activities present in preparations which are to be assayed for vit. B_{12}, and as a guide in separation and purification procedures.

E. gracilis as a tube assay organism, although satisfactory for the relatively pure materials used in this investigation, in our hands has not given the desired results in the assay of complex natural materials such as animal tissues and blood.

Summary. 1. A method for the use of *Euglena gracilis* var. *bacillaris* as a bioautograph organism for the paper chromatographic study of vit. B_{12} and related growth factors has been described. 2. Evidence has been presented to indicate the value of comparative bioautographs, using *E. gracilis* and *Lactobacillus leichmannii* 313, in the study of these growth factors.

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8. Woodruff, H. B., and Foster, J. C., *J. Biol. Chem.*, 1950, v183, 569.

9. Pierce, J. V., Page, A. C., Jr., Stokstad, E. L. R., and Jukes, T. H., *J. Am. Chem. Soc.*, 1949, v71, 2952.

10. Hotchkiss, R. D., *J. Biol. Chem.*, 1948, v175, 315.

A Simple Device For the Growth of Anaerobic Organisms in Liquid Media. (18249)

ARMINE T. WILSON.

From the Alfred I. du Pont Institute of the Nemours Foundation, Wilmington, Del.

The device described herewith (Fig. 1) is essentially a modification of the Theobald Smith fermentation tube(1). It consists of 2 test tubes differing in size which are fitted at the top with screw caps, and which are joined at the bottom with thick-walled capillary tubing. The plastic cap of the small tube is perforated with a drill-hole 2 mm in diameter, and both caps are supplied with solid heavy rubber gaskets so that air-tight seals are formed when they are screwed in place.

For the growth of anaerobic bacteria, the tubes are prepared in the following way: broth of the desired composition (tryptose phosphate; tryptisoy or a fresh meat infusion-

1. Smith, Theobald. The Fermentation Tube with Special Reference to Anaerobiasis and Gas Production Among Bacteria, Wilder Quarter Century Book, Comstock Publishing Co., Ithaca, N. Y., 1893.

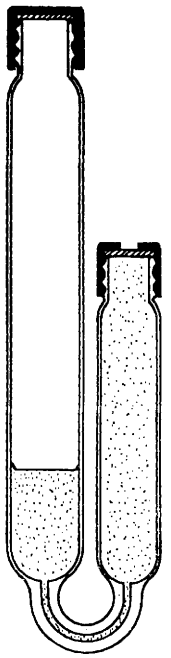

FIG. 1.
Anaerobic culture tube.

peptone broth containing a small amount of glucose) is added to fill the small arm. It is prevented from running into the large arm by tightening the cap on the latter. After the small arm is filled, its cap is screwed tightly in place, the cap in the large arm is removed and about 1 inch of broth is added to the large arm. The cap is replaced loosely on the large arm and the device with its contained broth is sterilized in an upright position in the autoclave by exposure to 121°C for 20 minutes. Pressure is then released slowly in the autoclave by shutting off the steam supply. When the tubes are taken from the autoclave and allowed to cool, the small arm will be found to be filled with broth. If methylene blue has been incorporated in the medium, the small tube will be seen to be in a reduced state, as shown by decoloration of the dye, whereas the medium in the large arm is oxidized, or becomes oxidized shortly from contact with the air which enters the arm. The reduced state in the small arm is maintained indefinitely (at least a month), whether the tubes are stored at room temperature or in the refrigerator.

The tubes are inoculated in one of 2 ways, depending on the quantity of inoculum available. When a large inoculum is used (*e.g.* approximately 0.1 ml of heavy broth culture), it may be added to the broth in the large arm by means of a sterile pipette. It is advantageous to deliver the inoculum at the orifice of the capillary and in many instances the cloudy inoculum can be seen to fall into the capillary. When a small inoculum must be used, it should be delivered into the small arm through the rubber gasket by means of sterile syringe and needle, care being taken not to introduce any air. Table I shows the smallest inoculum in terms of ml of original broth culture capable of initiating growth when introduced into the large arm by pipette, into the small arm by syringe and needle, and, for purposes of comparison, when grown in Brewer's thioglycollate medium.

Growth of the anaerobic organisms begins in the small arm, but when growth is particularly vigorous the medium in both arms will show growth and will be reduced as a result. Theobald Smith recognized the usefulness of his fermentation tube for the growth of anaerobic bacteria(1). The failure of his tube to come into general use for the purpose may be attributed to the inaccessibility of the anaerobic arm for inoculation or withdrawal of material, either of which can be done easily in the case of the modified tube. By means of this device, and larger models of it, any reasonable amount of anaerobic culture may be obtained in broth to which no agar, pieces of tissue or chemical reducing agents have been added. The tubes may be incubated in an ordinary incubator or water bath without the necessity of using an anaerobic jar, thus avoiding the disadvantage the latter has of exposing all its contained tubes to the air when a single tube must be withdrawn for study. Anaerobic bacteria which have been grown in the new device may be recovered from the broth culture by centrifugation, without sediment of tissue or agar to interfere. Furthermore, since the gas which forms during bacterial growth accumulates in the top of the small arm, to which access may be gained through the drill-hole and

TABLE I. Smallest Inoculum Required to Initiate Growth of Anaerobes.

Organism	Anaerobic device: Inoc. in large arm	Anaerobic device: Inoc. in small arm	Brewer's thioglycollate medium
Cl. perfringens	10^{-6}	10^{-9}	10^{-8}
Cl. lentoputrescens	10^{-6}	10^{-10}	10^{-10}
Cl. butyricum	10^{-4}	10^{-6}	10^{-7}
Cl. sporogenes	10^{-6}	10^{-8}	10^{-9}
Cl. tetani	10^{-1}	10^{-6}	10^{-6}
Cl. novyi	10^{-1}	10^{-3}	10^{-3}
Cl. bifermentans	10^{-1}	10^{-9}	10^{-9}
Cl. histolyticum	10^{-5}	10^{-11}	10^{-7}
Cl. septicum	10^{-4}	10^{-5}	10^{-6}
Cl. botulinum	10^{-4}	10^{-11}	10^{-8}

Serial dilutions of a heavy broth culture of the strains were made in 0.85% NaCl solution, so that the indicated inocula, in terms of original broth culture, were contained in 0.1 ml. The anaerobic device contained approximately 20 ml of broth of which 15 ml were in the small arm. Ten ml of Brewer's medium were used for testing. The endpoint was the smallest inoculum which produced visible growth on incubation.

rubber gasket, the gas may readily be removed by syringe and needle or other apparatus for analysis.

Motile variants of bacterial organisms may be isolated from mixed cultures by means of the device. The tubes are filled with semisolid medium and an inoculum is placed near the surface of one arm. During incubation the motile variants grow down the inoculated arm, through the capillary and up the opposite arm to the surface, where they may be recovered readily.

The anaerobic culture device can be made by a skillful glassblower by using Kimble Neutraglass culture tubes with plastic caps (No. 45066) and Kimble Neutraglass capillary tubing (N-51A, No. 46486), outside diameter 7 to 8 mm. The gaskets can be punched from rubber sheeting (heavy inner tubing will do) by means of a cork borer of appropriate size.

Summary. A simple device is described by means of which anaerobic bacteria can be grown in liquid medium without the addition of chemical reducing agents or pieces of tissue, and without the use of an anaerobic jar or similar apparatus.

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Response to Adrenocorticotrophic Hormone in Clinical Scurvy.* (18250)

HENRY S. TREAGER, GEORGE J. GABUZDA,† NORMAN ZAMCHECK AND CHARLES S. DAVIDSON.

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School.

A close relationship between ascorbic acid metabolism and adrenal cortical function is suggested by evidence derived chiefly from animal experiments(1-3). Diminished adrenal cortical activity might be expected, therefore, in human ascorbic acid deficiency. Accordingly, the change of the blood eosinophils and urinary uric acid following test doses of adrenocorticotrophic hormone (ACTH) was measured in 5 patients with clinical and laboratory evidence of scurvy before and after the administration of therapeutic amounts of ascorbic acid. Serum sodium and potassium concentrations likewise were measured before and after the specific antiscorbutic therapy.

Each of the 5 patients had typical scurvy as evidenced by perifollicular hemorrhages, ecchymoses, especially of the posterior thighs and buttocks, a positive tourniquet test, and

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† U. S. Public Health Service Postdoctorate Research Fellow.

1. Selye, H., Textbook of Endocrinology, Acta Endocrinoligica, 1947, Montreal University, Montreal, Canada.

2. Long, C. N. H. Recent Progress in Hormone Research, I, 1946, Academic Press, New York.

3. Sayers, G., and Sayers, M. A., *Ann. N. Y., Ac. Sc.*, 1949, v50, 522.