SCIENTIFIC APPARATUS AND LABORATORY METHODS

A CHEMICALLY DEFINED MEDIUM FOR THE CULTIVATION OF THE GONOCOCCUS[1]

A FLUID medium comprising 9 organic acids, 5 inorganic salts and glucose has been developed for the growth of the gonococcus. It contains 7 organic acids in addition to those required by the meningococcus as reported by Frantz.[2] The composition is as follows:

	Grams per liter
d-Glutamic acid[3]	1.3
dl-Leucine[3]	0.40
l-Arginine monohydrochloride[3]	0.25
l-Histidine monohydrochloride[3]	0.15
dl-Methionine[3]	0.15
l-Proline[3]	0.10
Glycine[4]	0.05
l-Cystine[4]	0.01
Indole-3-acetic acid[4]	0.10
NaCl[5]	6.0
$NaH_2PO_4 \cdot H_2O$[5]	2.5
NH_4Cl[5]	1.25
$Mg(NO_3)_2 \cdot 6H_2O$[5]	0.05
$FeSO_4$[5]	0.012
Glucose[4]	5.0

With the exception of glucose, indole acetic acid, ferrous sulfate and magnesium nitrate, the constituents are added to 950 ml of distilled water. The pH of the mixture is adjusted with normal sodium hydroxide to from 6.8 to 7.0, and then autoclaved at 121° C for 10 minutes in a pyrex-glass container. After cooling to room temperature, 25 ml of a sterile 20-per cent. glucose solution are introduced into the medium. One per cent. solutions of ferrous sulfate, magnesium nitrate and indole acetic acid are added separately in the following volumes, respectively; 1.2 ml, 5.0 ml and 10.0 ml. The pH is finally readjusted to from 7.0 to 7.2.

Five ml of the medium were inoculated with one loopful of washed gonococcal cells obtained by centrifugation from a 24-hour Douglas's broth culture. Incubation was carried out at 37° C in an atmosphere containing approximately 10 per cent. of carbon dioxide. The method of introducing 10 per cent. tank carbon dioxide as described by Leahy and Carpenter[6] gave better results than the method of burning a candle to self-extinction. The gaseous mixture was replaced at daily intervals.

Sixty strains of *Neisseria gonorrhoeae* were employed for the development and testing of this medium. Both recently isolated strains and those subcultured for several years were included. Not all strains grew equally well and approximately 25 per cent. did not grow either in the synthetic medium or in Douglas's broth. Growth was maximal after 2- to 3-days' incubation. At this time, 5.0 ml of the medium contained, on the average, 0.25 mg of bacterial nitrogen, which is equivalent to 2.0 mg of gonococcal cells. The growth was more than double that obtained in Douglas's broth under the same conditions. The cells remained viable for at least 5 days. Cultures transferred every third day have been maintained readily for 3 months.

The final concentration of each substance in the medium was determined on the basis of maximal growth of the majority of the strains tested. The concentrations of glycine, cystine, ferrous sulfate and of both ions of magnesium nitrate were critical. The amount of the other substances employed in the medium permitted of some variation. Divalent lead and trivalent iron salts in concentrations of 10 micrograms per ml favored the growth of certain strains. The manganous ion, in a concentration of 5 micrograms per ml was toxic for the gonococcus. The cupric ion was also toxic but only at concentrations greater than 5 micrograms per ml.

Growth of the strains which otherwise failed to grow in the medium above described was obtained in almost every instance when glutamine[7] and choline were incorporated in the medium in concentrations of 0.2 mg and 0.1 mg per ml, respectively.

Studies to determine the more rigid requirements of certain primary cultures of *Neisseria gonorrhoeae* are in progress.

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[1] Supported in part by grants from the John and Mary R. Markle Foundation and the United States Public Health Service.

[2] I. D. Frantz, *Jour. Bact.*, 43: 757, 1942.

[3] We are indebted to Merck and Company, Incorporated, Rahway, N. J., for certain of the synthetic amino acids.

[4] Eastman Kodak Company, Rochester, N. Y.

[5] J. T. Baker Chemical Company, Phillipsburg, N. J.

[6] A. D. Leahy and C. M. Carpenter, *Am. Jour. Syph., Gonor. and Ven. Dis.*, 20: 353, 1936.

[7] C. E. Lankford and E. E. Snell, *Jour. Bact.*, 45: 410, 1943.

BOOKS RECEIVED

DOBZHANSKY, TH. and CARL EPLING. *Contributions to the Genetics, Taxonomy and Ecology of Drosophila pseudoobscura and Its Relatives.* Illustrated. Pp. iii + 183. Carnegie Institution of Washington.

GUSTAFSON, A. F., C. H. GUISE, W. J. HAMILTON, JR. and H. RIES. *Conservation in the United States.* Illustrated. Pp. xi + 477. Comstock Publishing Company. $4.00.

STEWART, JOHN Q. and NEWTON L. PIERCE. *Marine and Air Navigation.* Illustrated. Pp. xii + 471. Ginn and Company. $4.50.