stimulate acid production either alone or in combination and in concentrations ranging from 20 to 0.01 $\mu\text{gm./ml.}$ are adenine, guanine, uracil, thymine, 4-methyl-5-ethoxymethyl uracil, 4-methyl-4:5-dihydro-uracil, 2-methyl-4-amino-5-aminomethyl pyrimidine, 4-methyl-5-amino-uracil and 4-methyl-uracil.

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- ¹ Snell, E. E., and Mitchell, H. K., *Proc. U.S. Nat. Acad. Sci.*, **27**, 1 (1941).
² Richardson, G. M. *Biochem. J.*, **30**, 2184 (1936).
³ Möller, E. F., *Z. physiol. Chem.*, **260**, 246 (1939).
⁴ Shull, G. M., Hutchings, R. L., and Peterson, W. H., *J. Biol. Chem.*, **142**, 913 (1942).
⁵ Lindner, M., and Pollack, M. A., *J. Biol. Chem.*, **143**, 655 (1942).
⁶ Feeney, R. E., and Strong, F. M., *J. Amer. Chem. Soc.*, **64**, 881 (1942).
⁷ Chattaway, F. W., Happold, F. C., and Sandford, M., *Biochem. J.*, **37**, 298 (1943).

Importance of Pyrimidine Derivatives in the Growth of Group C (Lancefield) Streptococci upon a Simplified Medium

RICHARDSON¹ showed that uracil (2.6 dihydroxy pyrimidine) is necessary for the anaerobic growth of *Staphylococcus aureus*, and more recently Snell and Mitchell² have reported that purine and pyrimidine derivatives stimulate the growth of certain lactic acid bacteria. Möller³ had previously claimed that adenine and guanine increase the growth of *Lactobacillus plantarum*. Pappenheimer and Hottle⁴ showed that uracil is not necessary for the growth of a strain of hæmolytic streptococcus Group A. The latter authors reported, however, that purine derivatives are necessary for this organism unless the carbon dioxide tension in the atmosphere above the culture is high. At a partial pressure of 40 mm. mercury of this gas, rapid and optimal growth occurs without the addition of purines to the medium.

It was found here that a strain of Group C (Lancefield) type 7 (Griffiths) hæmolytic streptococcus (called C7) gave only slight and variable growth upon a casein hydrolysate medium unless this was supplemented by uracil. The casein hydrolysate basal medium was similar to that designed by Bernheimer, Gillman, Hottle and Pappenheimer⁵, except that biotin, which is not necessary for the growth of C7 upon the casein medium, was omitted. Bicarbonate, and, of course, uracil were also not added. The growth-factors present were thiamine, nicotinic acid, pyridoxin, calcium pantothenate, riboflavine, adenine and glutamine. It is probable that not all these factors were necessary for the growth of the organism, but they were included in the medium for completeness. The glucose concentration was 0.25 per cent.

Two inocula were used: the first (I) was three drops of faintly opalescent suspension of bacterial cells in isotonic saline per 10 ml. of medium. The cells were from a 6-7 hour broth culture of C7 and were twice washed with saline. The second inoculum (II) was three drops of a 1/100 dilution of (I) per 10 ml. of medium.

Table 1 shows the mass of growth, as milligrams

of bacterial N per 50 ml. of culture, after twenty-four hours incubation at 37°C. in the medium supplemented by uracil or orotic acid (uracil-4-carboxylic acid).

TABLE 1. INFLUENCE OF ADDING VARIOUS AMOUNTS OF URACIL AND OROTIC ACID TO THE BASAL MEDIUM, UPON THE GROWTH OF STREPTOCOCCUS C7.

Mgm. of uracil or orotic acid added per 10 ml. of medium	Growth in mgm. bact. N per 50 ml. of medium					
	Inoculum I			Inoculum II		
	Uracil		Orotic acid	Uracil		Orotic acid
	Exp. a	Exp. b	Exp. a	Exp. a	Exp. b	Exp. a
0	5.9	2.3	6.6	3.8	0.7	3.9
0.005	—	3.4	6.1	5.2	0.7	3.5
0.02	9.2	6.0	7.4	8.1	3.5	7.5
0.10	12.5	—	11.5	—	—	10.8
0.20	10.0	8.6	10.2	10.0	10.0	11.2

The amount of growth which occurred in the media containing only small amounts of the pyrimidine derivatives was variable but did not increase during a further twelve days incubation. The addition to the medium of 10-20 $\mu\text{gm./ml.}$ of uracil or orotic acid, however, resulted in growth as good as that in peptone broth cultures.

Increased carbon dioxide tension up to 50 mm. mercury had no influence on the mass of growth that occurred in any of the media. Complete removal of carbon dioxide from the atmosphere above the culture inhibited the growth of inoculum II but not I, even in the presence of 20 $\mu\text{gm./ml.}$ of uracil.

Four out of five of the strains of Group C streptococci of various types which were tested also needed uracil or orotic acid for optimal growth. The two pyrimidine derivatives were equally effective in increasing both the rate and mass of growth of these organisms. Table 2 summarizes these results and includes C7 for comparison.

TABLE 2. INFLUENCE OF URACIL AND OROTIC ACID (EACH AT A CONCENTRATION OF 0.10 MG. PER 10 ML. OF MEDIUM) UPON THE RATE AND MASS OF GROWTH OF GROUP C STREPTOCOCCI. THE INOCULUM USED WAS OF STRENGTH II ABOVE.

Strain	Basal medium		Basal medium + uracil		Basal medium + orotic acid	
	M	T	M	T	M	T
Mare (2)	++	4 days	++++	40 hr.	++++	40 hr.
Mare (3)	+	5 "	++++	20 "	++++	20 "
Loewentahl M	+++	6 "	++++	60 "	++++	40 "
Loewentahl O	+++	40 hr.	+++	40 "	+++	40 "
Human (20)	+	5 days	++++	16 "	++++	24 "
C7	+ to +++	40 hr.	++++	20-40 "	++++	20-40 "

M equals final mass of growth; T equals time to reach maximum growth.

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- ¹ Richardson, G. H., *Biochem. J.*, **30**, 2184 (1936).
² Snell, E. E., and Mitchell, H. K., *Proc. U.S. Nat. Acad. Sci.*, **27**, 1 (1941).
³ Möller, E. F., *Z. physiol. Chem.*, **260**, 246 (1939).
⁴ Pappenheimer, A. M., and Hottle, G. A., *Proc. Soc. Exp. Biol. and Med. N.Y.*, **44**, 645 (1940).
⁵ Bernheimer, A. W., Gillman, W., Hottle, G. A., and Pappenheimer, A. M., *J. Bact.*, **43**, 495 (1942).