The response of *S. cremoris* H1–2 to a purified preparation of POF is shown in table 1. The data presented are for 24 hours' incubation, but very little change is observed on further incubation. Growth has never been observed in tubes containing no added POF. This strain of *S. cremoris* differs in this respect from other lactic acid bacteria that are used for the assay of this group of factors, for, as stated by Guirard *et al.* (Arch. Biochem., **9**, 361, 1946) and amply confirmed in this laboratory, these organisms will frequently grow in media containing no added acetate or factor, especially if incubation is prolonged. *Corynebacterium* sp. (Stokstad *et al.*: Proc. Soc. Exptl. Biol. Med., **74**, 571, 1950) resembles *S. cremoris* in having an absolute requirement for this group of factors.

TABLE 1

The response of S. cremoris to POF

POF*	GROWTH	POF*	GROWTH
mμg/5 ml	Optical density	mμg/5 ml	Optical density
0	.001	90.0	.182
7.5	.009	105.0	.186
15.0	.013	120.0	.189
30.0	.092	150.0	.194
45.0	.126	300.0	.202
60.0	.158	750.0	.209
75.0	.174		

Twenty-four hours' incubation at 30 C. Growth measured by an Evelyn colorimeter with a 660 filter.

* Purified 2,300 times from yeast extract. From Dr. I. C. Gunsalus.

The range of response of *S. cremoris*, 15 to 100 mμg, is similar to that found for *Lactobacillus casei* (Snell and Broquist, *loc. cit.*) with a POF concentrate of the same order of purity. Thus it seems probable that POF represents the active fraction of reticulogen.

GASEOUS REQUIREMENTS FOR THE CULTIVATION OF FUSOBACTERIA

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Although the difficulties in the cultivation of strains of *Fusobacterium* are widely recognized, the factors responsible have not yet been precisely established.

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Repeated attempts by us to use the classic pyrogallol anaerobic technique of Buchner (Centr. Bakt. Parasitenk., **4**, 149, 1888) proved satisfactory for obtaining primary isolations but subcultures failed consistently. In an attempt to determine the factors responsible, it became evident after some preliminary observations that the gaseous requirements are of crucial importance. An examination of eight strains confirmed the report of Schwabacher and Lucas (J. Gen. Microbiol., **1**, 109, 1947) that some fusobacteria require a CO_2 concentration higher than that present in the atmosphere. In addition, it was found that for all strains there was a critical concentration of CO_2 below which no growth occurred. In most cases this concentration was less than 0.03 per cent. An attempt was then made to modify Buchner's pyrogallol method accordingly. Sodium carbonate solution was substituted for potassium hydroxide as the alkalinizing agent, and a 6 per cent CO_2 concentration was provided for by means of the citric acid and sodium bicarbonate reaction. Nevertheless, *Fusobacterium* produced only water-clear, barely perceptible colonies, although a control with *Clostridium* in another pyrogallol jar grew quite well and *Fusobacterium* in a Brewer anaerobic jar control showed optimum growth.

The reason for the failure has not been elucidated by us, but it appears most probable that the demonstrated evolution of CO upon the oxidation of pyrogallol (Nicol: Biochem. J., **23**, 324, 1929) is the responsible factor. Strictly anaerobic microorganisms are not known to be CO-sensitive, but, although we have not observed aerobic fusobacteria, such forms have been reported (Málek and Málková: Zentr. Bakt. Parasitenk., I, Orig., **143**, 126, 1939) and it is therefore possible to postulate that the anaerobic fusobacteria may have retained essential respiratory enzymes that are CO-sensitive. Successful primary cultivations with the pyrogallol method may have been possible because of selective absorption of the CO by the associated organisms.