THE RELATION OF AEROBIOSIS TO THE FERMENTATION OF MANNITOL BY STAPHYLOCOCCI

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While studying the fermentative reactions of a number of strains of *Staphylococcus aureus*, it was noticed that the mannitol tubes differed in appearance from those of the fermentable carbohydrates glucose, lactose, and sucrose. In the mannitol tubes (1 per cent mannitol semi-solid agar with Andrade indicator) only the top of the medium to a depth of $\frac{1}{8}$–$\frac{1}{4}$ inch was pink after 24–48 hours incubation, while in the other tubes the medium was uniformly pink. This observation led to the study of the fermentation of mannitol by staphylococci from various sources with special reference to their oxygen requirements. Six strains of mannitol-fermenting streptococci were tested in comparison.

The fermentation of mannitol by staphylococci has been studied by a number of investigators, Hine (1922), Dudgeon and Simpson (1928), Julianelle (1937), Thompson and Khorazo (1937), and others, and their conclusions are in close agreement that the pathogenic strains, (*Staphylococcus aureus* and *Staphylococcus albus*), are, in the main, mannitol-positive, while the nonpathogenic ones (usually *S. albus*) are, with few exceptions, mannitol-negative. With regard to the oxygen requirements for the growth of staphylococci, the test books list these organisms as facultative anaerobes which grow best in the presence of oxygen. Fildes and his co-workers (1936) found that a synthetic nutrient mixture that supported aerobic growth of staphylococci was inadequate for anaerobic growth without the addition of three other substances, namely pyruvic acid, CO_2, and a

"staph factor" which was shown later by Knight (1937) to consist of nicotinamide and aneurin (Vitamin B_1).

METHODS

Solutions of the test substances glucose, lactose, sucrose, and mannitol (Pfanstiehl), sterilized by Berkefeld filtration, were added in 1 per cent amounts to the basic media, 1 per cent Difco Bacto peptone, pH 7.0, and semi-solid agar containing 0.5 per cent peptone, pH 7.1. Andrade and brom-cresol-purple were used as indicators. After inoculation of a small loop of 18-hour slant growth, free access of oxygen was excluded from certain tubes by a layer of sterile vaseline on top of the medium.

SOURCE OF CULTURES

Six of the staphylococcus cultures from boils, all from normal throats, normal skin, and cream filling were freshly isolated and tested within two weeks. The rest were stock cultures isolated within five years. All colonies fished from each individual source were identical by mannitol fermentation.

Only cultures of staphylococci which were found to ferment mannitol aerobically are included in this study. Other cultures were tested which failed to ferment under both aerobic and anaerobic conditions; they included 4 *S. aureus* strains (normal throat and normal skin) and 10 *S. albus* (normal throat, normal skin and infected sebaceous gland).

The results of the fermentation tests of 25 *Staphylococcus aureus*, 5 *Staphylococcus albus*, and 6 *Streptococcus* cultures in mannitol, glucose, lactose, and sucrose mediums in test tubes with and without a layer of vaseline are summarized in the table. All of the staphylococcus cultures fermented mannitol within 48 hours when grown aerobically at 37°C. The semi-solid medium showed the indicator change only at the top of the medium during 24–48 hours incubation except for two cultures, H and C, which were slightly acid in the butt as well; by the third day of incubation all tubes were uniformly acid. When grown anaerobically, 28 of the 30 cultures were negative up to the 5th day, but after 6–14 days small and irregular amounts of indicator change were

noted. The 6 cultures of *Streptococcus* fermented mannitol within 24 hours under both aerobic and anaerobic conditions.

The carbohydrates glucose, lactose, and sucrose were fermented promptly by all of the cultures of *Staphylococcus* and *Streptococcus* when grown with and without vaseline seal.

Other basic media, namely extract broth and 10 per cent horse-serum water containing mannitol were tested with a limited

TABLE 1

Fermentation of mannitol, glucose, lactose, and sucrose by staphylococci and streptococci when tested aerobically and anaerobically (vaseline seal)

ORGANISM	SUB-GROUP	SOURCE	NUMBER	MANNITOL			GLUCOSE, LACT., SUCROSE	
				Aerobic	Anaerobic		Aerobic	Anaerobic
				48 hours	48 hours	5 days	48 hours	48 hours
Staphylococcus	*Aureus*	Boils, etc.	16	+	−	−	+	+
		Boil (H)	1	+	±	+	+	+
		Blood	1	+	−	−	+	+
		Skin (normal)	2	+	−	−	+	+
		Throat (normal)	2	+	−	−	+	+
		Throat (normal) (C)	1	+	±	±	+	+
		Cream filling	2	+	−	−	+	+
	Albus	Boil	1	+	−	−	+	+
		Skin (normal)	1	+	−	−	+	+
		Throat (normal)	3	±−+	−	−	+	+
Streptococcus	*Alpha*	Feces	2	+	+	+	+	+
		Milk	2	+	+	+	+	+
	Beta	Milk	2	+	+	+	+	+

Explanation of symbols: +, acid; ±, weakly acid; − no change in indicator.

number of cultures. The results coincided with those obtained in the peptone and semi-solid media.

More concise data on the relation of free access of oxygen to the fermentation of mannitol by staphylococci resulted from a comparison of the pH readings on a culture incubated in a shallow layer of mannitol-peptone medium in a flask and in test tubes, with and without vaseline seal. pH readings were made by the

colorimetric method. After 24 hours the pH of the growth in the flask was 4.9, in the open tube 6.0, and in the closed tube 7.0; after 48 hours the readings of the growth in the respective environments were 4.5, 5.2, 7.0; and after 5 days 4.5, 4.5, 6.8. Four additional cultures were grown under vaseline seal in duplicate for 5 days. The pH readings ranged from 6.3 to 6.9. Duplicate tubes, in some instances, gave different pH readings, which were usually associated with slight differences in cloudiness.

GROWTH OF STAPHYLOCOCCUS UNDER ANAEROBIC CONDITIONS

The small changes in pH reading and the occasional slight increased cloudiness after 5 days incubation under vaseline seal would indicate that the organisms had grown to some extent. More definite proof was obtained when serial dilutions of culture in semi-solid mannitol-agar were incubated with and without vaseline seal. Observations made after 24 hours incubation showed that, in the highest culture dilution, approximately the same number of colonies had developed in the aerobic and anaerobic tubes, but the colonies in the closed tube were much smaller than in the aerobic one and they did not increase in size upon incubation for 5 days.

FERMENTATION OF GLYCEROL AND SORBITOL

Because of the observed difference in the fermentative reactions of staphylococci in the alcohol mannitol as compared with the carbohydrates glucose, lactose, and sucrose, it seemed of interest to test reactions in the alcohols glycerol and sorbitol. The result of this test showed that 8 mannitol-positive cultures, including strain H, fermented glycerol aerobically in 48 hours to 5 days but failed to ferment within 14 days when incubated under vaseline seal. A mannitol-negative strain of *Staphylococcus* was also negative in glycerol.

Sorbitol was not fermented by 10 cultures of *Staphylococcus* incubated aerobically for 12 days. Ten per cent horse-serum water with 1 per cent sorbitol was used in addition to the peptone and semi-solid mediums. Sorbitol was fermented within 24 hours by 2 cultures of beta-hemolytic streptococcus of Lancefield (1933) group C when grown aerobically and under vaseline seal.

DISCUSSION

The results of these experiments, which show distinct differences in the anaerobic actions of staphylococci on mannitol and glycerol on the one hand and on glucose, lactose, and sucrose on the other, but not in the aerobic actions on these same test substances, are presented without attempting to enter further into the chemical, metabolic, or enzymatic factors involved. A possible explanation of these findings may be based on difference in chemical composition, the former compounds being alcohols and the latter carbohydrates; and the probability that under anaerobic conditions the alcohols are not utilized as readily as the carbohydrates by an organism which grows feebly anaerobically. The studies of Gladstone, Fildes, and Richardson (1935) may have some bearing on this work. They found that "in an anaeroboid CO_2-free atmosphere, *Staphylococcus aureus* did not grow in 45 hours but that if glucose or lactate were present growth occurred in 24 hours."

SUMMARY

Twenty-eight cultures of *Staphylococcus* (23 *S. aureus* and 5 *S. albus*) which fermented mannitol in 24–48 hours aerobically, when tested under vaseline seal showed no change of indicator after 5 days incubation, but small and irregular amounts on longer incubation. pH readings on a limited number of cultures, incubated for 5 days under vaseline seal, ranged from 6.3 to 6.9. Two cultures of *Staphylococcus aureus* showed slight to moderate fermentation of mannitol after 48 hours incubation under vaseline seal.

Eight cultures of mannitol-fermenting staphylococci fermented glycerol aerobically in from 48 hours to 5 days, but failed to ferment when grown anaerobically for 14 days.

The fermentation of glucose, lactose, and sucrose by the 30 cultures of *Staphylococcus* took place within 48 hours aerobically and under vaseline seal.

Unlike the staphylococci, 6 *Streptococcus* cultures (4 alpha and 2 beta) fermented mannitol as well as glucose, lactose, and sucrose within 48 hours whether grown under aerobic or anaerobic conditions.

The marked inhibition or lack of fermentation of mannitol anaerobically by staphylococci is dependent, to some extent at least, upon the limited growth activity in an anaerobic environment.

It is suggested as a possible explanation that staphylococci cannot utilize the alcohols mannitol and glycerol as readily as the carbohydrates glucose, lactose, or sucrose in order to obtain substances essential for vigorous growth under anaerobic conditions.

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