The effect of carbohydrates on the production of staphylococcal pigment

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SYNOPSIS The effect of the presence of carbohydrates on the pigmentation of *Staphylococcus aureus* was investigated. It was shown that pigmentation is greatly increased in the presence of those carbohydrates which are fermented by the organism. This effect may be due to the products of fermentation.

This work was initiated by the observation of one of us, which was that the colonies of *Staphylococcus aureus* adjacent to a penicillin sensitivity testing tablet showed a golden pigmentation whereas those on the remainder of the blood agar plate were white. This effect had been noticed but not investigated by Coackley (1951). Preliminary tests indicated that this phenomenon was not due to the penicillin but to the lactose, which formed part of the base substance of the sensitivity tablet. Chapman (1943) had demonstrated increased staphylococcal pigment in the presence of lactose, Kooistra and Sellers (1957) an increase with mannite, Allegra, Niutta, and Giuffrida (1955) an increase with glucose, and Sevag and Green (1944) had shown a similar effect with glucose, d-galactose, d-mannitol, d-mannose, and trehalose but could not demonstrate an increase with l-arabinose, dulcitol, or rhamnose. Johnston (1956), however, when, working to try to explain why milk increased staphylococcal pigmentation, failed to demonstrate an increased pigmentation with glucose, lactose, or mannite. The present investigation, therefore, was undertaken to confirm that the pigmentation of staphylococci was increased by the presence of carbohydrates and if this was so to ascertain which carbohydrates had this effect and to elucidate the underlying mechanism of this phenomenon.

MATERIALS AND METHODS

Fifty unselected coagulase-positive strains of *Staphylococcus aureus* were tested. The organism was inoculated heavily onto a plain agar plate and a blank Sentest tablet (lactose base only) placed on the surface. The plates were incubated overnight at 37°C., followed by six hours at room temperature, and then the observations

were made. At the same time in order to confirm that any increased pigmentation was due to the lactose in the tablet and not to some impurity or binding agent, five of the strains of *Staphylococcus aureus* were plated on plain agar and 1% lactose agar and incubated as before.

As 49 of the 50 strains of staphylococci tested showed increased pigmentation round the tablet and the colonies on lactose agar were markedly more pigmented than those on plain agar, the fermentation reactions of each strain were tested. At the same time the effect of the carbohydrates, which were used in the fermentation reactions, on the pigmentation of the staphylococci was investigated. Twenty-eight of the strains showed a 'ring' effect, which is an area of unpigmented growth of approximately 2 cm. round the tablet surrounded by a ring of deeply pigmented colonies. As this reaction might arise if an optimum concentration of lactose is required by staphylococci to increase their pigmentation, gradient plates were prepared by the method of Szybalski (1952). Ten per cent lactose agar was used as the top layer. These were inoculated with streaks of 10 strains of staphylococci and incubated as before.

RESULTS

Forty-nine out of 50 strains of staphylococci showed an increased pigmentation round the lactose tablet and the colonies on lactose agar were much more pigmented than those on plain agar.

Ten per cent lactose gradient plates demonstrated an increase of pigmentation with a diminishing concentration of lactose, whereas control plates showed no alteration in the depth of pigmentation.

The fermentation reaction of the 50 strains of staphylococci and the effect of these carbohydrates on the pigmentation of these organisms is recorded in Table I. It is thus evident that the pigmentation of these strains of staphylococci is enhanced by the presence of those carbohydrates which are fermented by them.

TABLE I
FERMENTATION REACTION AND PIGMENTATION OF 50 STRAINS OF STAPHYLOCOCCI

Carbohydrate	Glucose	Lactose	Dulcitol	Mannitol	Maltose
No. of strains showing fermentation	50	49	0	49	50
No. of strains showing enhancement of pigmentation	50	49	0	49	50

CONCLUSIONS

The pigmentation of *Staphylococcus aureus* is enhanced by the presence of those carbohydrates fermented by the organism and this may be due to a product of fermentation.

In the case of some strains of *Staphylococcus aureus* this effect is shown most clearly at a low concentration of the carbohydrate.

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