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EFFECT OF SODIUM CHLORIDE ON GROWTH OF STAPHYLOCOCCUS AUREUS IN SYNTHETIC TISSUE CULTURE MEDIUM

(Staphylococcus aureus/sodium chloride)

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Staphylococcus aureus Smith was cultured in a synthetic tissue culture medium containing various concentrations of sodium chloride. The growth was at a higher level in the media containing sodium chloride of concentrations of 2.0 and 4.0% than in those of less than 2.0% and more than 4.0%. The result suggests that the bacteria grow more vigorously in the fairly high concentrations of sodium chloride than in the physiological concentration of it, in the synthetic medium. It may be considered that the high incidence of staphylococcal skin infections under hot conditions is due to the high level of growth of Staphylococcus aureus in fairly high concentrations of sodium chloride produced by hypersweating on the skin.

Staphylococcus, especially Staphylococcus aureus is a frequent cause of bacterial skin infections (1). The incidence of staphylococcal skin infections is high in tropical countries (2), and hot conditions result in an increased incidence in them (3, 4). These facts indicate that the frequency of staphylococcal skin infections is increased by hypersweating. However, it is not yet clearly known why hypersweating increases the frequency of the diseases. As the growth of Staphylococcus, including Staphylococcus aureus is possible in high concentrations of sodium chloride (5) which is one of the main components of sweat (6), it may be assumed that the bacteria grow under hypersweating more vigorously than the other sodium chloride-sensitive organisms, and therefore, infectious lesions are formed on the skin. Staphylococcus aureus has been stated to be resistant to, and not dependent upon, high concentrations of

sodium chloride (7-10). This conclusion, however, has been obtained from the observation of its growth in non-synthetic and semi-synthetic media, both of which contained high amounts of peptides and/or proteins. Sweat contains not so high concentrations of peptides and proteins (11). Therefore, it may be necessary to study whether the growth of Staphylococcus aureus is resistant to, dependent upon or sensitive to high concentrations of sodium chloride in a medium without peptides and proteins, in order to know the mode of its colonization on the skin through hypersweating. For this purpose, the growth of Staphylococcus aureus Smith was observed in the present study in Medium 199 as a synthetic medium without peptides and proteins, and in nutrient broth and a semi-synthetic medium which contained various concentrations of sodium chloride, as controls.

MATERIALS AND METHODS

Staphylococcus aureus Smith (diffuse type) was used in this study. The media used were Medium 199 (Gibco Laboratories, Grand Island) containing 30 mM Tris-HCl buffer (pH 7.4) as a synthetic tissue culture medium, nutrient broth (Nissui Pharmaceuticals, Tokyo) and a semi-synthetic medium containing the following components (per l): $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 13.9 g; KH_2PO_4 , 2.1 g; NH_4Cl , 1.0 g; CaCl_2 , 0.0146 g; MgSO_4 , 0.22 g; $\text{FeCl}_3 \cdot 4\text{H}_2\text{O}$, 0.24 mg; glucose, 1.0 g; peptone, 5.0 g; yeast extract, 1.0 g (10) as controls. To each medium, sodium chloride was, initially, not added, and then added in final concentrations of 1.0, 2.0, 4.0, 7.0, 10.0 and 15.0%, respectively. Staphylococcus aureus Smith grown and stocked on a fresh brain heart infusion agar slant was cultured at 37°C overnight in 20 ml of each medium without addition of sodium chloride in a 200ml Erlenmeyer flask. A part of the cultured bacteria was added to 50 ml of each of the same media as that of the preculture containing a series of various concentrations of sodium chloride in a 500-ml Erlenmeyer flask. They were then cultured in a temperature-regulated reciprocal shaker (37°C, 100 rev/min). The growth of the bacteria was measured according to optical density of Klett Summerson photoelectric colorimeter (green filter, 540 μm) at intervals of every one hour (12). At the same time, a small part of the culture was taken and diluted serially with saline, and the viable cells were counted on nutrient agar plates.

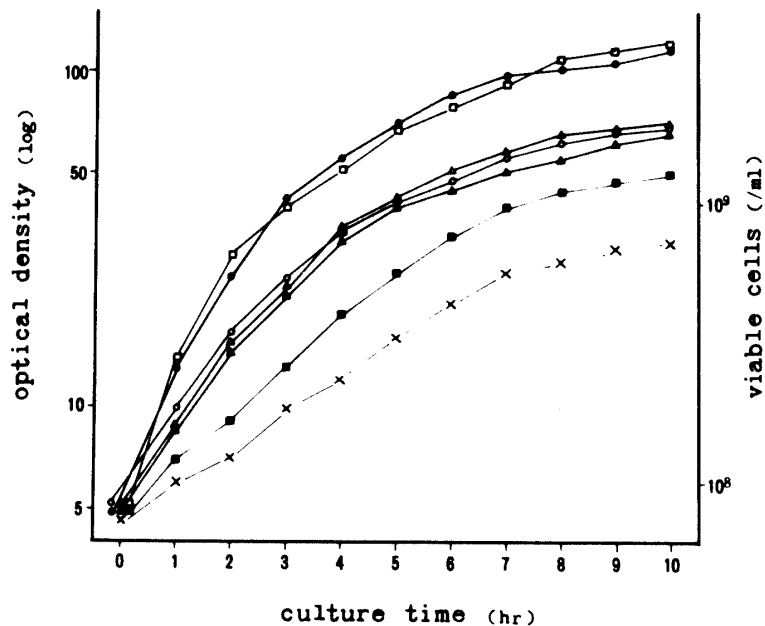


Fig. 1. Growth of *Staphylococcus aureus* Smith in Medium 199 containing various concentrations of sodium chloride, O: not added, Δ: 1.0%, □: 2.0%, ●: 4.0%, ▲: 7.0%, ■: 10.0%, X: 15.0%.

RESULTS

The growth of *Staphylococcus aureus* Smith in Medium 199, nutrient broth and the semi-synthetic medium, containing various concentrations of sodium chloride is shown in Figs. 1-3, respectively. In nutrient broth and the semi-synthetic medium, the more sodium chloride was added, the less the bacteria grew. In Medium 199, however, they grew more vigorously with the addition of sodium chloride in final concentrations of 2.0 and 4.0% than they did without the addition of sodium chloride and more vigorously than they did with the addition of it in final concentration of 1.0%. When sodium chloride was added in final concentrations of more than 4.0%, the more sodium chloride was added, the less the bacteria grew.

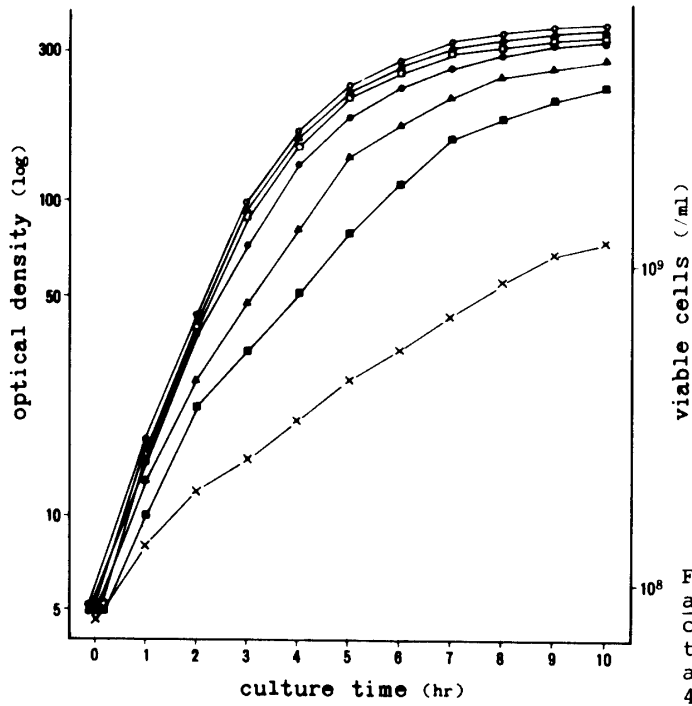


Fig. 2. Growth of *Staphylococcus aureus* Smith in nutrient broth containing various concentrations of sodium chloride, O: not added, Δ : 1.0%, \square : 2.0%, \bullet : 4.0%, \blacktriangle : 7.0%, \blacksquare : 10.0%, X: 15.0%.

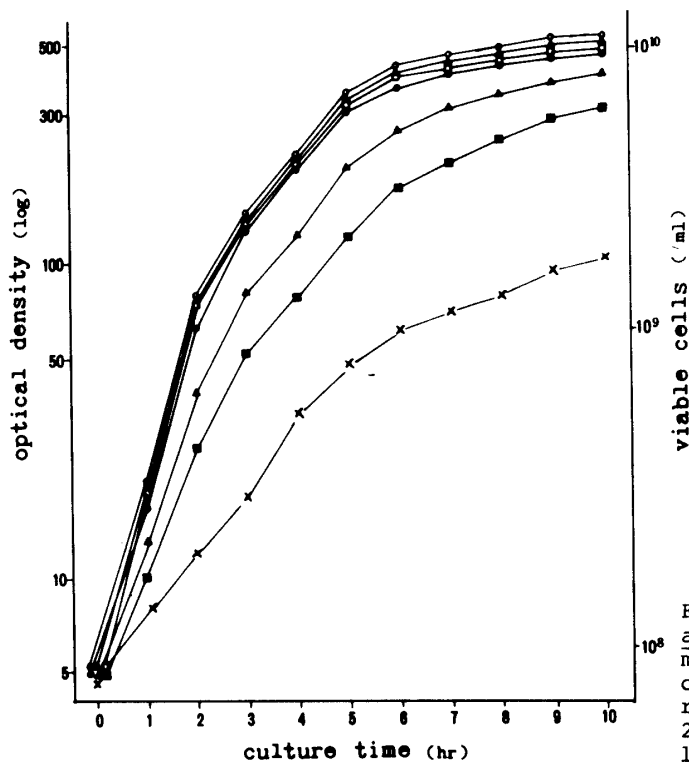


Fig. 3. Growth of *Staphylococcus aureus* Smith in semi-synthetic medium containing various concentrations of sodium chloride, O: not added, Δ : 1.0%, \square : 2.0%, \bullet : 4.0%, \blacktriangle : 7.0%, \blacksquare : 10.0%, X: 15.0%.

DISCUSSION

The results reported above indicate that the growth of Staphylococcus aureus Smith in Medium 199 containing fairly high concentrations of sodium chloride was dependent upon sodium chloride, in contrast to that of the physiological concentration. The growth of the bacteria in nutrient broth and the semi-synthetic medium possessed a mode of relative resistance to high concentrations of sodium chloride, which is the same result as reported by many workers (7-10). Any bacteria other than Staphylococcus strains show almost any growth in media containing more than 3% of sodium chloride (10). Non-synthetic components are beef extract and peptone in nutrient broth, and yeast extract in the semi-synthetic medium. Vitamins are present in beef extract and yeast extract, and also in Medium 199. Therefore, it may be suggested that the growth of Staphylococcus aureus is dependent upon fairly high concentrations of sodium chloride under conditions without peptides and proteins.

In man, a plasma-like isotonic precursor fluid is secreted by the secretory coil and the sodium chloride is reabsorbed in excess of water by the duct, in the eccrine sweat gland (13, 14). As the ductal sodium chloride reabsorption is a rate-limiting process, at lower sweat rates the final sodium chloride concentration in sweat is low, and at higher sweat rates it approaches isotonicity. Under hot conditions, hypersecretion of sweat with nearly isotonic concentrations of sodium chloride occur. After vaporizing of water in the secreted sweat, the concentrations of sodium chloride on the skin may become fairly higher than the physiological concentration.

The results of the present study indicate that the growth of Staphylococcus aureus in the synthetic tissue culture medium depends upon fairly high concentrations of sodium chloride. Therefore, it may be possible that the high incidence of staphylococcal skin infections is due to the high degree of growth of Staphylococcus aureus on the skin due to fairly high concentrations of sodium chloride after hypersweating.

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