

THE PRESENCE OF DEXTRAN-FORMING BACTERIA, RESEMBLING *STREPTOCOCCUS BOVIS* AND *STREPTOCOCCUS SANGUIS*, IN HUMAN DENTAL PLAQUE

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SEVERAL investigators have reported the presence of extracellular polysaccharide producing bacteria in the oral cavity of man (CARLSSON, 1965; KRASSE, 1966; GIBBONS *et al.*, 1966; VAN HOUTE 1967) and of animals (GUGGENHEIM *et al.*, 1966; KRASSE, 1966). The polysaccharide, which is formed mainly from sucrose, proved to be a dextran-like polymer (GUGGENHEIM *et al.*, 1966; WOOD and CRITCHLEY 1966; GIBBONS and BANGHART, 1967; DE STOPPELAAR, unpublished data). The importance of dextran in relation to plaque-formation has been suggested (GIBBONS *et al.*, 1966; WOOD and CRITCHLEY, 1966).

In the present study it was possible to isolate two types of dextran-forming streptococci from human dental plaque, using colonial morphology on sucrose agar as a basis for recognizing these organisms. Plaque samples from 13 year-old subjects were homogenized in 5 ml thioglycollate broth by sonic oscillation, serially diluted in 0.067 M phosphate buffer pH 7.2 and plated on blood agar plates and in duplicate on 5% sucrose agar. One litre of the latter medium (TYC—medium; DE MOOR, personal communication) contains trypticase B.B.L. 15 g, yeast extract Difco 5 g, L-cystine 0.2 g, Na₂SO₃ 0.1 g, NaCl 1g, Na₂HPO₄·12 aq. 2 g, NaHCO₃ 2g, sodium acetate, 3 aq. 20 g, sucrose 50 g; pH 7.3. The plates were incubated anaerobically in an atmosphere of 90% H₂ and 10% CO₂ for 4–5 days.

On the sucrose agar three different colony types (called A, B and C; see Plate 1) of dextran-forming bacteria could be recognized. Type A: heaped, yellow colony, 1–3 mm in diameter, granular surface, irregular edge, agar somewhat deformed. Type B: exactly like Type A, but gray-white instead of yellow. Type C: convex, gray-white colony, 1–3 mm, glossy surface, edge less irregular, agar deformed; colony was very hard (rubbery) and could not be cut with a wire loop. Sometimes, after prolonged incubation this C-type colony became very large (up to 5 mm), round, flat, with a raised translucent centre and radiary structure. All colony types often showed a glistening drop, presumably the polysaccharide.

Eighty strains with typical morphology were studied; all were tested for their ability to form dextran and to ferment mannitol and sorbitol. After growth in 5% sucrose TYC-medium, dextran was detected by observing increased viscosity of the

medium and by microprecipitation of the diluted (1 in 100) culture fluid with pneumococcus Type II antiserum (Wellcome Laboratories, Beckenham, England).

All eighty strains were dextran-forming streptococci. Strains with colony types A and B were microaerophilic and fermented mannitol and sorbitol; those with C-type colonies never fermented mannitol and sorbitol. Twelve randomly selected strains were studied in greater detail (Table 1). Strains with A and B type colonies (dextran

TABLE 1. PROPERTIES OF DEXTRAN-FORMING STREPTOCOCCI FROM HUMAN DENTAL PLAQUE

Strains	UTB 1	UTB 2	UTB 3	UTB 5	UTS 1	UTS 2	UTS 3	HS 6
		UTB 4	UTB 6			UTS 5	UTS 4	GS 5
							UTS 6	
Mannitol	+	+	+	+	--	--	--	+
Sorbitol	+	+	+	+	--	--	--	+
NH ₃ from arginine	--	--	--	--	+	+	--	--
Raffinose	--	+	+	--	--	--	+	+
Inuline	+	+	+	--	--	+	--	+
Bile 40%	+	+	+	+	+	+	--	+
Action in blood	γ	γ	γ	γ	γ	γ	α	γ
Morphologic type of colony on sucrose agar	A	A	B	A	C	C	C	B

All strains fermented glucose, sucrose, maltose, lactose, trehalose; none fermented arabinose or glycerol; none of the strains hydrolysed sodium hippurate, tolerated 0.1% methylene blue, grew at 45°C or survived 60°C for 30 min.

positive, mannitol+, sorbitol+, arginine not split) closely resembled *Streptococcus bovis* (SHERMAN, 1937; NIVEN *et al.*, 1948; COWAN and STEEL, 1965). However, none of these strains reacted with any of several group D antisera. Strains with C type colonies have the characteristics of *Streptococcus sanguis* (dextran positive, mannitol neg., sorbitol neg. and arginine variable). Some strains reacted with *Strep. sanguis* Type I or Type II antiserum (prepared against NCTC-London no 7863 and 7864 resp.).

Strains without the typical colonial morphology were also regularly studied; they never formed dextran.

Twenty-four plaque samples were analyzed quantitatively. Total viable counts were obtained from blood agar plates and the three colony types (A, B and C) were counted separately on the sucrose plates. In three of the twenty-four plaques, no dextran-forming bacteria were found. *Strep. bovis* was found in eleven and *Strep. sanguis* in seventeen samples; seven plaques contained both organisms. In the twenty-one positive plaque samples dextran-forming streptococci (bovis+ sanguis) ranged from 1 to 40 per cent of the total flora (average 11.6 per cent). In another series only the yellow colony-variety of *Strep. bovis* was counted. In twenty-six out of

fifty-seven cases these yellow colonies were found forming 0·2–32 per cent of the flora (average 6·8 per cent). Other dextran formers were not looked for in this last series.

Strain HS 6, cariogenic for conventional hamsters (FITZGERALD and KEYES, 1960) and the human strain GS 5, cariogenic for gnotobiotic rats (GIBBONS *et al.*, 1966) were also found to resemble *Strep. bovis* (Table 1). KRASSE (1966) describes human and hamster streptococci which also resemble *Strep. bovis*. The regular occurrence of *Strep. sanguis* in our material confirms the findings of CARLSSON (1965). Also this investigator's oral strains of groups I:i and II (CARLSSON, 1967), and the so-called *Streptococcus mutans* described by CLARKE (1924) have all characteristics in common with our *Strep. bovis*-like isolates.

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DEXTRAN-FORMING STREPTOCOCCI IN DENTAL PLAQUE

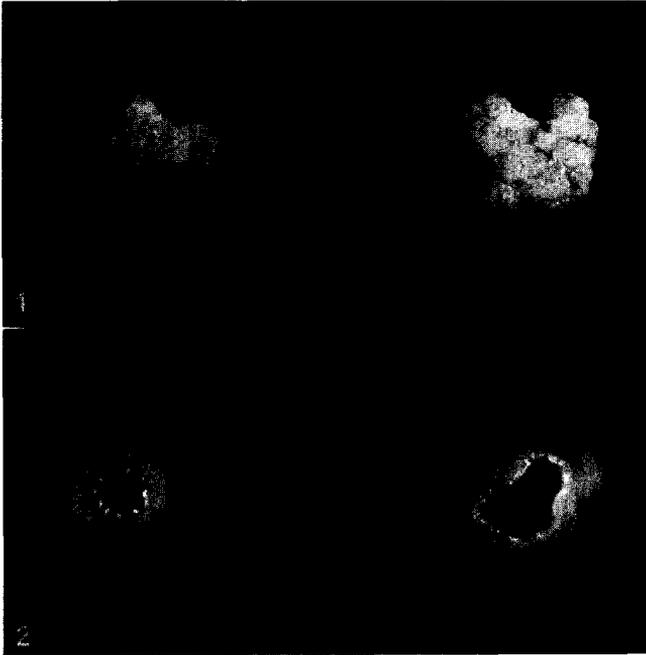


FIG. 1. Colonial morphology on sucrose agar (type A and B, see text) of strains considered to be *Strep. bovis*.

FIG. 2. Colonial morphology on sucrose agar (type C, see text) of strains with the characteristics of *Strep. sanguis*.