Differentiation of *Streptococcus sanguis* and *S. mitior* by whole-cell rhamnose content and possession of arginine dihydrolase

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Summary. Whole-cell rhamnose concentrations were measured in 48 strains of streptococci resembling *Streptococcus sanguis* and *S. mitior*. Physiological characteristics were tested by the API-20/Strep system, and it was found that "typical" *S. sanguis* (arginine positive, aesculin positive) contained significant amounts of rhamnose, while "typical" *S. mitior* (arginine negative, aesculin negative) contained very low or undetectable amounts of rhamnose. Both groups contained dextran-positive and dextran-negative strains. Organisms that were more difficult to speciate (those giving positive results in the arginine or the aesculin test, but not in both) could also be divided into a rhamnose-positive and a rhamnose-negative group; with one exception, all of the rhamnose-positive strains gave a positive result with arginine in the API-20/Strep test.

There were several discrepancies between the results of conventional tests for arginine and aesculin hydrolysis and those of the corresponding API test. The results of conventional tests for arginine hydrolysis did not correlate closely with rhamnose content, and conventional tests for aesculin hydrolysis were less sensitive than API tests. With the API-20/Strep system, *S. sanguis* can almost always be distinguished from *S. mitior* by its ability to hydrolyse arginine.

Introduction

White and Niven (1946) isolated a group of α -haemolytic streptococci from cases of endocarditis and named them *Streptococcus* s.b.e. These organisms produced dextran from sucrose (Niven *et al.*, 1946) and are now known as *S. sanguis*. There is general agreement on the identification of typical *S. sanguis* strains, which are usually α -haemolytic, hydrolyse arginine and aesculin, ferment lactose, sucrose, trehalose and inulin, produce hydrogen peroxide, form dextran from sucrose, and grow in the presence of 40% bile. However, many authors have broadened the species definition to include in *S. sanguis* strains that are dextran negative (Colman and Williams, 1972; Facklam, 1977), arginine negative (Facklam, 1977), or aesculin negative (Colman and Williams, 1972) (table I).

Washburn *et al.* (1946) described two type anti-

gens of *S. sanguis*: I (represented by strain ATCC 10556/NCTC 7863) and II (represented by strain ATCC 10557/NCTC 7864). A few strains carried both antigens, and were designated I/II (represented by strain ATCC 10558/NCTC 7865). Members of serotypes I and I/II were found to belong to Lancefield-group H but *S. sanguis* type II did not (Hare, 1935; Porterfield, 1950). Porterfield found that type-II strains were physiologically distinct and this has been confirmed more recently by Hamada *et al.* (1980).

Streptococci differ in the amounts of glucose, galactose, rhamnose and other sugars in their cell-walls (Cummins and Harris, 1956; Roberts and Stewart, 1961; Slade and Slamp, 1962; Colman and Williams, 1965). Colman and Williams (1965) noted that amongst α -haemolytic streptococci resembling *S. sanguis*, strains of Lancefield-group H contained rhamnose but not ribitol, whereas those not of this group contained little or no rhamnose and large amounts of ribitol. This was confirmed by Rosan (1976), and Colman and Williams (1972) later called these rhamnose-deficient strains *S. mitior*. Rosan and Applebaum (1979) believe that the group-H antigen of *S. sanguis* is a glycerol teichoic acid, and

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Table I. Physiological reactions of organisms resembling *Streptococcus sanguis* reported in earlier publications

Test	Percentage of strains giving a positive reaction in the stated test in						
	1* (42)†	2 (25)	3 (35)	4 (29)	5 (81)	6 (202)	7 (97)
Hydrolysis of: arginine	100	100	91	90	91	64	99
aesculin	86	72	57	100	84	77	91
Production of dextran	95	96	57	93	72	72	100

* 1 = White and Niven (1946); 2 = Carlsson (1968) (group IB); 3 = Colman and Williams (1972); 4 = Hardie and Bowden (1976) (*S. sanguis* I); 5 = Parker and Ball (1976); 6 = Facklam 1977 (*S. sanguis* I); 7 = Hamada *et al.* (1980) (*S. sanguis* serotypes I, III, IV, UT).

† Number of strains examined.

that this is replaced in *S. mitior* by a ribitol teichoic acid.

Transformation and DNA-homology studies have confirmed that *S. mitior* and *S. sanguis* are genetically distinct (Colman, 1969; Coykendall and Specht, 1975; Coykendall and Munzenmaier, 1978). The name *S. mitior* was used by Schotmüller (1903) and pre-dates the description of similar organisms as *S. mitis* by Andrewes and Horder (1906). The names *S. sanguis* II, *S. mitis* and *S. mitior* tend to be used interchangeably in the literature.

Organisms resembling *S. mitior* of Colman and Williams (1972) are typically arginine and aesculin negative, form hydrogen peroxide, ferment sucrose and lactose but rarely mannitol, sorbitol or inulin,

and are relatively bile and salt tolerant. The *S. mitior* strains of Colman and Williams (1972) were mainly dextran negative but *S. sanguis* II and *S. mitis* strains reported by other workers show considerable variations in this character (table II). Parker and Ball (1976) recognised dextran-positive and dextran-negative varieties of *S. mitior*, and Coykendall and Munzenmaier (1978) did not find dextran production to be a genetically significant characteristic. Facklam (1977) divided his aesculin-negative *S. mitior*-like strains arbitrarily into the raffinose-positive *S. sanguis* II and the raffinose-negative *S. mitior*, (table II) but Coykendall and Munzenmaier (1978) could find no genetic justification for this.

Table II. Physiological reactions of organisms resembling *Streptococcus mitior* reported in earlier publications

Test	Percentage of strains giving a positive reaction in the stated test in						
	2* (15)†	3 (46)	4 (10)	5 (120)	6a (231)	6b (177)	7 (16)
Hydrolysis of: arginine	0	7	0	0	21	16	0
aesculin	0	4	0	0	30	33	0
Production of dextran	100	11	100	30	43	10	100

* 2 = Carlsson (1968) (Group IA); 3 = Colman and Williams (1972) (*S. mitior*); 4 = Hardie and Bowden (1976) (*S. sanguis* II); 5 = Parker and Ball (1976); 6a = Facklam (1977) (*S. sanguis* II); 6b = Facklam (1977) (*S. mitis*); 7 = Hamada *et al.* (1980) (*S. sanguis* serotype II).

† Number of strains examined.

Because various authors allow different reactions within both *S. sanguis* and *S. mitior*, their differentiation in the clinical laboratory is often difficult. Parker and Ball (1976) classified as *S. sanguis* organisms that gave positive results in two or more of the three tests for arginine and aesculin hydrolysis and dextran production, whereas strains that were negative for all three tests they called *S. mitior*. These authors isolated in addition a number of dextran-negative organisms that had other reactions intermediate between those of *S. sanguis* and *S. mitior*; these they were unable to classify.

In summary, it appears that most authorities recognise typical *S. sanguis* and *S. mitior* (the latter sometimes being referred to as *S. mitis* or *S. sanguis* II), but that atypical or intermediate strains are allocated arbitrarily between these two groups. Typical *S. sanguis* is arginine and aesculin positive, belongs to Lancefield-group H and Washburn-serotype I and has moderate to large amounts of rhamnose but no ribitol in the cell wall. Typical *S. mitior* is arginine and aesculin negative, is not of group H, but is of serotype II, and contains ribitol but little or no rhamnose in the cell wall. The serological and chemical tests are difficult to perform, and are unlikely to be used routinely for identification in a clinical laboratory.

To determine which tests are significant for the identification of these organisms, we performed physiological characterisation tests on streptococci resembling *S. mitior* and *S. sanguis*, and measured their whole-cell rhamnose content. Rhamnose has previously been estimated only in cell walls (Colman and Williams, 1965; Rosan, 1976; Roberts *et al.*, 1979). We measured whole-cell rhamnose, which is a simpler procedure, and we have performed characterisation tests both by conventional methods and by means of the API-20/Strep system.

Materials and methods

Bacterial strains

The origins of the 48 streptococcal strains examined are specified in tables III–V. In these we give, in addition to our own serial number, the strain designation of the laboratory from which it had been obtained, and coded information about the donor of the strain JH = Dr J. M. Hardie, Department of Oral Microbiology, The London Hospital Medical College; LH = Department of Microbiology, The London Hospital Medical College; MS = Dr M. S. Shafi, Department of Microbiology, Central Middlesex Hospital; NC = National Collection of Type Cultures, Central Public Health Laboratory; RL = Streptococcus and Staphylococcus Reference Laboratory,

Central Public Health Laboratory; TH = Clinical isolates from Department of Microbiology, St Thomas' Hospital), and about its site of isolation (BC = blood culture; WS = wound swab; O = oral; CSF = cerebrospinal fluid; SP = sputum; ? = site unknown).

Characterisation tests

Organisms were tested by the API-20/Strep system and by conventional methods. API-20/Strep (API-System S.A., France) is a commercial test kit specially designed for the identification of streptococci and consisting of a gallery of 20 physiological and enzymatic tests: for acetoin production; hydrolysis of hippurate and aesculin; fermentation of ribose, arabinose, mannitol, sorbitol, lactose, trehalose, inulin, raffinose, starch and glycogen; and for possession of the enzymes pyrrolidonylarylamidase, α -galactosidase, β -galactosidase, β -glucuronidase, alkaline phosphatase, leucine aminopeptidase and arginine dihydrolase. The detection of the latter enzyme was taken as indicating arginine hydrolysis. The kit was used according to the manufacturer's instructions.

Conventional characterisation tests were performed according to the methods of Cowan (1974) and Waitkins *et al.* (1980). These included tests for: colonial morphology and haemolysis on horse-blood agar; the production of catalase and hydrogen peroxide; the hydrolysis of arginine, aesculin, starch and urea; and the production of dextran and laevan from sucrose (tested by the ethanol-precipitation method). When conventional and API results differed, the tests were repeated.

Estimation of rhamnose in whole cells

Todd-Hewitt broth (150 ml) was inoculated from a purity plate and incubated at 37°C for 24 h, or until heavy growth was visible. The broth was then centrifuged at 1500 *g* for 15 min and the supernate discarded. The deposit was placed in a weighed test tube and dried at 80°C in a BT3 heater unit (Grants Instruments, Basingstoke, Hants). The tube was reweighed and the dry weight of the deposit calculated.

A modification of the hydrolysis method described by Cummins and Harris (1956) was used: 2 ml of 2N H₂SO₄ was added to the dried cells and heated at 100°C for 2 h in a sealed tube. The extract was neutralised with 1 N NaOH and then centrifuged at 1500 *g* for 10 min. The supernate was then pipetted off and its volume was recorded; it was stored at 4°C until required.

The hydrolysed sample was desalted with Duolite MB5113 mixed resin (BDH Chemicals Ltd) which had been converted to the acetate form (Menzies *et al.*, 1978); this left almost all the sugar in the supernate. Thin-layer chromatography was performed according to the method of Menzies *et al.* (1978) on Merck 60 chromatography plates: 2.5 μ l of the hydrolysed sample was applied and the chromatogram developed with an ethyl acetate:pyri-

dine: glacial acetic acid: water (60:30:10:10) solvent system. A *p*-aminobenzoic acid reagent (Menzies *et al.*, 1978) was used to locate rhamnose on the chromatogram. Sorbose was selected as the internal marker sugar as it was not detected in any of the samples, nor found to interfere with any of the chromatographic bands.

Each series of zones produced from a sample was cut into strips and scanned in the axis of chromatographic development by reflective densitometry against an opaque matt white Formica backing. A Chromoscan 200 double-beam recording and integrating densitometer with a quartz-iodine light source, blue filter and 5×0.5 mm light slit, and sample holder drive in 1:1 gear ratio (Joyce-Loebl and Co. Ltd, Team Valley, Gateshead, Durham) was found to be suitable for this purpose. Peak heights were measured from the scan profile and were then corrected by applying an "internal marker" factor which compensated for variation in application volume and other variables.

The amount of rhamnose in each sample was measured in the Chromoscan and recorded as height (mm) of the relevant peak. Seven chromatograms were run, each having four rhamnose standards. The internal marker correction factor was applied, calibration curves constructed, and the rhamnose concentration in $2.5 \mu\text{l}$ of each sample calculated.

Results

Cultural and biochemical tests

All the organisms were gram-positive cocci in chains that were catalase-negative, produced hydrogen peroxide and possessed leucine aminopeptidase. All but one were α -haemolytic on blood agar, the exception being the non-haemolytic dextran-negative *S. sanguis* strain 166. None hydrolysed urea or hippurate. Only one (the dextran-positive *S. sanguis* strain 91) hydrolysed starch in the conventional test, though a number of others showed starch hydrolysis in the API test. None produced laevan from sucrose. All strains failed to ferment mannitol, arabinose and glycogen; only one fermented sorbitol (the "intermediate" strain 110) and all but one (no. 167, a dextran-negative *S. sanguis*) fermented lactose. No strain possessed pyrrolidonylarylamidase or β -glucuronidase, and only two possessed β -galactosidase (strain 12, a dextran-negative *S. mitior*, and no. 94, an intermediate strain).

Table III. Characters of strains identified as *Streptococcus sanguis**

Serial no.	Donor's code	Origin†	API-20/Strep tests‡										Conventional tests			Percentage of rhamnose in whole cell
			Arg	Aes	Raf	In	Tre	Rib	St	α -G	Pho	Arg	Aes	Dex		
7	R79/334	RL:WS	+	+	—	—	+	—	—	—	+	+	+	+	3.25	
16	L/Sang	JH:O	+	+	—	+	+	—	—	—	+	—	+	+	3.40	
18	BC:1770	TH:BC	+	+	—	+	+	—	—	—	+	+	+	+	2.05	
24	H730	JH:O	+	+	—	—	+	—	—	—	+	+	+	+	1.76	
37	BC:1746	TH:BC	+	+	—	+	+	—	—	—	+	+	+	+	1.75	
44	NCTC7865	NC:BC	+	+	—	—	+	—	—	—	+	—	+	+	1.74	
46	Carlsson 903	JH:O	+	+	—	—	+	—	—	—	+	—	+	+	3.61	
56	M517	JH:O	+	+	+	+	+	—	+	+	—	+	+	+	1.65	
66	R79/3247	RL:BC	+	+	—	+	+	—	—	—	+	+	+	+	1.99	
91	BC:1821	TH:BC	+	+	—	+	+	—	+	—	—	+	+	+	2.96	
55	BC:1736	TH:BC	+	+	—	+	+	—	—	—	+	—	+	—	3.05	
96	L24/80BC	LH:BC	+	+	—	—	+	—	—	—	—	+	—	—	2.22	
166	Kendall	TH:BC	+	+	—	—	—	—	+	—	—	+	+	—	1.41	
167	M371	JH:O	+	+	—	—	+	—	—	+	—	+	+	—	1.26	

* Gave a positive reaction in API-20/Strep tests for fermentation of arginine and aesculin.

† Donor: site of isolation (see *Materials and methods*).

‡ Hydrolysis of: Arg = arginine, Aes = aesculin; fermentation of Raf = raffinose, In = inulin, Tre = trehalose, Rib = ribose, St = starch; formation of: α -G = α -galactosidase, Pho = alkaline phosphatase, Dex = dextran; + = positive reaction, — = negative reaction.

Table IV. Characters of strains identified as *Streptococcus mitior**

Serial no.	Donor's code	Origin†	API-20/Strep tests‡										Conventional tests‡				Percentage of rhamnose in whole cell
			Arg	Aes	Raf	In	Tre	Rib	St	α-G	Pho	Arg	Aes	Dex			
34	NCTC7864	NC:BC	-	-	+	-	-	-	+	+	+	-	-	+	+	ND§	
68	Svir616	RL:?	-	-	+	-	-	-	-	+	+	-	-	+	+	ND	
103	B88	JH:O	-	-	+	-	-	-	+	+	+	-	-	+	+	ND	
112	S5732	JH:?	-	-	+	-	+	-	+	+	+	-	-	+	+	ND	
115	H240	JH:O	-	-	+	-	-	-	+	+	+	-	-	+	+	ND	
123	OPA1	JH:O	-	-	+	-	-	-	+	+	+	-	-	+	+	ND	
171	1.80/187.79	TH:BC	-	-	+	-	-	-	+	+	+	-	-	+	+	ND	
172	6.78/198a.77	TH:BC	-	-	+	-	-	-	+	+	+	-	-	+	+	ND	
12	R79/3386	RL:?	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	
27	Mays 1892	TH:WS	-	-	+	-	+	-	-	+	+	-	-	-	-	ND	
31	R75/1027	RL:?	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	
41	R79/3328	RL:WS	-	-	+	-	-	-	-	-	-	-	-	-	-	ND	
51	Strep 351	TH:BC	-	-	-	-	-	+	-	-	-	-	-	-	-	ND	
87	Bury Gen	TH:BC	-	-	-	-	-	-	+	-	+	-	-	-	-	ND	
92	BC:1800	TH:BC	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	
101	LHO23/81BC	LH:BC	-	-	-	-	-	-	-	-	+	-	-	-	-	ND	
161	Welbeloved	TH:CSF	-	-	-	-	-	-	-	-	-	-	-	-	-	0.68	
182	NCTC10712	NC:SP	-	-	+	-	-	-	+	+	-	-	-	-	-	ND	

* Gave a negative reaction in API-20/Strep tests for hydrolysis of arginine and aesculin.

† } See corresponding footnotes of table III.

‡ } ND = not detectable.

Table V. Characters of "intermediate" strains*

Serial no.	Donor's code	Origin†	API-20/Strep tests‡										Conventional tests‡			Percentage of rhamnose in whole cell
			Arg	Aes	Raf	In	Tre	Rib	St	α-G	Pho	Arg	Aes	Dex		
<i>Probable S. sanguis strains</i>																
15	STH23	JH:BC	+	-	-	+	+	-	-	+	+	-	-	+	+	2.71
54	T427	JH:O	+	-	-	+	+	-	-	+	+	-	-	-	-	2.14
88	BC:1805	TH:BC	+	-	-	-	+	-	-	+	+	-	-	-	-	2.54
110	M19	JH:O	+	-	+	+	+	-	-	+	+	+	-	+	+	1.99
173	6.77/77a.77	TH:BC	+	-	-	+	+	+	-	+	-	-	-	+	+	1.51
<i>Unidentified strain</i>																
135	J	MS:BC	-	+	-	-	+	-	-	-	-	-	-	-	+	2.12
<i>Probable S. mitior strains</i>																
21	R79/2188	RL:O	-	+	+	-	+	+	-	+	+	+	-	-	-	ND§
38	Svir 975	RL:?	-	+	-	-	+	+	-	-	-	-	-	-	-	ND
58	BC:1780	TH:BC	-	+	+	-	+	-	-	+	+	+	+	-	-	0.45
61	Strep 527	TH:BC	-	+	+	-	+	+	-	-	-	-	-	+	+	ND
73	Svir 722	RL:?	-	+	+	-	+	+	+	+	+	+	+	-	-	ND
94	LH537/80BC	LH:BC	-	+	-	-	-	-	-	-	-	-	-	-	-	ND
98	LH833/80BC	LH:BC	-	+	+	-	-	+	-	-	-	-	-	-	-	ND
100	LH134/80BC	LH:BC	-	+	+	-	-	+	+	+	+	-	-	-	-	ND
104	B159	JH:O	-	+	+	-	-	-	-	+	+	+	-	-	+	ND
107	H362	JH:O	-	+	+	-	-	+	-	+	+	+	-	+	+	ND

* Gave a positive reaction in API-20/Strep test for hydrolysis of arginine or of aesculin, but not in both.
+ } See corresponding footnotes of table III.
+ }
§ ND = not detectable.

The organisms could be divided by the results of API-20/Strep into 14 strains of typical *S. sanguis* (aesculin and arginine positive), 18 strains of typical *S. mitior* (aesculin and arginine negative), and 16 "intermediate" strains (either aesculin or arginine positive, but not both) (tables III-V). In all three groups there were dextran-positive and dextran-negative strains. Although *S. sanguis* strains tended to be more reactive than *S. mitior* in the other API tests, none of these helped to classify the "intermediate" group.

However, eight strains that were arginine positive by API-20/Strep test were arginine negative by the conventional method. Four of these were otherwise typical *S. sanguis* strains, and included three reference strains that are said to be arginine positive by other workers (table III). The other four isolates were aesculin-negative "intermediate" strains that would have been classified as *S. mitior* had the arginine result been taken as negative (table V).

There were also nine strains that were aesculin positive by the API test but aesculin negative by the conventional method. One of these was the *S. sanguis* strain 96 (table III), but the remaining eight were "intermediate" strains (table V). If these eight strains had been regarded as aesculin negative, they would have been classified as typical *S. mitior*.

Rhamnose content of whole cells

The lowest concentration of rhamnose detectable by the examination of 2.5 µl samples was 75 µg/ml. Only one strain classified as typical *S. mitior* by the API tests yielded detectable amounts of rhamnose; that was the dextran-negative strain 101 which contained 143.3 µg/ml, a percentage concentration of 0.68% (table IV). In contrast, typical strains of *S. sanguis* contained 385.2–1356.5 µg/ml, corresponding to whole-cell rhamnose concentrations of 1.26–3.61% (table III).

"Intermediate" strains showed concentrations of rhamnose varying from undetectable to 2.71% (table V). With one exception, strains that yielded > 1% of rhamnose hydrolysed arginine when tested by the API method. The exception was strain 135, a blood-culture isolate that was dextran and aesculin positive, and which contained 2.12% rhamnose.

Thus all 19 strains that hydrolysed arginine when tested by the API method had high whole-cell concentrations of rhamnose (1.26–3.61%), whereas only one of 29 strains that did not hydrolyse arginine contained a concentration of rhamnose greater than 0.68%. In 26 of 29 arginine-negative strains, rhamnose was undetectable.

Discussion

The hydrolytic procedure based on that described by Cummins and Harris (1956) was found to be suitable for the production of monosaccharides from the whole streptococcal cell, and yielded results comparable with those reported for isolated cell-walls.

With the API test results, typical strains of *S. sanguis* and *S. mitior* were easily identified, and it was confirmed that all strains of *S. mitior* contained undetectable or very low levels of rhamnose, while *S. sanguis* strains contained significant amounts of this sugar. Of the 16 "intermediate" strains, six yielded significant amounts of rhamnose, and all but one of these hydrolysed arginine when tested by the API method and could be classified as *S. sanguis*. The remaining ten "intermediate" strains contained undetectable or low levels of rhamnose, and could be classified as aesculin-positive *S. mitior*. All ten strains failed to hydrolyse arginine. Both the *S. sanguis* and the *S. mitior* groups contained dextran-positive and dextran-negative strains; this character does not appear to be useful in defining the species.

There were a number of discrepancies between conventional and API results for arginine and aesculin hydrolysis. This had previously been noted for aesculin hydrolysis by Bridge and Sneath (1983). We preferred the API results for arginine hydrolysis to those obtained by the conventional method for the following reasons: (1) three *S. sanguis* reference strains were positive for arginine hydrolysis by the API test but were repeatedly negative by the conventional method; (2) there was a direct relationship between API arginine-hydrolysis and the possession of significant amounts of rhamnose by presumed *S. sanguis* strains; and (3) four aesculin-negative "intermediate" strains that would have been classified as arginine-negative *S. mitior* by conventional methods had a high rhamnose content and were better classified as API arginine-positive *S. sanguis*.

The API test for aesculin hydrolysis also gave more positive results than the conventional method. Seven "intermediate" strains that were arginine negative by both methods were aesculin positive by API but aesculin negative by conventional methods (table V). These seven strains did not contain significant amounts of rhamnose and were therefore presumed to be *S. mitior* strains. By conventional testing they would be classified as typical *S. mitior*, but by the API method they are *S. mitior*, aesculin positive.

If it is accepted that *S. sanguis* can be aesculin negative and *S. mitior* can be aesculin positive, then, with the exception of one strain, the only physiological characteristic that can reliably distinguish between these two species is the ability of *S. sanguis* to hydrolyse arginine when tested by the API method.

Table I shows that previous workers, with the exception of Facklam (1977), have found that at least 90% of *S. sanguis* strains hydrolyse arginine. It is notable that all 42 of White and Niven's (1946) original "s.b.e." strains and 96 of the 97 *S. sanguis* non-serotype-II strains of Hamada *et al.* (1980) were arginine positive. Most strains are also reported to hydrolyse aesculin, but this property is more variable (table I).

Again, with the exception of Facklam (1977), previous workers have found that strains resembling *S. mitior* usually do not hydrolyse arginine or aesculin (table II). Colman and Williams (1972)

allow more variation in both these reactions than do other authors.

With the API-20/Strep system, we found that amongst strains resembling *S. sanguis* the correlation of arginine hydrolysis with high levels of whole-cell rhamnose is almost perfect. We suggest therefore that *S. sanguis* may be distinguished from *S. mitior* in the clinical laboratory by the possession of arginine dihydrolase, and that strains of both *S. sanguis* and *S. mitior* may hydrolyse aesculin or produce dextran from sucrose or give both reactions. The API-20/Strep system is more reliable and sensitive than the conventional method for detecting arginine and aesculin hydrolysis.

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