Transport, Nutritional and Metabolic Studies of Taurine in Staphylococci

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A specific, Na⁺-dependent, energy-requiring transport system for taurine has been reported recently in the *Staphylococcus aureus* M strain. Taurine was taken up vigorously by all *S. aureus* strains tested. The system was Na⁺-dependent, and Na⁺ decreased the K_m but had no effect on the V_{max} of the transport system. Among coagulase-negative staphylococci, the *Staphylococcus epidermidis* group (a taxonomically related group of species associated with humans or other primates) and the free-living, wide-ranging species *Staphylococcus sciuri* showed vigorous taurine uptake. Somewhat lower rates were found in the *Staphylococcus saprophyticus* group. Low or barely detectable uptake rates were noted in other staphylococcal species that were primarily of animal origin. No taurine uptake was detected in a variety of other bacterial species tested. Taurine uptake, which was not Na⁺-dependent, occurred in a *Pseudomonas aeruginosa* strain grown on taurine as sole energy, carbon, nitrogen, and sulphur source, but not when it was grown in a gluconate/salts medium. In nutritional studies we were unable to demonstrate a role for taurine as a sulphur source for *S. aureus*. [1,2-¹⁴C]- and [³⁵S]taurine were taken up during overnight growth of cells, and radioactivity was distributed similarly among cellular fractions, indicating that the carbon and sulphur atoms of taurine were not cleaved and had the same fate. We were unable to demonstrate any catabolism of taurine in radiorespirometric experiments to detect evolution of ¹⁴CO₂ by cells incubated with [1,2-¹⁴C]taurine. Thus, we found no evidence for a role of taurine in the energy, carbon and sulphur metabolism of *S. aureus*.

INTRODUCTION

The occurrence of the sulphonated β -amino acid taurine was first described in staphylococci as a component of the capsular polysaccharide of the encapsulated *Staphylococcus aureus* M strain (Liau *et al.*, 1974). Later, it was shown that a variety of *Staph. aureus* strains took up radiolabelled taurine from the growth medium during overnight growth, and appeared to carry out some limited metabolism of taurine (Smiley & Wilkinson, 1983). This led to the discovery of an Na⁺-dependent, energy-requiring, highly specific uptake system for taurine in the M strain (Bieber & Wilkinson, 1984). This previous work prompted a number of questions about the role of taurine in staphylococci. How widespread is the uptake system in staphylococci and other bacteria? What is the role, if any, of the taurine that is taken up in the nutrition of staphylococci? What are the fates of the sulphur and carbon atoms of taurine? In this report we present some of our attempts to answer these questions.

METHODS

Strains used. The sources of the bacteria studied are shown in Table 2 and below. '*Micrococcus lysodeikticus*', *Bacillus megaterium* KM and *Paracoccus denitrificans* ATCC 13543 were from the culture collection of this

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Table 1. *Components of defined media for growth of staphylococci*

The media, designed to study the role of taurine in sulphur nutrition, were assembled from various combinations of the stock solutions.

Stock solution	Final concn in medium (mg l ⁻¹)	Stock solution	Final concn in medium (mg l ⁻¹)
<i>Major carbon source</i>		L-Cystine	20
Glucose	5000	L-Methionine	3
or		Taurine	950
Glutamate	4090	<i>Amino acids</i>	
<i>Salts A</i>		L-Ala	60
K ₂ HPO ₄	7000	L-Arg	50
NaH ₂ PO ₄ ·2H ₂ O	2300	L-Asp	90
(NH ₄) ₂ SO ₄	1000	L-Glu	100
MgCl ₂	50	Gly	50
<i>Salts A¹</i>		L-His	20
K ₂ HPO ₄	7000	L-Ile	30
NaH ₂ PO ₄ ·2H ₂ O	2300	L-Leu	90
NH ₄ Cl	800	L-Lys	50
MgCl ₂	50	L-Phe	40
<i>Salts B</i>		L-Pro	80
FeCl ₃	128	L-Ser	30
Citric acid·H ₂ O	210	L-Thr	30
<i>Vitamins A</i>		L-Trp	10
Thiamin	1	L-Tyr	40
Niacin	1·2	L-Val	80
<i>Vitamins B</i>			
Calcium pantothenate	0·25		
Biotin	0·005		

laboratory. Endocarditis isolates of *Streptococcus faecalis*, '*Streptococcus viridans*', *Streptococcus bovis*, *Streptococcus mutans* and *Serratia marcescens* were supplied by W. R. Wilson. *Strep. faecalis* ATCC 9790 was supplied by F. Harold, *Escherichia coli* K12 W3110 by D. P. Brunner and *Pseudomonas aeruginosa* TAU-5 by R. S. Berk. All strains were maintained on Tryptic Soy Agar slants (Difco) at 4 °C.

Transport studies. Staphylococcal strains, '*M. lysodeikticus*', *B. megaterium* and *Serr. marcescens* were grown in PYK medium (100 ml per 250 ml Erlenmeyer flask), consisting of Phytone peptone (BBL), 5 g l⁻¹; glucose, 2 g l⁻¹; Bacto Yeast extract (Difco), 5 g l⁻¹; and K₂HPO₄, 3 g l⁻¹; the pH was adjusted to 7·2–7·4 (Bieber & Wilkinson, 1984). The inoculum was 1 ml of an overnight culture and cultures were usually grown at 37 °C with shaking at 200 r.p.m. for about 4 h to an OD₅₈₀ of 0·5–1·0 (exponential phase) (Bieber & Wilkinson, 1984). Two *Staph. aureus* strains were also grown in a defined medium somewhat modified from that described by Pattee & Neveln (1975) (Table 1). Glucose or glutamate was used as the major carbon source and the medium was sterilized by filtration. Streptococcal strains were grown in PYK medium containing 2% (w/v) glucose autoclaved separately. Cultures were inoculated with a loopful of bacteria from a fresh slant and were incubated stationary for 6–8 h to an OD₅₈₀ of 0·5–1·0. *Pa. denitrificans* was grown in succinate/salts medium (Chang & Morris, 1962). *E. coli* was grown in medium containing E salts (Vogel & Bonner, 1956), with 0·5% (w/v) glucose or glycerol as carbon source (Witholt *et al.*, 1976). *Ps. aeruginosa* TAU-5 was grown either with taurine as sole energy, carbon, nitrogen and sulphur source in the medium of Shimamoto & Berk (1979), or in a gluconate/salts medium. When an organism was found not to take up taurine, or take it up poorly, it was retested after growth in medium supplemented with 10 mM-NaCl and 1 mM-aurine in an attempt to induce the uptake system. Transport of taurine by bacterial suspensions in buffered glucose in the presence and absence of 10 mM-NaCl was measured by membrane filtration at intervals as described by Bieber & Wilkinson (1984). An OD₅₈₀ versus dry weight curve prepared for the *Staph. aureus* M strain was used to estimate bacterial dry weight. An OD₅₈₀ of 1·0 was equivalent to 0·45 mg dry wt ml⁻¹.

Nutritional studies. A loopful of bacteria from a slant was used to inoculate 10 ml defined medium in 50 ml Erlenmeyer flasks which were then incubated with shaking (200 r.p.m.) at 37 °C for 18 h. Samples of 5 ml were collected aseptically by centrifugation at 10000 g for 15 min at 4 °C. The cells were washed by resuspension and centrifugation in sterile, cold distilled water. The washed cells were resuspended in 5 ml sterile water and 0·1 ml was used to inoculate 10 ml amounts of the various test media. Growth was measured by determining the OD₅₈₀ of 1:10 diluted samples in a Spectronic 20 spectrophotometer (Bausch & Lomb).

Uptake and distribution of [¹⁴C]- and [³⁵S]taurine in overnight grown cells. [1,2-¹⁴C]Taurine (specific activity 56·08 mCi mmol⁻¹; 2·08 GBq mmol⁻¹; New England Nuclear) and [³⁵S]taurine (88·1 mCi mmol⁻¹;

3.2 GBq mmol⁻¹; Amersham) were used in these studies. Cells were grown in PYK medium minus glucose (Smiley & Wilkinson, 1983) and defined medium (25 ml per 50 ml Erlenmeyer flask). Unlabelled taurine was supplied at 0.1 mM and 1 µCi radiolabelled taurine was added. Uptake and distribution of radioactivity among cellular fractions were determined as described by Smiley & Wilkinson (1983).

Respirometry. Cells were grown to mid-exponential phase in PYK minus glucose medium and were washed in distilled water. Oxygen uptake was studied in 20 ml flasks using a Gilson differential respirometer (Gilson Medical Electronics), at 37 °C (Umbreit *et al.*, 1964). Reaction mixtures consisted of cells (2 ml; 1 mg dry wt ml⁻¹) in 0.05 M-Tris/HCl pH 7.5 containing 10 mM-NaCl. Experimental additions were 1 or 10 mM-aurine or 30 mM-glucose.

Radiorespirometry. These studies were done in a similar manner to the respirometric studies. Washed cells were grown in PYK minus glucose medium and the incubations were carried out in sealed 20 ml Gilson respirometer flasks at 37 °C for 4 h. One µCi of [1,2-¹⁴C]taurine or D-[U-¹⁴C]glucose (3.9 mCi mmol⁻¹; 0.14 GBq mmol⁻¹; New England Nuclear) was included with the washed cells (2 ml) in 0.05 M-Tris/HCl pH 7.5 containing 10 mM-NaCl and unlabelled taurine (0.1 mM) or D-glucose (0.1 mM). Hyamine hydroxide (1 M-methylbenzethonium hydroxide in methanol, Sigma) (0.2 ml) was placed in the centre well to absorb any evolved ¹⁴CO₂, which was detected by liquid scintillation counting. Samples were kept in the dark before counting. This method resembles that described by Hackstadt & Williams (1981).

RESULTS

Additional characteristics of taurine transport in the Staph. aureus M strain

Previous studies characterized the taurine transport system in the M strain (Bieber & Wilkinson, 1984). Before investigating the occurrence of the taurine transport system in other bacteria, we examined some additional characteristics of the system in the M strain. Uptake was more rapid by cells harvested from the exponential phase than from the stationary phase of growth (Table 2). Initial uptake rates were similar in cells grown in PYK, PYK minus glucose and defined media (Table 2). A variety of Na⁺ salts were comparably efficient to NaCl in supplying the Na⁺ requirement of the system, including phosphate, nitrate, sulphate, citrate and acetate salts (data not shown). This indicates that the anion is unimportant in taurine uptake.

Our previous studies of the kinetic parameters of taurine uptake in the M strain revealed a K_m of 42 µM and a V_{max} of 4.6 nmol min⁻¹ (mg dry weight)⁻¹ in the presence of 10 mM-NaCl. Decreasing concentrations of NaCl increased the K_m of the system and appeared to decrease the V_{max} . Study of the kinetic parameters of taurine uptake in the presence of 10 mM-NaCl by *Staph. aureus* strains Lafferty, Endo and Oughterson revealed K_m values of 38.5, 40.0 and 41.6 µM, and V_{max} values of 7.2, 8.9 and 6.2 nmol min⁻¹ (mg dry weight)⁻¹, respectively. Decreasing the NaCl concentration increased the K_m values but had essentially no effect on the V_{max} values (data not shown).

Survey of taurine transport

The results of the survey for the taurine transport system are shown in Table 2. Unless stated otherwise, strains were grown in PYK medium to the exponential phase of growth. Results are expressed as the initial rate of uptake in the presence and absence of Na⁺ and the uptake attained after 15 min (Bieber & Wilkinson, 1984). In all staphylococcal strains there was much more taurine uptake in the presence of Na⁺ than in its absence. All 20 *Staph. aureus* strains tested took up taurine vigorously. The mean initial uptake rate in the presence of Na⁺ was 1.27 and 1.71 nmol min⁻¹ (mg dry wt)⁻¹ in non-endocarditis and endocarditis isolates, respectively. The uptake of taurine by coagulase-negative species was slower than that by *Staph. aureus*. Of six *Staph. epidermidis* endocarditis isolates tested, three had relatively high uptake rates and three relatively low rates. Vigorous uptake was noted by *Staph. simulans*, *Staph. capitis*, *Staph. haemolyticus*, *Staph. hominis*, *Staph. warneri* and *Staph. sciuri*. Somewhat lower initial rates of uptake were noted in a group comprised of *Staph. saprophyticus*, *Staph. cohnii* and *Staph. xylosus*. Low, or very low, taurine uptake activity was detected in *Staph. hyicus*, *Staph. lentus*, *Staph. auricularis*, *Staph. hyicus* subsp. *chromogenes* and *Staph. intermedius*. Thus, the possession of an Na⁺-dependent taurine uptake system was a general property of several staphylococcal strains, although some coagulase-negative species showed very low activities.

Table 2. *Taurine transport among various species of Staphylococcus*

Uptake was measured in exponential-phase cells grown in PYK medium unless stated otherwise. The initial rate of uptake and the uptake attained after 15 min are shown. The values represent the results of one experiment.

Organism (medium and growth phase)	Source and comments	Taurine uptake [nmol (mg dry wt) ⁻¹]			
		After 1 min		After 15 min	
		+ Na ⁺	- Na ⁺	+ Na ⁺	- Na ⁺
<i>S. aureus</i> M (PYK, exponential)	This lab., encapsulated	1.20	0.11	3.80	1.50
<i>S. aureus</i> M (PYK, stationary)		0.60	0.03	1.70	0.20
<i>S. aureus</i> M (PYK minus glucose, exponential)		1.21	0.16	3.66	1.54
<i>S. aureus</i> M (defined, exponential)		1.36	0.19	4.30	1.36
<i>S. aureus</i> Lafferty	L. J. Wheat	1.50	0.11	3.80	1.50
<i>S. aureus</i> Smith diffuse	This lab., encapsulated	2.27	0.21	4.40	1.40
<i>S. aureus</i> SA222	This lab., encapsulated	1.02	0.19	4.03	1.29
<i>S. aureus</i> Oxford	This lab.	1.99	0.20	3.51	1.40
<i>S. aureus</i> Wood 46	This lab.	0.80	0.06	3.30	0.49
<i>S. aureus</i> NS58D	This lab., encapsulated	1.40	0.25	3.20	1.40
<i>S. aureus</i> Cowan I	This lab.	0.70	0.02	3.10	0.62
<i>S. aureus</i> Copenhagen	D. J. Tipper	0.90	0.03	3.00	0.32
<i>S. aureus</i> M variant	This lab., unencapsulated derivative of M strain	1.22	0.07	2.60	0.96
<i>S. aureus</i> DU4916	This lab., methicillin-resistant	0.70	0.01	2.23	0.17
<i>S. aureus</i> H	This lab.	1.42	0.39	2.16	1.40
<i>S. aureus</i> ATCC 6538P	S. Silver	0.76	0.02	3.10	0.06
<i>S. aureus</i> Mastitis	This lab., bovine mastitis isolate	1.96	0.05	3.62	0.21
<i>S. aureus</i> Pelezinski	T. E. West, endocarditis isolate	2.20	0.21	3.40	1.35
<i>S. aureus</i> ENDO	R. Procter, endocarditis isolate	1.62	0.19	3.33	1.10
<i>S. aureus</i> Kurch	T. E. West, endocarditis isolate	1.53	0.16	3.23	1.00
<i>S. aureus</i> Mah	T. E. West, endocarditis isolate	1.60	0.19	3.10	1.00
<i>S. aureus</i> Pope	T. E. West, endocarditis isolate	1.60	0.12	3.10	0.79
<i>S. aureus</i> Stoiber	T. E. West, endocarditis isolate	1.70	0.20	3.00	1.20
<i>S. epidermidis</i> II-5	This lab., methicillin-resistant	0.70	0.03	3.30	0.40
<i>S. epidermidis</i> ATCC 14990	This lab.	0.41	0.01	2.51	0.21
<i>S. epidermidis</i> Burk	T. E. West, endocarditis isolate	0.27	0.00	4.39	0.05
<i>S. epidermidis</i> Grooms	T. E. West, endocarditis isolate	0.44	0.00	3.30	0.07
<i>S. epidermidis</i> Gregg	T. E. West, endocarditis isolate	0.83	0.02	2.87	0.07
<i>S. epidermidis</i> Oster	T. E. West, endocarditis isolate	1.00	0.01	3.30	0.05
<i>S. epidermidis</i> Oughterson	T. E. West, endocarditis isolate	1.13	0.01	4.14	0.12
<i>S. epidermidis</i> Rambo	T. E. West, endocarditis isolate	0.35	0.00	3.76	0.04
<i>S. xylosus</i> TPC 1	This lab.	0.30	0.04	2.00	0.84
<i>S. xylosus</i> SE 4	W. E. Kloos	0.30	0.05	2.13	0.61
<i>S. simulans</i> ML 26	This lab.	0.81	0.02	3.51	0.47
<i>S. simulans</i> ATCC 27848	W. E. Kloos	0.60	0.02	2.90	0.54
<i>S. capitis</i> LH A3	This lab.	0.50	0.03	2.13	0.23
<i>S. capitis</i> ATCC 27840	W. E. Kloos	0.90	0.22	3.20	1.53
<i>S. haemolyticus</i> KH 11	W. E. Kloos	0.70	0.06	3.74	1.40
<i>S. haemolyticus</i> DSM 20263	W. E. Kloos	0.50	0.05	2.60	1.00
<i>S. hominis</i> ATCC 27844	W. E. Kloos	0.93	0.06	3.20	1.25
<i>S. hominis</i> DGS 73	W. E. Kloos	0.30	0.01	2.02	0.21
<i>S. cohnii</i> BB 3	W. E. Kloos	0.30	0.03	2.44	0.44
<i>S. cohnii</i> DSM 20260	W. E. Kloos	0.20	0.01	1.70	0.16
<i>S. saprophyticus</i> ATCC 15305	This lab.	0.21	0.01	1.64	0.28
<i>S. saprophyticus</i> GH 196	W. E. Kloos	0.51	0.02	2.88	0.05
<i>S. sciuri</i> 29059	W. E. Kloos	0.84	0.22	3.20	1.50
<i>S. sciuri</i> GV 234	W. E. Kloos	0.31	0.00	1.50	0.01
<i>S. lentus</i> FB 2	This lab.	0.01	0.00	0.20	0.00
<i>S. lentus</i> K6	W. E. Kloos	0.60	0.10	3.44	1.20
<i>S. warneri</i> KL 105	W. E. Kloos	1.02	0.05	4.60	0.71
<i>S. intermedius</i> CF 238	W. E. Kloos	0.12	0.00	0.60	0.00
<i>S. auricularis</i> ATCC 33757	W. E. Kloos	0.05	0.00	0.40	0.00
<i>S. hyicus</i> NCTC 10350	W. E. Kloos	0.32	0.00	1.40	0.03
<i>S. hyicus</i> subsp. <i>chromogenes</i> LSU 89LR	W. E. Kloos	0.08	0.00	0.43	0.00

Table 3. Influence of taurine on the growth of *Staph. aureus* with various sulphur sources

Each medium contained either glucose (Glc) or glutamate (Glu) as major carbon source plus salts B, vitamins A, vitamins B and amino acids (see Table 1). In addition the various media contained: 1, salts A, cystine, methionine; 2, salts A, cystine, methionine, taurine; 3, salts A¹, cystine, methionine; 4, salts A¹, cystine, methionine, taurine; 5, salts A; 6, salts A, taurine; 7, salts A¹; 8, salts A¹, taurine.

Strain	Medium and growth (OD ₅₈₀)															
	1 Glc	1 Glu	2 Glc	2 Glu	3 Glc	3 Glu	4 Glc	4 Glu	5 Glc	5 Glu	6 Glc	6 Glu	7 Glc	7 Glu	8 Glc	8 Glu
M	3.7	2.9	2.9	2.2	3.6	2.2	2.6	2.2	0.10	0	0.07	0	0.05	0	0.05	0
ENDO	4.8	3.4	4.1	3.6	4.6	3.6	4.2	3.6	0	0	0	0	0	0	0	0

We were unable to detect taurine uptake in *Strep. faecalis*, '*Strep. viridans*', *Strep. bovis*, *Strep. mutans*, *Serr. marcescens*, *B. megaterium*, *Pa. denitrificans*, '*M. lysodeikticus*' or *E. coli*, whether or not the strains had been grown in the presence of taurine, which indicated that the system was not inducible. Several of the strains tested were endocarditis isolates.

Ps. aeruginosa TAU-5 took up taurine when the cells were grown in a medium where taurine served as C, N, S and energy source. However, the system was not dependent on Na⁺, and the absence of glucose from the assay mixture did not diminish uptake, in contrast to the finding with *Staph. aureus* (Bieber & Wilkinson, 1984). Shaking increased uptake by 25%. Negligible uptake occurred at 0 °C, indicating that we were not observing simple binding of taurine to cells. However, no uptake occurred when the strain was grown in gluconate/salts medium, which indicates that the taurine uptake system was inducible.

Nutritional studies of taurine in *Staph. aureus*

The defined medium used was modified from that described by Pattee & Neveln (1975). We attempted to observe any stimulatory effect taurine might have on growth, and focussed on whether taurine could serve as a sulphur source for staphylococci (Smiley & Wilkinson, 1983). In most experiments glucose served as the major carbon source and sulphur was supplied in the form of (NH₄)₂SO₄, cystine and methionine, and in minor amounts in the form of the vitamins thiamin and biotin. *Staph. aureus* strains M and ENDO were used in these experiments.

Both strains grew well in complete defined medium. Omission of methionine decreased the OD₅₈₀ achieved in overnight growth and omission of cystine resulted in even less growth. Omission of both amino acids simultaneously led to poor growth and supplementation with taurine did not lead to increased growth. This indicates that *Staph. aureus* is auxotrophic for cystine in particular. Similar results were obtained when glutamate served as the major carbon source.

With regard to major sulphur sources the following combinations were investigated (Table 3): 1, (NH₄)₂SO₄, cystine, methionine; 2, (NH₄)₂SO₄, cystine, methionine, taurine; 3, cystine, methionine; 4, cystine, methionine, taurine; 5, (NH₄)₂SO₄; 6, (NH₄)₂SO₄, taurine; 7, none; 8, taurine. Taurine did not stimulate growth when (NH₄)₂SO₄ + cystine + methionine and cystine + methionine served as major sulphur sources. In fact growth was somewhat less when taurine was present. When (NH₄)₂SO₄ or taurine alone was the sulphur source, negligible or no growth occurred, even on prolonged (42 h) incubation. Similar results were obtained with glutamate as major carbon source (Table 3). Thus we were unable to provide any evidence that taurine was able to serve as a source of sulphur for staphylococci.

Respirometric and radiorespirometric studies with taurine

The data of Smiley & Wilkinson (1983) had shown a discrepancy between uptake of taurine measured by disappearance from growth medium, and as cell-associated radioactivity. It seemed possible that catabolism of taurine to CO₂ could account for the missing radioactivity. Taurine did not stimulate the oxygen consumption of *Staph. aureus* strain NS58D above the endogenous rate. In previous studies this strain had shown the largest discrepancy in taurine uptake measured by disappearance versus cell-associated radioactivity (Smiley & Wilkinson,

Table 4. *Radiorespirometric studies of taurine and glucose metabolism by three Staph. aureus strains*

The values are the results of a representative experiment.

Conditions	Radioactivity (c.p.m.) in hyamine hydroxide		
	M	ENDO	NS58D
Cells alone	442	273	715
No cells, 1 μ Ci [1,2- 14 C]taurine + 1 μ Ci [U- 14 C]glucose	2667	2941	3344
Cells + 1 μ Ci [1,2- 14 C]taurine	1090	1406	2622
Cells + 1 μ Ci [U- 14 C]glucose	536980	583878	715157

Table 5. *Uptake and distribution of radioactivity from [1,2- 14 C]- and [35 S]taurine among cellular fractions by three Staph. aureus strains grown overnight in complex and defined media*

The values are the means of two separate experiments.

Fraction	PYK minus glucose medium						Defined medium					
	M		ENDO		NS58D		M		ENDO		NS58D	
	14 C	35 S	14 C	35 S	14 C	35 S	14 C	35 S	14 C	35 S	14 C	35 S
	Percentage uptake*											
Spent medium	25	30	34	41	54	76	41	21	35	24	82	51
Harvested cells	23	36	33	40	51	66	35	5	32	9	74	22
	Percentage distribution in harvested cells†											
Pool metabolites	94	94	97	97	88	81	85	83	95	95	76	35
Lipid	4.7	3	3	2	2	4	14	10	4	4	3	26
Nucleic acid-teichoic acid-capsular polysaccharide	<1	3	<1	<1	8	12	<1	5	<1	<1	14	36
Protein	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Peptidoglycan	<1	<1	<1	<1	2	2	<1	2	<1	<1	7	2

* Percentage of total radioactivity supplied taken up by cells calculated by difference between uninoculated and spent medium or by direct counting of cells (Smiley & Wilkinson, 1983).

† The starting radioactivity in harvested cells (100%) was 10^5 – 10^6 c.p.m.

1983). Substantial oxygen uptake was observed when glucose was supplied as a substrate (data not shown). Because of these negative results we tested for the evolution of $^{14}\text{CO}_2$ from [1,2- ^{14}C]taurine as a more sensitive assay for the catabolism of taurine.

No release of $^{14}\text{CO}_2$ was detected upon incubation of washed cell suspensions of *Staph. aureus* strains M, ENDO and NS58D with [1,2- ^{14}C]taurine, whereas substantial release was noted from D-[U- ^{14}C]glucose, which was used as a control (Table 4). We conclude that no substantial catabolism of the taurine carbon skeleton occurred under the conditions studied.

Uptake and distribution of [1,2- ^{14}C]- and [^{35}S]taurine in overnight grown cells

In order to obtain information on the fate of the sulphur atom of taurine taken up by *Staph. aureus*, the uptake and distribution of radioactivity from [^{35}S]taurine among fractions in cells grown in PYK minus glucose and defined media were compared with the uptake and distribution of [1,2- ^{14}C]taurine (Table 5). Substantial amounts of both [1,2- ^{14}C]- and [^{35}S]taurine were taken up by the three strains studied from both PYK minus glucose and defined media. Similar amounts of [1,2- ^{14}C]- and [^{35}S]taurine were taken up by the strains grown in PYK minus glucose medium (Table 5). Similar percentage uptake values were obtained by measuring uptake by counting either spent medium or harvested cells. The strains appeared to take up somewhat less [^{35}S]taurine than [1,2- ^{14}C]taurine when grown in defined

medium. Measuring uptake of [^{35}S]taurine by counting harvested cells indicated substantially less uptake than by counting spent medium in the case of defined medium. In PYK minus glucose medium radioactivity from [1,2- ^{14}C]- and [^{35}S]taurine was similarly distributed among the cellular fractions. In this medium most of the radioactivity remained in the pool metabolites fraction in all strains with either isotope. Strain NS58D incorporated radioactivity into other cellular fractions, consistent with previous observations (Smiley & Wilkinson, 1983). Basically similar results were seen when defined medium was used, i.e. the distribution of radioactivity from [1,2- ^{14}C]- and [^{35}S]taurine was similar, indicating that the carbon and sulphur atoms of taurine had similar fates. In strain NS58D grown in defined medium, more radioactivity from [1,2- ^{14}C]taurine appeared to be present in the pool metabolites fraction than from [^{35}S]taurine. Overall, because of the similarity of the distribution of radioactivity from [1,2- ^{14}C]- and [^{35}S]taurine, the results indicated that cleavage of the carbon and sulphur atoms of taurine had not occurred.

DISCUSSION

A taurine transport system of high activity was present in *Staphylococcus aureus* [mean 1.40, range 0.80–2.27 nmol min $^{-1}$ (mg dry wt) $^{-1}$]. Relatively high activities were present in *S. epidermidis*, *S. hominis*, *S. warneri*, *S. simulans*, *S. capitis*, *S. haemolyticus* [mean of all strains 0.67, range 0.30–1.02 nmol min $^{-1}$ (mg dry wt) $^{-1}$] and *S. sciuri* [mean 0.58, range 0.31–0.84 nmol min $^{-1}$ (mg dry wt) $^{-1}$]. *S. epidermidis* and the other coagulase-negative species above, except *S. simulans* and *S. sciuri*, compose the *S. epidermidis* species group as defined by phenotypic characteristics and DNA–DNA hybridization studies (Kloos, 1980). Staphylococci of the *S. saprophyticus* species group, composed of *S. saprophyticus*, *S. xylosus* and *S. cohnii*, all showed lower initial uptake rates than the aforementioned species [mean 0.30, range 0.20–0.51 nmol min $^{-1}$ (mg dry wt) $^{-1}$]. The staphylococcal species showing poor uptake were of animal origin, with the exception of *S. auricularis* [mean 0.08, range 0.01–0.60 nmol min $^{-1}$ (mg dry wt) $^{-1}$]. *S. sciuri*, like *S. xylosus*, is a free-living, wide-ranging species (Kloos, 1980). In the absence of knowledge of the function of the taurine uptake system it is difficult to appreciate the significance of the different levels of activities in the different groups, which appear to be reflecting the different evolutionary histories of the species. Taurine is present in high concentrations in the tissues of man and other animals, in particular heart tissue (Jacobsen & Smith, 1968).

A taurine transport system was not detected in a variety of other bacterial species, several of which were endocarditis isolates. Again without knowledge of the function of the system it is difficult to explain its apparent staphylococcal specificity. The taurine uptake system in *Ps. aeruginosa* had different properties from the staphylococcal one, notably in its Na $^{+}$ -independence.

In view of the finding that a taurine transport system is a characteristic of a variety of staphylococcal species it would appear likely that the taurine is taken up for some purpose. However, we have not yet discovered the role of the taurine transport system in staphylococcal physiology. One possibility is that it is not a taurine transport system at all, but exists for the uptake of some other molecule. However, we consider this to be unlikely because the system is highly specific for taurine. None of the common amino acids tested competed with taurine for uptake, and only two compounds closely related to taurine showed any inhibition of taurine uptake (Bieber & Wilkinson, 1984).

We had previously speculated (Smiley & Wilkinson, 1983) that if taurine were cleaved to sulphite, as it is in *Ps. aeruginosa* (Shimamoto & Berk, 1979), then taurine might be able to feed into a postulated staphylococcal sulphate assimilation pathway by analogy to that in other bacteria. However, we were unable to demonstrate a contribution of taurine to the sulphur requirements of *S. aureus* through our nutritional studies. It is possible, but we consider it unlikely, that the nutritional requirement for cystine is obscuring a contribution of taurine to *S. aureus* sulphur nutrition. Also, studies of the uptake and distribution of [^{14}C]- and [^{35}S]taurine, which gave very similar results, showed no evidence for cleavage of the carbon–sulphur bond.

We have been unable to demonstrate a role for taurine in the carbon and energy metabolism of *S. aureus*. No evolution of $^{14}\text{CO}_2$ by cells incubated with [^{14}C]taurine could be detected. The discrepancy between taurine uptake measured by counting spent medium and harvested cells noted previously (Smiley & Wilkinson, 1983) was probably due to different degrees of quenching in the samples. In support of this, a similar discrepancy was noted in measuring the uptake of [^{35}S]taurine in cells grown in defined medium.

It is possible that taurine could provide a source of nitrogen for staphylococci by participating in transamination reactions, similar to those known to occur in other micro-organisms (Shimamoto & Berk, 1979). Such a role would be difficult to demonstrate given the multiple amino acid auxotrophies of *S. aureus*.

Rather, it appears that taurine is relatively metabolically inert once it has been taken up by staphylococci, although chromatographic evidence indicates that it may undergo a limited transformation (Bieber & Wilkinson, 1984; Smiley & Wilkinson, 1983). Metabolic inertness is characteristic of taurine in a wide range of organisms (Jacobsen & Smith, 1968). A possible role for taurine, or a closely related metabolite, would be as an osmoregulatory molecule. Taurine is known to have an osmoregulatory function in a variety of organisms (Huxtable, 1980; Jacobsen & Smith, 1968). Staphylococci are halotolerant, and proline is a known osmoregulator in these organisms, as it is in a variety of other bacteria (Koujima *et al.*, 1978; Measures, 1975). Taurine is present in high concentrations in animal tissues and it would appear logical that staphylococci might have evolved the ability to utilize taurine as an osmoregulator. In preliminary experiments increases in the medium NaCl concentration increased the amount of taurine taken up during overnight growth by the *S. aureus* M strain (T. J. Giehl & B. J. Wilkinson, unpublished observations). Future experiments will be devoted to examining this possible role for taurine in staphylococci.

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