Effect of food deprivation on serum gastrin concentration and gastric histidine decarboxylase activity[a]

	Serum gastrin concentration (pg eqv SHG/ml)		Histidine decarboxylase activity (pmoles CO_2/mg/h)	
Unoperated rats				
Fed ad libitum	144 ± 14	(15)[d]	56.2 ± 9.8	(15)[d]
Fasted 6 h	123 ± 16	(8)[d]	38.9 ± 6.7	(14)[d]
8 h	98 ± 5	(5)[d]	29.4 ± 4.1	(5)[d]
10 h	91 ± 16	(5)[c]	28.6 ± 5.1	(5)[c]
12 h	74 ± 10	(14)[d]	18.2 ± 3.0	(9)[c]
18 h	62 ± 8	(8)[c]	13.9 ± 3.0	(9)[b]
24 h	43 ± 8	(16)	10.8 ± 1.4	(18)[c]
36 h	34 ± 6	(8)	6.8 ± 1.1	(10)
48 h	27 ± 5	(12)	5.8 ± 0.5	(4)
Antrectomized rats				
Fed ad libitum	42 ± 6	(8)	6.6 ± 0.9	(8)
Fasted 24 h	—		7.3 ± 0.6	(4)
48 h	35 ± 7	(4)	6.6 ± 1.1	(11)

[a] Mean ± SEM (n). Significance of difference compared to the 48 hour values is given by [b] $0.05 > p > 0.01$; [c] $0.01 > p > 0.001$ and [d] $0.001 > p$; Student's t-test.

order to prevent coprophagia. They had free access to water. Upon sacrifice, the abdomen was opened under diethyl ether anesthesia and blood was drawn from the aorta. Serum was lyophilized and kept at −25 °C until analysis. Gastrin was determined by radioimmunoassay, using antibodies raised in rabbits against synthetic human gastrin I. The technique, its accuracy and reliability on rat serum has been described elsewhere[3]. The oxyntic mucosa was scraped off the stomach wall and homogenized in 0.1 M phosphate buffer, pH 7.0, to a final concentration of 100 mg (wet weight) per ml. After incubation of 0.5 ml of the homogenate with 1-^{14}C-L-histidine (4×10^{-4} M; 1.3 mCi/mM) in the presence of pyridoxal-5-phosphate (10^{-5} M) and reduced glutathione (5×10^{-4} M) at 37 °C under nitrogen for 1 h (final reaction volume 0.53 ml), the $^{14}CO_2$ produced during the reaction was collected and measured by liquid scintillation counting. The results were corrected for non-enzymatic decarboxylation by incubating boiled samples. Enzyme activities are expressed as pmoles $^{14}CO_2$ produced per mg mucosa per h[3].

The results are summarized in the Table. In the unoperated, freely fed rats, the serum gastrin concentration was high. It decreased after withdrawal of food, to reach a minimum at between 24 and 48 h of fasting. In the antrectomized, freely fed rats, the serum gastrin concentration was low and was not further lowered by fasting for 48 h. Following food deprivation, the gastric histidine decarboxylase activity in normal rats was markedly but slowly reduced. After 24 h of fasting, the basal enzyme activity was not yet established. After 36–48 h, the enzyme activity appeared to be maximally reduced. In freely fed antrectomized animals, the histidine decarboxylase activity was low and it was not further reduced by fasting. It is evident from the results that a fasting period of 18 h, as employed by ROSENGREN and SVENSSON[13], SVENSSON[14] and LUNDELL[15], is too short to establish a basal level of histidine decarboxylase activity. Any difference in enzyme activity between unoperated and radically antrectomized rats fasted for less than 36–48 h is therefore probably due to incomplete fasting.

The results of the present investigation emphasize the importance of adequate fasting in attempts to determine basal gastric histidine decarboxylase activity[16].

Summary. The serum gastrin concentration and the gastric histidine decarboxylase activity are high in freely fed, unoperated rats but low in antrectomized rats. Following food deprivation the serum gastrin level and the enzyme activity are reduced simultaneously in the unoperated rats. After fasting for 36–48 h – but not before – the enzyme activity drops to the same low levels as in antrectomized rats.

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Invertase in Cell-Free Culture Fluids of *Streptococcus mutans* Strain SL-1

Intracellular invertases have been purified and characterized from several strains of the cariogenic organism *Streptococcus mutans*[1–4]. FUKUI[5] has recently reported that invertase found extracellularly for *S. mutans* strain HS-6 has properties that are distinct from those of the intracellular invertases. The current investigation was directed at the purification and characterization of invertase from cell-free culture fluids of *S. mutans* strain SL-1.

Materials and methods. Cultures were grown anaerobically 10–14 h in a chemically-defined medium (Table). All subsequent procedures were performed at 3–7 °C unless otherwise stated. Extracellular protein was precipitated from the cell-free culture solutions, drawn at intervals during organism growth, by addition of ammonium sulfate to 80% saturation, followed by overnight settling. After centrifugation at 13,000 × g, the protein fraction was taken up in 0.020 M potassium phosphate at pH 6.7 (150 ml per l of original culture) and dialyzed against this buffer for 40 h, against distilled water for 4–5 h and lyophilized.

Extracellular protein preparations were dissolved in 0.025 M potassium phosphate buffer (pH 6.7) containing 0.04% NaN_3, and were chromatographed on a Sephadex G-100 column (2.5 × 40 cm) which had been equilibrated and washed in the same buffer. Eluted fractions with

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Composition of chemically-defined medium for *Streptococcus mutans*

Component	Quantity/l	Component	Quantity/l
Tissue Culture Medium 199 in Earle's Base, dehydrated	As prescribed by manufacturer (BBL)	Vitamins	(mg)
		p-aminobenzoic acid	0.20
		Riboflavin	0.60
Amino Acids	(g)	Pyridoxine · HCl	0.60
L-Arginine	0.175	Nicotinic acid · HCl	1.00
L-Cystine	0.050		
L-Histidine	0.180	Nitrogenous bases	(g)
L-Isoleucine	0.262	Guanine · HCl	0.01
L-Leucine	0.131	Adenine sulfate	0.01
L-Lysine	0.150	Uracil	0.01
DL-Methionine	0.185		
L-Phenylalanine	0.083	Salts and glucose	(g)
L-Threonine	0.120	$MgSO_4$	0.11
DL-Tryptophan	0.130	$FeSO_4 \cdot 7H_2O$	0.01
L-Tyrosine	0.181		
DL-Valine	0.117	$MnSO_4 \cdot H_2O$	0.009
L-Alanine	0.090	$CH_3COONa \cdot 3H_2O$	2.50
DL-Asparagine	0.240	K_2HPO_4	–[b]
L-Aspartic acid	0.133	KH_2PO_4	–
Glycine	0.175		
L-Glutamic acid	2.000	Glucose	20.0
L-Proline	0.065	$(NH4)_2SO_4$	0.1
L-Serine	0.105	$NaHCO_3$	15.0
L-Hydroxyproline	0.100		
L-Cysteine · HCl	0.050		
Vitamin concentrates[a], × 100	(ml)		
Minimum essential medium	20		
Basal medium	20		

[a] Microbiological Associates, Bethesda, Md. [b] Potassium phosphate was added in quantities sufficient to bring medium pH to 4.9–5.0 and provide final phosphate concentration of 0.08 *M*. The final pH was adjusted to and maintained at 6.3–6.4., and the organisms were cultured in a fermentor (Magnaferm model 107, New Brunswick, Pa.).

enzymic activity were pooled, dialyzed 4–5 h against water, and lyophilized.

Intracellular invertase preparations were obtained from the supernatants of mechanically disrupted cells[3] by dialysis for 4–5 h against water and lyophilization.

Electrophoresis was conducted for 6 h at 7–10 °C, following in general the horizontal flat-bed technique of MEYER and LAMBERTS[6]. Except where noted, samples of lyophilized protein or enzyme preparations were dissolved in 0.020 *M* sodium phosphate (pH 8.5) in a 1% protein concentration, clarified by centrifugation, and electrophoresed with the phosphate solution in 5% gel slabs of Cyanogum-41 (Fisher Scientific Co., Fair Lawn, N.J.).

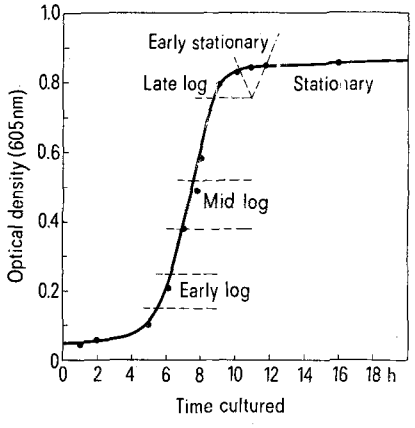

Fig. 1. Typical growth curve for strain SL-1 showing locations of cell harvesting.

After electrophoresis the gels were incubated at 37 °C in 0.05 *M* phosphate buffer (pH 6.5) containing 10% sucrose. Sites of polysaccharide synthesis were observed in the gels[7] after 16 h. In another procedure[8], gels were incubated 40 min and then soaked in 2, 3, 5-triphenyltetrazolium chloride. Red bands were produced in the gel at sites of enzymic release of reducing sugar from sucrose. A nonpolysaccharide-synthesizing, tetrazolium-positive gel site indicated the presence of invertase-like activity.

Molecular weights were estimated with Sephadex G-100 in accordance with the procedures of ANDREWS[9], as described by KURAMITSU[2], except that the eluting buffer was 0.025 *M*, 6.8 pH potassium phosphate and protein standards were albumin (bovine), myoglobin (horse heart) and cytochrome c (horse heart).

Enzyme activity was determined as the mg of reducing sugar released per h at 37 °C from 6% sucrose in 0.05 *M* potassium phosphate of pH 6.2. Reducing sugar was determined by the method of SOMOGYI[10], glucose by glucose oxidase (Reagent Kit, Fisher Scientific Co., Fair Lawn, N.J.) and protein by the method of LOWRY et al.[11].

Results and discussion. Samples of extracellular protein preparations from points designated along the growth

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curve (Figure 1) were electrophoresed, and enzyme patterns delineated by the tetrazolium procedure. Figure 2 shows some comparisons of the invertase-like activity found in preparations by use of the tetrazolium reagent. Only faint amounts of activity were observed from mid-log phase preparations. However, invertase-like activity was found in steadily increasing proportions as growth of the SL-1 cultures progressed into the early stationary phase or beginning of the stationary phase.

To permit further characterization, the invertase-like enzyme was separated from other sucrose-metabolizing enzymes by chromatography of the extracellular protein preparations on Sephadex G-100. The recovered activity represented about 8% of the total sucrose-metabolizing enzyme activity found prior to the chromatography. The enzyme acted on sucrose as substrate to produce reducing sugar, 49.4% of which was found to be glucose. No polysaccharide synthesis could be demonstrated. The enzyme had a pH optimum of 6.2, temperature optimum of 38–39 °C and molecular weight of 48,000. Since trehalose, an α-glucoside, did not serve as substrate, the enzyme was not an α-glucosidase.

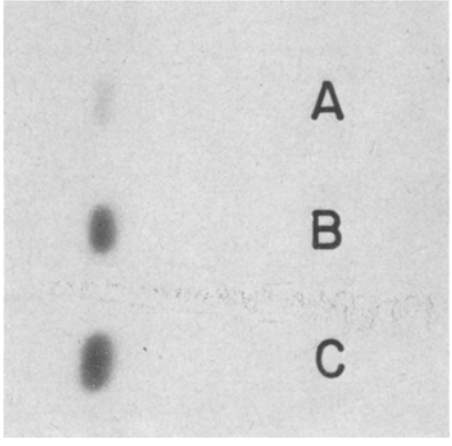

Fig. 2. Polyacrylamide gel electrophoresis showing invertase activity from extracellular protein preparations. Samples were from A) late log phase B) early stationary phase and C) stationary phase.

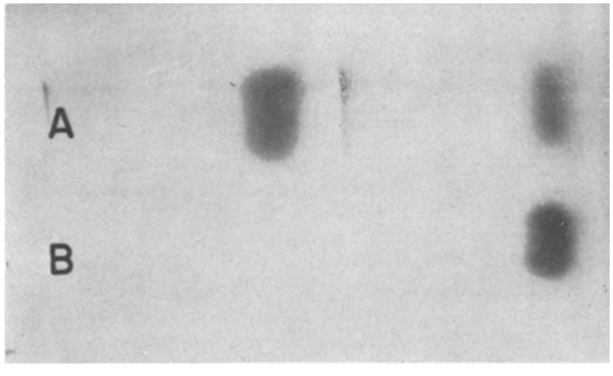

Fig. 3. 5% gel electrophoresis of A) extracellular protein preparation and B) intracellular invertase preparation at pH 7.3. Migration is to the right toward the (+) electrode and enzyme components were delineated by the tetrazolium procedure. The two components with the same migration rate near the right edge of the figure are invertase. Similar results were obtained under other electrophoretic conditions.

Intracellular invertase preparations also generated equimolar fructose and glucose from sucrose without polysaccharide synthesis, and did not generate reducing sugar from trehalose.

Intracellular invertase preparations and extracellular protein preparations were compared electrophoretically under several different conditions (5% gel concentrations at pH 6.7, 7.3 and 8.5; 7% gel concentrations at pH 8.5). A typical result is shown in Figure 3. In every instance both intracellular invertase and the invertase-like enzyme in the extracellular protein preparations produced single activity bands which had the same migration rates.

Thus, the enzyme characteristics of the invertase-like enzyme from the culture fluids were very similar to those for intracellular invertase, as found in this study or as previously reported[3] for strain SL-1. The data therefore indicated that the invertase-like enzyme is intracellular invertase which is released into culture fluids primarily during late log and stationary phases of growth[12].

Summary. Invertase from extracellular culture fluids of *S. mutans* strain SL-1 was shown to have the same characteristics as intracellular invertase from the same strain. The data indicate that intracellular invertase is released into the culture fluids primarily during the late log and stationary phases of growth.

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Penetration of Phospholipases A_2 and C into the Squid (*Loligo pealii*) Giant Axon

Phospholipase A_2 and C (PhA_2, PhC) are used as enzymatic probes, to study the structural organization of biological membranes and to search for specific functions of phospholipids (see[1] for references). In studies on axons, however, connective tissue, Schwann cell and myelin may interfere with the access of externally applied phospholipases to the axolemma where processes associated with generation of the action potential occur. Lack of an effect following the application of phospholipases may reflect a non-involvement of phospholipids or may be due to an inability of the phospholipase to reach the site

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