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.

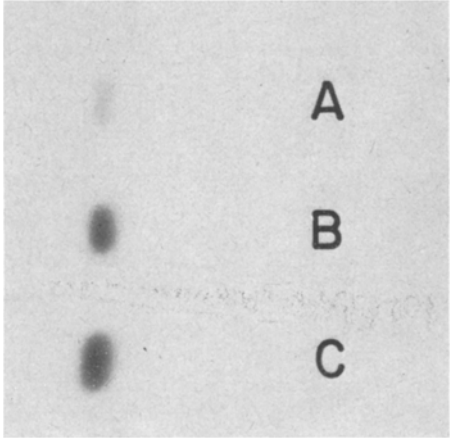

Fig. 2. Polyacrylamide gel electrophoresis showing invertase activity from extracellular protein preparations. Samples were from A) late log phase B) early stationary phase and C) stationary phase.

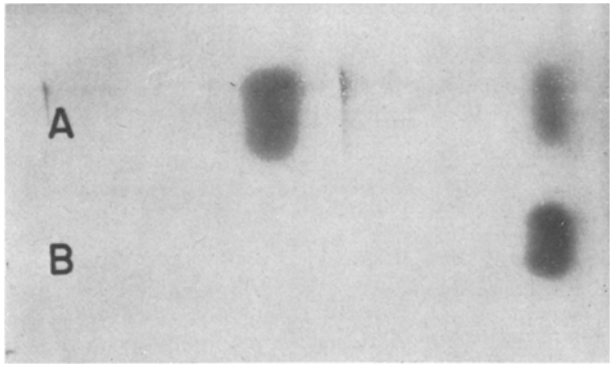

Fig. 3. 5% gel electrophoresis of A) extracellular protein preparation and B) intracellular invertase preparation at pH 7.3. Migration is to the right toward the (+) electrode and enzyme components were delineated by the tetrazolium procedure. The two components with the same migration rate near the 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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Penetration of phospholipases A and C (PhA_2, PhC) into axoplasm of the squid giant axon

Enzyme		Enzymatic activity[a]			Penetration (%)[b]
Type	(mg/ml)	Fresh solution	Stored incubation solution	Axoplasm	
PhA_2	20	7.41	5.28	18.47	350
	20	6.90	6.32	13.90	220
	20		4.16	14.51	349
	1	0.31	0.32	1.03	322
	1	0.28	0.20	0.68	340
	1		0.26	0.35	135
	1		0.41	0.44	107
PhC	10	0.330	0.270	0.048	18
	10	0.336	0.254	0.052	20
	1	0.030	0.032	0.010	31
	1	0.036	0.034	0.008	24

Axoplasm was extruded following exposure of axons for 1 h to incubation solutions containing indicated concentrations of phospholipases. Incubation and axoplasm containing solutions were stored in liquid nitrogen for about 1 month prior to assay. 'Fresh solution' was made up within 30 min prior to assay. Each of the 11 experiments is based upon axoplasm pooled from 4 axons. The weight of axoplasm extruded per axon ranged from 1.1 to 3.2 mg. [a] PhA, μEquiv. free fatty acids released per 10 min per μl PhC, increase in O.D. per 60 min per 10μl. For purposes of calculation it is assumed that 1 mg of axoplasm equals. 1 μl. [b] Activity in axoplasm × 100 divided by activity in incubation solution.

of interest. It thus is important to determine whether these enzymes can penetrate into the axoplasm of an axon following external application.

Materials and methods. PhC, (phosphatidylcholine: choline phosphohydrolase; EC 3.1.4.3) a partially purified preparation from *Clostridium perfringens* was obtained from Schwarz-Mann (Orangeburg, New York); the preparation (lot No. X 3452) has an activity of 15 units/mg. Cottonmouth moccasin (*Agkistrodon piscivorus piscivorus*) venom (lot No. 102065) was obtained from Ross Allen Reptile Institute (Silver Springs, Florida). A solution of this venom boiled for 15 min at 100 °C and pH 5 was used as the source of PhA_2 (phosphatide acyl hydrolase; EC 3.1.1.4) after adjustment of the supernatant fluid to pH 8.0 before use. PhA_2 is the only enzyme present in snake venoms which is resistant to boiling at acid pH[2,3]. Both acid-boiled venom and commercially obtained PhC are devoid of proteolytic activity[4–7]. We confirmed that these preparations had no proteolytic activity as judged by determinations[8] on supernatants of TCA precipitated incubation mixtures.

Giant axons of the squid (*Loligo pealii*), containing some adhering small nerve fibres were dissected as previously described[9]. Only uninjured axons were used, the ends being carefully ligated to avoid leakage of axoplasm. For each experiment, 4 axons (~60 mg) were incubated together for 1 h, with or without addition of phospholipase, in 10 ml of an artificial sea water solution (m*M* composition: NaCl, 449; KCl, 10; $CaCl_2$ 50; *Tris*, 3; pH 8.0). The axons were then removed from the chamber and axoplasm collected as described previously[9]. 1 ml samples of axoplasm in sea water and the 10 ml of incubation solutions were immediately placed in liquid nitrogen until phospholipase analyses.

PhA_2 activity was determined by titrating with 0.02 *N* NaOH according to the method of DOLE[10] the free fatty acids liberated at 0, 5, 10, 15 and 20 min from a 1:10 dilution, in 0.9% saline, of egg yolk. The results are expressed as microequivalents free fatty acid released per 10 min per μl of freshly prepared phospholipase solution, incubation solution, or extruded axoplasm. PhC activity was determined by following the development of turbidity in egg yolk suspensions[11]. An egg yolk is emulsified in 200 ml of 0.9% NaCl, placed in the refrigerator for 24 h, the supernatant filtered and NaCl added to a final concentration of 2%. Enzyme containing solution is added to this substrate (2.5 ml) and absorbance readings (540 nm) taken every 5 min for 30 min to 3 h; the increase in optical density being linear with time.

Results and discussion. Axoplasm from axons exposed to artificial sea water alone for 1 h showed no detectable level of endogenous PhA_2 or PhC activity (4 experiments). To determine whether phospholipases were stable during incubation and storage, we compared the activities of freshly prepared solutions of these enzymes with the activities in the incubation solutions (Table). The enzymatic activities of the freshly prepared and incubation solutions were similar. In 5 experiments with PhA_2 there was a 3-fold accumulation of the enzyme in the axoplasm and in the remaining 2 experiments the enzymatic activities in the axoplasm and in the incubation solutions were about equal. The PhC activity in the axoplasm was 20 to 30% of that in the incubation solution (Table). Using conditions identical to those used in the present study, it was found that after exposure of control squid axons to impenetrable radioactive compounds less than 1% penetrated into the axoplasm[12]. Contamination is thus not a serious source of error in our studies.

Our present results show that PhA_2 and PhC are able to penetrate through connective tissue, Schwann cell and

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axolemma, which is in agreement with earlier data where we found splitting of axoplasmic phospholipids following exposure of intact squid giant axons to PhA_2 or PhC[1,13,14]. Lack of penetration may therefore not be the explanation for the finding[15] that pancreatic PhA has no effect following external application, but blocks the action potential of the squid giant axon following internal perfusion. Unfortunately they only used externally one concentration of PhA, which was equal to the lowest concentration which had an effect internally.

PhA_2, externally applied, causes vesiculation and disruption of the Schwann cell of the squid axon[16] along with a large release of free amino acids[1] indicative of a breakdown of phospholipid-protein binding, correlating well with our present findings that PhA_2 is highly penetrable. The apparent penetration of PhA_2 was often above 100% which may indicate axoplasmic binding or assymetric permeability properties. The high percent penetration of PhA_2 may not only be due to direct disruption of phospholipids but may be due to the concomitant production of lysophosphatides, which have a detergent action and are responsible for block of conduction following PhA_2 application to the squid giant axon[17].

It is difficult to explain the apparent discrepancy between our finding that PhC does not block squid axon conduction following external application[13,17] even though we now know that it penetrates into the axoplasm, and the finding that internal injection (1.5 µl of a 0.5 mg/ml solution) or perfusion (5 mg/ml) of phospholipase C into the axoplasm causes a loss of excitability in 20 to 30 min[18] (I. TASAKI, 1972, personal communication).

Summary. Following 1 h exposure, the level of phospholipase A_2 penetration into the axoplasm of the squid giant axon was 107 to 350% of that in the external media; corresponding values for phospholipase C were 18 to 31%. Phospholipases can therefore be used to study phospholipid function in axons since they can penetrate through connective tissue and Schwann cell to reach the axolemma.

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Evolution et contrôle de l'activité de l'argininosuccinate synthétase, de l'argininosuccinase et de l'arginase du foie foetal de rat

Control Activities and Regulation by the Glucocorticoids of Three Urea-Cycle Enzymes in Rat Foetal Liver: Argininosuccinate Synthetase, Argininosuccinase and Arginase

Au cours des derniers jours de la vie fœtale, de nombreuses enzymes s'accumulent dans le foie et il est souvent possible de déceler très tôt leur activité. De récentes recherches ont montré que la régulation de ces enzymes obéit, bien avant la naissance, à un déterminisme endocrinien. Ainsi, les travaux de JOST et JACQUOT[1-3], de GREENGARD[4], de HOLT et OLIVER[5], de RÄIHÄ et SUIHKONEN[6], ont révélé le rôle important que jouent les glucocorticoïdes, le glucagon et d'autres hormones sur l'activité enzymatique de diverses voies métaboliques. Au cours d'une précédente note[7], les activités des deux premières enzymes du cycle de l'urée, la carbamyl phosphatase synthétase, (E.C. 2, 7, 2, 2) et l'ornithine transcarbamylase (E.C. 2, 1, 3, 3) ont été mesurées chez le fœtus et leur contrôle par les glucocorticoïdes a été mis en évidence. Le présent travail rapporte les résultats obtenus en étudiant, dans les mêmes conditions expérimentales que précédemment[7], trois autres enzymes impliquées dans la synthèse de l'urée, l'argininosuccinate synthétase (E.C. 6, 3, 4, 5), l'argininosuccinase (E.C. 4, 3, 2, 1) et l'arginase (E.C. 3, 5, 3, 1).

Matériels et méthodes. L'étude porte sur des rats blancs Sherman. L'âge du fœtus est déterminé par rapport au moment supposé de la fécondation (1 heure du matin). La surrénalectomie de la mère est faite à 14,5 jours. Les animaux opérés reçoivent à volonté de l'eau salée (NaCl 9 g l^{-1}). L'hypophysectomie des fœtus est faite dans les conditions habituelles, par décapitation in utero[8,9] à 18,5 jours. Certains fœtus décapités reçoivent aussitôt après l'opération 0,1 mg d'acétate de cortisol (Roussel) par voie intrapéritonéale au travers du muscle utérin. D'autres fœtus entiers provenant de mères non surrénalectomisées reçoivent aussi cette préparation hormonale au même âge (18,5 jours) et des fœtus témoins de l'autre corne, un volume équivalent de NaCl à 9 g l^{-1}. Au stade de gestation à étudier, la mère est assommée et les fœtus sont retirés de l'utérus et saignés. Les foies sont rapidement prélevés et homogénéisés dans du bromure de N. cétyl-N, N, N, triméthylammonium à 0,1% pour la mesure de l'activité de l'argininosuccinate synthétase et de l'argininosuccinase et dans un milieu constitué de 9 g l^{-1} de NaCl et de 6 g l^{-1} de $MnSo_4$, 1 H_2O pour la mesure de l'activité de l'arginase. Les activités enzymatiques sont mesurées in vitro selon RATNER[10] pour

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