at 55° for 5 min. The inactivation could be prevented almost completely by the presence of 15 mM aspartate. Fumarate and malate were also effective, whereas oxaloacetate, PEP and CoASAc were not so effective. Therefore, the form of the enzyme bound with the inhibitor seems more heat-stable.

Phosphoenolpyruvate carboxylase from spinach leaves prepared according to the method of BANDURSKI[11] was neither activated nor inhibited by aspartate. Therefore, a special device for controlling the activity is supposed to be inherent in the enzyme from Enterobacteriaceae.

From the facts presented above, aspartate seems to cause an inhibition of the enzyme by occupying the site(s) distinct from the active site.

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Comparative effects, *in vitro*, of various detergents on liver glucose-6-phosphate phosphohydrolase, inorganic pyrophosphate–glucose phosphotransferase, and acid inorganic pyrophosphatase activities

A variety of detergents previously have been demonstrated to affect the hydrolytic activity of classical liver glucose-6-phosphate phosphohydrolase (EC 3.1.3.9) (see, for example, refs. 1–6). Recent studies[7–13] indicate that this enzyme also catalyzes the hydrolysis of PP_i and the transfer of a phosphoryl group from PP_i to the hydroxyl group attached to the number six carbon atom of glucose. Deoxycholate was employed as activating detergent in these studies which were carried out in our laboratory[7–11], while Triton X-100 was employed elsewhere[12,13]. The effects of a variety of other anionic, cationic, and non-ionic detergents on the various hydrolytic

and synthetic activities of this enzyme recently have been investigated. Results of these studies, which indicate the rather general nature of detergent effects on these enzymic activities, are described in this communication.

Sources of detergents were as follows: Anionic detergents—deoxycholic acid (Mann Research Laboratories), cholic acid (Calbiochem), and sodium lauryl sulfate (Fisher Scientific Co.). Non-ionic detergents—Triton X-100 (Rohm and Haas), Tween 20 and Tween 80 (Atlas Chemical Co.). Cationic detergent—cetyltrimethyl ammonium bromide ("cetrimide", Eastman Organic Chemicals). Detergent solutions were neutralized with dilute NaOH solution where necessary. Sources of other chemicals were as previously described[7,9]. Young, male, albino rats, obtained from Sprague–Dawley, Inc., Madison, Wisc., and maintained on stock Purina Lab Chow and tap water, *ad libitum*, were employed. Animals were decapitated, and livers were removed, blotted, homogenized in ice-cold 0.25 M sucrose solution for 1.5 min in a Potter–Elvehjem homogenizer operating at 600 rev./min, and diluted with the sucrose solution to 15 ml per g liver. Aliquots (8.0 ml) of the homogenates then were supplemented with 2.0 ml of deionized water or of detergent solutions of appropriate concentrations, and were allowed to stand at 0° for 30 min before assays were carried out with 0.1-ml aliquots. Assay mixture compositions were as follows: PP_i–glucose phosphotransferase mixtures, pH 5.5, contained 40 mM sodium cacodylate buffer, 20 mM PP_i, and 180 mM D-glucose. Inorganic pyrophosphatase mixtures, pH 5.5, contained 40 mM sodium cacodylate buffer and 10 mM PP_i. Glucose-6-phosphate phosphohydrolase mixtures,

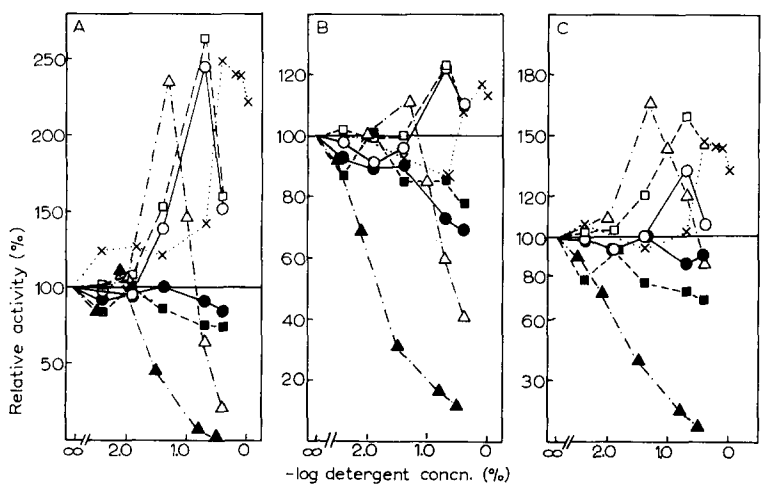

Fig. 1. Effects of various detergents on (A) PP_i–glucose phosphotransferase, (B) glucose-6-phosphate phosphohydrolase, and (C) inorganic pyrophosphatase activities. Enzymic activity values are expressed in terms of per cent of activity observed in the absence of added detergent. Results obtained with various detergents are designated as follows: ○—○, deoxycholate; □— —□, Triton X-100; △—·—△, cetrimide; ×- - - - -×, cholate; ●—●, Tween 20; ■— — —■, Tween 80; ▲—·—▲, sodium lauryl sulfate. For simplicity of presentation, detergent concentrations are recorded on the axis of abscissas in terms of per cent (v/v) for Triton X-100 and Tweens 20 and 80, or as per cent (w/v) for all other compounds. Molar concentrations may be calculated, where molecular weights of detergents are known with certainty, on the basis of the relationship that a 0.1% solution of the following compounds corresponds with the indicated molar concentration: sodium deoxycholate 2.55 mM; sodium cholate 2.45 mM; sodium lauryl sulfate 3.46 mM, and cetyltrimethyl ammonium bromide 2.75 mM. Concentration values in all instances refer to detergent concentrations in liver homogenates.

pH 6.5, contained 40 mM sodium cacodylate buffer and 20 mM glucose-6-*P*. The volume of all assay mixtures was 1.5 ml; incubations were carried out for 10 min at 30 ± 0.1°, with shaking. Other details on assay procedures were as described previously[7,9].

Results obtained are presented in Fig. 1. The various detergents appear to fall into two groups on the basis of their effects on these enzymic activities—those which activate (deoxycholate, cholate, Triton X-100, and cetrimide), and those which inhibit progressively with increasing concentration (sodium lauryl sulfate and Tweens 20 and 80). The effects of the various detergents on glucose-6-phosphate phosphohydrolase activity, including the polyphasic action of the activating compounds[2], are in agreement with earlier reports[2,4]. The effects of the stimulating group of compounds on the various activities of the enzyme also resemble generally those obtained with deoxycholate in this and earlier[9,10] studies in that (a) each activity is stimulated by optimal concentrations of the various detergents to approximately the same maximal extent, and (b) with each detergent the degree of stimulation of phosphotransferase > inorganic pyrophosphatase > glucose-6-phosphate phosphohydrolase. The rather general nature of stimulation of these activities by surface-active agents is apparent from the observation that anionic (deoxycholate and cholate), non-ionic (Triton X-100), and cationic (cetrimide) detergents all activated, while other anionic and non-ionic detergents (sodium lauryl sulfate, Tween 20 and 80) inhibited and did not stimulate. Deoxycholate, cholate, cetrimide, and Triton X-100 all appear from these studies to be satisfactory activating agents for studies of the various activities of this multifunctional enzyme, although the last-mentioned compound occasionally produced mild turbidity in P_i assay mixtures when the higher concentrations of this detergent were employed.

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