This phenomenon is not due to disaccharide interaction, because addition of osmotically equivalent amounts of other solutes (glucose, galactose, fructose, xylose, mannitol, urea, NaCl and KCl) also increased the lactosuria after a standard lactose load (40 g/133 ml). The excretion of lactulose and raffinose, not hydrolysed in human small intestine (Dahlqvist & Gryboski, 1965; M. Udupihille, unpublished work), also increased when oral loads were made hyperosmotic by solute addition. Decrease in systemic catabolism is excluded, since recovery of these disaccharides from the urine after intravenous administration was almost quantitative (above 80% of 0.5 g within 5 h).

There remains the probable explanation that gastrointestinal permeability to disaccharide and trisaccharide (raffinose) is increased after the ingestion of hyperosmotic solutions. Similar observations have been made on the permeability of frog skin and toad bladder (Ussing, 1966; Urakabe *et al.*, 1970).

Consecutive administration of raffinose and lactulose indicated that the permeability increase produced by hyperosmotic urea (21 g/100 ml) was temporary, returning to normal within 2½ h (two normal adults).

Lactulosuria after hyperosmotic loading (lactulose 5 g/100 ml + sucrose 40 g/100 ml) ranged from 14 to 39 mg/5 h for ten normal adults, showing little increase above the response to iso-osmotic loading (sucrose omitted). In nine subjects with coeliac disease hyperosmotic loading produced greater lactulosuria (60–180 mg/5 h), although several showed normal responses after iso-osmotic loading.

These results stress the relation of sugar-load osmolality to disacchariduria in normal and especially coeliac subjects, and suggest that raffinose and lactulose may be useful as indicators *in vivo* of gastrointestinal permeability.

- Menzies, I. S. & Seakins, J. W. T. (1969) in *Chromatographic and Electrophoretic Techniques* (Smith, I., ed.), p. 310, Heinemann, London
 Moncrieff, A. (1960) *Maandschr. Kindergeneesk.* **28**, 51
 Dahlqvist, A. & Gryboski, J. D. (1965) *Biochim. Biophys. Acta* **110**, 635
 Urakabe, S., Handler, J. S. & Orloff, J. (1970) *Amer. J. Physiol.* **218**, 1179
 Ussing, H. H. (1969) *Quart. Rev. Biophys.* **4**, 365

The Interaction of Aliphatic and Aromatic Dicarboxylic Acids with Aspartate Aminotransferase

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The interaction of aliphatic dicarboxylic acids with soluble aspartate aminotransferase from pig heart results in spectroscopic changes (associated with the protonation of the pyridoxal phosphate coenzyme) from which the dissociation constants for the dicarboxylate, K_a , may be determined (Jenkins & Taylor, 1965; Jenkins & D'Ari, 1966). These values correlate with the values of K_i for competitive inhibition. Strongest binding is shown by C_4 and C_5 dicarboxylates (succinate, glutarate, oxaloacetate, α -oxoglutarate, maleate), with K_a of the order of $1 \times 10^{-3} M$ at pH 8 (Michuda & Martinez-Carrion, 1970). The ability of the aromatic dicarboxylic acids *o*-, *m*- and *p*-phthalate to act as competitive inhibitors was noted by Velick & Vavra (1962), though the K_i values were in general greater than those for aliphatic dicarboxylic acids by one order of magnitude.

We have now compared *o*-, *m*- and *p*-phthalate with glutarate and α -oxoglutarate for their effectiveness in producing changes in absorption spectra after binding to the enzyme. The measurements were made in carefully buffered solutions at pH 6.5 and 7.5 in 0.02 M-phosphate at 20°C, with preparations of pig heart aspartate aminotransferase (α -form, prepared according to method A of Martinez-Carrion *et al.*, 1967) at concentrations in the range 2.0–4.0 mg/ml. The results obtained were treated by the method of Jenkins & Taylor (1965) or by Scatchard plots (Tanford, 1961).

All the dicarboxylates have the same qualitative effect, namely the increase in absorption in the region of 430 nm and a decrease in the region of 360 nm. The systems are in general closely isosbestic. The isosbestic point and the maximum of the longer-wavelength band have different values for individual ligands, in the ranges 386–395 nm and 426–436 nm respectively. The characteristic values are maintained at both pH 6.5 and 7.5.

Good agreement was found for K_a obtained by both the above methods, and for values at both wavelengths. Values for glutarate and α -oxoglutarate were 0.1 mM (pH 6.5) and 1–2 mM (pH 7.5). By contrast, the values for the phthalates were within the range 20–90 mM for all three isomers (*o*-, *m*- and *p*-) at both pH values. The Scatchard treatment brought out in addition a stronger binding process for the aromatic

dicarboxylic acids. This was best resolved for *m*-phthalate at both pH values, as indicated by biphasic plots, and was characterized by K_a approx. 1 mM.

Significantly different values for the absorption at the long-wavelength maximum of the enzyme at infinite ligand concentration are obtained from the intercepts of the plots. Thus at pH 6.5, relative to glutarate as 1.0, the values are 0.85 for α -oxoglutarate and 0.8 for *o*-, *m*- and *p*-phthalate; at pH 7.5 these values are 0.73 for α -oxoglutarate and 0.2, 0.35 and 0.4 for *o*-, *m*- and *p*-phthalate respectively. These differences may be in part due to differences in protonic dissociation constant of the protonated enzyme-ligand complex, $E-H-L^+$, since conversion of all the enzyme into this form does not necessarily occur. In addition, contributions to these differences from conformational or environmental factors in the region of the pyridoxal coenzyme may be inferred from the fine differences in the characteristic points of the spectrum of the $E-H-L^+$ complex.

The affinity for a given ligand, the change in apparent pK of the enzyme, the relative disposition of the binding sites for the two carboxyl groups and the conformational adaptability of the enzyme are evidently interrelated properties. The analogy between these properties and those of the enzyme-substrate complex provides the basis for obtaining structural information about the active site.

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Jenkins, W. T. & D'Ari, L. (1966) *J. Biol. Chem.* **241**, 2845

Jenkins, W. T. & Taylor, R. T. (1965) *J. Biol. Chem.* **240**, 2907

Martinez-Carrion, M., Turano, C., Chiancone, E., Bossa, F., Giartosio, A., Riva, F. & Fasella, P. (1967) *J. Biol. Chem.* **242**, 2397

Michuda, C. M. & Martinez-Carrion, M. (1970) *J. Biol. Chem.* **245**, 262

Tanford, C. (1961) *The Physical Chemistry of Macromolecules*, Chapter 8, Wiley, New York

Velick, S. F. & Vavra, J. (1962) *J. Biol. Chem.* **237**, 2109

Inhibitors of Shikimate Dehydrogenase as Potential Herbicides

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The shikimate biosynthetic pathway, which occurs in higher plants but not in animals, leads to several essential amino acids and also to a variety of other metabolites. It was hoped therefore that an inhibitor

of the pathway might have a damaging effect on plant metabolism and possibly be a useful commercial herbicide with low mammalian toxicity. Shikimate dehydrogenase (EC 1.1.1.25), the enzyme that catalyses the conversion of dehydroshikimate (I) into shikimate (II), was chosen as the target for inhibition because it occurs early in the pathway and because of the accessibility of synthetic substrate analogues.

It was our intention to prepare active-site-directed irreversible enzyme inhibitors for the enzyme, and as a starting point for this we prepared the novel dehydroshikimate analogue 1,6-dihydroxy-2-oxoisonicotinic acid (III). When tested against shikimate dehydrogenase from pea (assayed in the direction shikimate $\rightarrow$ dehydroshikimate) this had K_i 0.13 mM (K_m 0.17 mM). A hydrophobic area exists near the active site of the enzyme, since a series of 6-*O*-alkyl derivatives of compound (III) bound as well as or better than the parent compound. Maximum binding was obtained with the isopropyl ether (K_i 0.07 mM). We think this area may also have been instrumental in helping to complex the 6-*O*-phenyl analogue (K_i 0.12 mM) and the related potential active-site-directed inhibitors.

It is of interest that these compounds, all of which are more like dehydroshikimate than shikimate, are very weak inhibitors when the enzyme is assayed in the direction dehydroshikimate $\rightarrow$ shikimate (Dowsett *et al.* 1971).

Although chemical synthesis was difficult, we have prepared some iodoacetamidophenoxy derivatives of compound (III) that appear to cause progressive irreversible inhibition directed at the active site. The most powerful of these caused 50% inhibition after 48 min when tested at 1 mM.

The compounds were inactive as herbicides *in vivo* when screened on a range of representative plants, and possible reasons for this will be discussed.

Dowsett, J. R., Corbett, J. R., Middleton, B. & Tubbs, P. K. (1971) *Biochem. J.* **123**, 23 P

Metabolism and some Biochemical Effects of *N*-Nitrosomorpholine

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The hypothesis that the carcinogenic and other biological activities of *N*-nitroso compounds could be mediated by their conversion *in vivo* into alkylating agents has received support from studies on structure-activity relationships by Druckrey *et al.* (1967) and from direct evidence of alkylation of nucleic acids or proteins *in vivo* by several nitrosamines and nitrosamides (see Magee, 1969). However, Argus *et al.*