tissues[3], dermatan sulphate is associated closely with collagen fibres since collagenase digestion removes more of this glycosaminoglycan than either salt extraction or digestion with elastase, a less specific protease. By this criterion heparan sulphate is closely associated with neither collagen nor elastin and its distribution is clearly different to that of heparin, being least effectively solubilized by salt solution. As has been noted with the product from human aorta[13], an appreciable fraction of the heparan sulphate of bovine lung is eluted from Dowex 1×2 by relatively low concentrations of NaCl (38% from parenchyma and 57% from pleura eluted with 0.8 *M* NaCl). Also, a significant fraction is not precipitated by cetylpyridinium chloride in the presence of 0.5 *M* NaCl. This could indicate a variable degree of sulphation (CIFONELLI and DORFMAN[14]) or the presence of heparan sulphate of low molecular weight.

Discussion. The low levels of heparan sulphate found in the pleura can probably be attributed to the vascular layer since this glycosaminoglycan is found in arteries[15]. When found in bovine aorta[16] heparan sulphate is accompanied by much higher levels of glycosaminoglycans containing galactosamine. These are present in parenchyma but in insufficient quantities for the heparan sulphate there to be attributed solely to residual blood vessels which are difficult to remove from the tissue.

Thus bovine lung parenchyma contains large quantities of a readily-extractable, heterogenous, heparan sulphate fraction which could have a specific function in the connective tissue components involved in gas exchange.

Résumé. On décrit et compare les glycosaminoglycanes du parenchyme et de la plèvre du poumon bovin. Tous les glycosaminoglycanes principaux, sauf le kératan-sulfate, y ont été trouvés, mais il y a beaucoup plus d'héparan-sulfate dans le parenchyme.

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Genetic Evidence for the Tetramer Structure of Glyceraldehyde-3-Phosphate Dehydrogenase

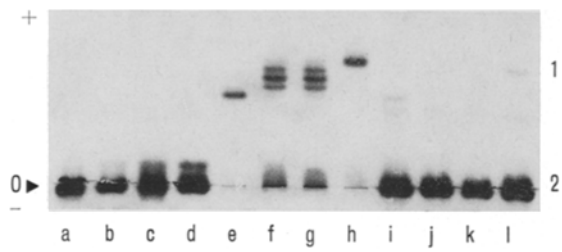
Crystallographic[1], molecular weight[2] and amino acid[3] studies have suggested that the active enzyme form of glyceraldehyde-3-PO_4 dehydrogenase (G3PD) is a tetramer, composed of 4 polypeptide subunits. Here we present genetic evidence using starch-gel electrophoresis to support that hypothesis.

If 2 electrophoretically different polypeptide chains (A and A′) are produced in cells and if these subunits combine randomly to form tetramer molecules, molecules AAAA, AAAA′, AAA′A′, AA′A′A′, and A′A′A′A′ should be produced in a 1:4:6:4:1 ratio[4].

Starch gel electrophoresis of tissue extracts[5] of *Xiphophorus maculatus* strain 163A, platyfish[6], and *X. helleri strigatus*, swordtail, followed by histochemical staining for G3PD[7] reveals 2 isozymes of G3PD in each species, a slow migrating form G3PD-2 present in liver and muscle and a faster migrating form G3PD-1 present in eye and brain (Figure). While G3PD-2 is the same in both species there is an electrophoretic difference in the G3PD-1 isozyme of the two species. These facts plus the observed tissue specificity indicate the 2 G3PD isozymes are specified by separate genetic loci.

In the F_1 hybrids[8] which contain 1 G3PD-1 allele specifying the platyfish subunit and 1 for the swordtail subunit, the expected 5 banded pattern consistent with the tetramer structure of G3PD was obtained (Figure). F_1 hybrids when backcrossed to swordtails produced 15 fish displaying the 5 banded hybrid pattern and 16 fish the 1 fast band pattern of the swordtail. This agrees with the expected 1:1 ratio ($\chi^2 = 0.03$, $P > 0.8$).

Five membered sets of G3PD bands have been reported as being usually seen following zone electrophoresis on



Glyceraldehyde-3-PO_4 dehydrogenase isozymes in platyfish, swordtails and their hybrids. a–d) Liver extracts of a) platy, b) and c) F_1 hybrids and d) swordtail. e–h) eye extract of e) platy, f) and g) F_1 hybrids and h) swordtail. i–l) muscle extracts of i) platy, j) and k) F_1 hybrids, l) swordtail. The hybrid eye patterns (channels f and g) were cut from the starch gel and the activity in each band was estimated using a Densicord recording densitometer. The ratio of the bands in the hybrid pattern was approximately 1:4:6:4:1.

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cellulose acetate strips of certain tissues of turtle, perch, trout, spinach and yeast[9]. Lack of symmetry in the banding patterns and loss of activity of certain of the bands in different tissues complicated the analysis yet it was suggested that the results could be explained on the basis of random formation of tetramers from subunits which were coded by different genes. The genetic data presented here demonstrating a symmetrical 5 banded pattern in cells with 2 different G3PD-1 alleles at the same locus not only confirms the tetramer structure of G3PD but indicates that the irregular patterns seen by the former workers[9] were possibly produced by genes at 2 different loci under separate genetic control. The lack of intermediate isozymes between the 2 G3PD loci of platyfish and swordtails might be accounted for by the strict tissue specificity or a restriction in subunit aggregation[10].

Zusammenfassung. Es wurden bei *Xiphophorus maculata* und *Xiphophorus helleri* zwei Isozyme von Glyceraldehyd-3-phosphat-Dehydrogenase nachgewiesen. Die biochemisch-genetischen Untersuchungen deuten auf eine Tetrameren-Struktur.

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Antiviral Properties of 1-Allyl and 1-Crotyl Derivatives of 2-(α-Hydroxybenzyl) benzimidazole

The importance of the lipophilic characters of 1-substituted derivatives of 2-(α-hydroxybenzyl)benzimidazole (HBB) in relation to their antiviral activities is indicated by an initial increase in activity with increase in carbon chain length of the 1-substituent[1,2] and by a rough parallelism between log (activity) and Hansch π value[3] for small substituents as illustrated by activities against poliovirus type 1 in Table I. When the unbranched chain equals or exceeds 4 carbon atoms, reduction in antipoliovirus activity accompanies further increase in chain length[2,4]. This reduction might be caused by increase in strength of hydrophobic binding between the substituted benzimidazole and cellular lipids and proteins, resulting in restriction of access of the active molecules to specific receptor sites as the lipophilic character of these molecules is increased beyond its optimum level. Hansch et al.[5] have discussed examples where log (biological response) is a quadratic function of appropriate π values giving rise to maxima in the structure-activity patterns.

Introduction of a double bond into an alkyl substituent greatly increases some biological responses. In such cases, the influence of factors other than hydrophobic interactions may predominate. Thus, although the corresponding propyl compounds have little or no activity, certain allyl acetamide and barbiturate derivatives produce hepatic porphyria[6] with loss of cytochrome P-450 and haem[7], various 1-allyluracils have diuretic, appetite inhibiting, antisecretory, anti-iritic and smooth muscle relaxant properties[8], and 1-allyl-3, 5-diethyl-6-chloro-uracil and other 1-allyl-5-alkyluracils possess inhibitory activity against herpes and vaccinia viruses[9]. The preparation of 1-allyl and 1-crotyl derivatives of HBB has been reported[10] and further details are now given of their antiviral properties.

The compounds were tested for their inhibiting effect on the multiplication of poliovirus type 1 (*L Sc 2 ab*), type 2 (*P 712 Ch 2 ab*) and type 3 (*Leon 12 ab*) and coxsackievirus A 21 in ERK (human) cell monolayers, coxsackievirus A 9 and ECHO virus 11 in primary monkey kidney cell monolayers, and neurovaccinia virus in HeLa

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Table I. Virus inhibitory concentrations (VIC) and Log_{10} (activities) of HBB and its derivatives with 1-phenyl and small 1-alkyl substituents and the corresponding Hansch substituent constants π

Substituent	H	Me	Et	Pr	Ph
VIC[a]	160	120	100	9	6
log (activity)[b]	0.80	0.92	1.0	2.0	2.2
π value	0	0.5	1.0	1.5	1.8[c]

[a] Micromolarity of compound required to reduce type 1 poliovirus yield in ERK cells by 75% in 16 h. [b] Defined as $\log_{10}$ (1000/VIC) in order to permit ready comparison with the π value. [c] A value that allows for some dipolar interaction involving the phenyl substituent[3].

Table II. Effect of introducing a double bond on the virus inhibitory concentrations[a] of 1-alkyl-HBB derivatives

Poliovirus	Substituent (and its Hansch π value)			
	allyl (1.2)	Crotyl (1.7)	Pr (1.5)	Bu (2.0)
1	8.25[b]	10.75[c]	9.0	10
2	5.0	7.75	7.5	5.5
3	9.75	10.75	22.5	25

[a] VIC values are quoted to the nearest 0.25 μM. [b] $Log_{10}(1000/VIC) = 2.1$. [c] $Log_{10}(1000/VIC) = 2.0$.