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.

D. A. Wright, M. J. Siciliano and J. N. Baptist

The University of Texas Graduate School of Biomedical Sciences, and M.D. Anderson Hospital and Tumor Institute, Houston (Texas 77025, USA),
8 February 1972.

[10] This research was supported in part by research grant No. GM 15597 and training grant No. CA 5047, both from NIH.

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

[1] D. G. O'Sullivan, C. Ludlow, D. Pantic and A. K. Wallis, *Antimicrobial Agents and Chemotherapy* 1969 (American Society for Microbiology, Washington, D.C. 1970), p. 153.
[2] D. G. O'Sullivan, D. Pantic and A. K. Wallis, Experientia *23*, 704 (1967).
[3] C. Hansch, A. R. Steward and J. Iwasa, Molec. Pharmac. *1*, 87 (1965). – C. Hansch and S. M. Anderson, J. med. Chem. *10*, 745 (1967).
[4] D. G. O'Sullivan, D. Pantic and A. K. Wallis, Nature, Lond. *203*, 433 (1964).
[5] C. Hansch, E. J. Lien and F. Helmer, Arch. Biochem. Biophys. *128*, 319 (1968).
[6] A. Goldberg and C. Rimington, Proc. R. Soc. B *143*, 257 (1955).
[7] F. De Matteis, Fedn. Europ. biochem. Soc. Lett. *6*, 343 (1970).
[8] C. C. Cheng and B. Roth, *Progress in Medicinal Chemistry*, (Eds. G. P. Ellis and G. B. West; Butterworths, London, 1970), vol. 7, p. 309.
[9] K. K. Gauri and B. Rohde, Klin. Wschr. *47*, 375 (1969). – K. K. Gauri, German Patent 1,248,665 (1967); Chem. Abstr. *68*, 21948j (1968).
[10] D. G. O'Sullivan and A. K. Wallis, J. med. Chem. *15*, 103 (1972).

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] $Log_{10}(1000/VIC) = 2.0$.

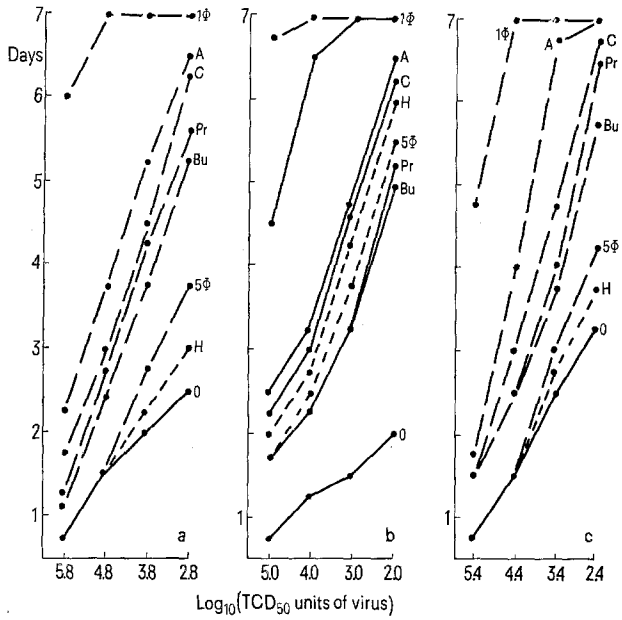

Fig. 1. Time between infection with poliovirus [a) type 1; b) type 2, and c) type 3] and 50% CPE plotted against virus dose (in 2 ml medium) per tube. In uninfected tubes, 50% cell death occurred in 7 days. Lettering of each line codes for the HBB derivative as follows: - O, no compound; H, HBB itself; 5Φ, 5-phenyl; Bu, 1-butyl; Pr, 1-propyl; C, 1-crotyl; A, 1-allyl; 1Φ, 1-phenyl. Concentrations are indicated as follows: - - -, half maximum tolerated concentration (MTC); — — —, $^1/_4$ MTC; ——, $^1/_8$ MTC (and infected controls).

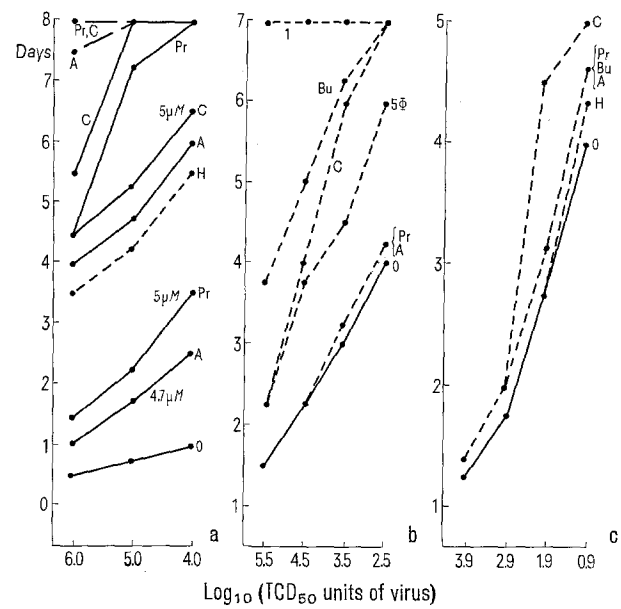

Fig. 2. Time between infection with a) coxsackievirus A9; b) coxsackievirus A21, and c) vaccinia virus and 50% CPE plotted against virus dose (in 2 ml medium) per tube. In uninfected tubes, 50% cell death occurred in a) 8 days; b) 7 days; and c) 5 days. Compounds and concentrations are identified as in Figure 1, but additionally, in Figure 2a, concentrations are quoted for data obtained at $^1/_{16}$ MTC.

cell monolayers. The experimental methods used for ERK and MK cells have been described briefly[11]. HeLa cells were dispersed in Eagle's minimum essential medium containing foetal bovine serum (10% v/v), $NaHCO_3$ (0.112% w/v), glutamine (1% w/v), benzyl-penicillin (100 U/ml) and streptomycin (100 μg/ml). The suspension was allowed to stand in inclined tubes (1 ml per tube) for 3 days at 37 °C and the medium was then replaced from the cell layers by fresh medium (2 ml per tube) with the serum content reduced to 2%, the $NaHCO_3$ content increased to 0.224%, and containing vaccinia virus and test compound at appropriate concentrations. Tubes were then slowly revolved and incubated at 37 °C. Remaining details were as previously described[11].

The concentrations required to produce 75% inhibition of poliovirus growth in a 16 h period (Table II) suggest that, although lipophilic character, as represented by the π value, is an important component of the effect of the allyl and crotyl substituents, other factors may be involved. Fit into a simple parabolic relationship[5] is poor.

The Figures compare the cell protective effects of 1-allyl, 1-crotyl, 1-propyl, 1-butyl, 1-phenyl[2] and 5-phenyl derivatives of HBB at appropriate fractions of the maximum concentrations of the compounds tolerated[11] by ERK cells (75, 80, 80, 60, 140 and 70 μ*M*, respectively). Time intervals between infection and 50% cytopathic endpoint are portrayed for infection with different virus titres, the compound being added simultaneously with virus. Under the test conditions, the order of activity[12] 1-Ph > Allyl > Crotyl > Pr > Bu > 5-Ph > HBB applies to poliovirus 1 and 3 (Figure 1a, and c). The only modification of this order for poliovirus 2 is the reversal of the positions of HBB and its 5-phenyl derivative (Figure 1b). Thus, unsaturated compounds appear more active than saturated compounds in relation to cell protection from the effects of poliovirus infection. Sequence Crotyl > Pr > Allyl applies to coxsackievirus A 9 (Figure 2a). Separate experiments showed the 1-phenyl to be less effective than the 1-propyl with this virus. The order of activity[12] (Figure 2b) for coxsackievirus A 21 [1-Ph > Bu > Crotyl > 5-Ph > Pr, Allyl > HBB (inactive)], when compared with that for the HANSCH π values of the 1-substituents (Tables I and II), suggests that the lipophilic influence of the 1-substituent may be relatively more important for cell protection against this virus. This suggestion is still valid even if allowance is made for the different molar concentrations used in the test. At half their maximum tolerated concentrations, the 1-allyl, 1-crotyl and 1-propyl derivatives completely protected MK cells against the cytopathic effect (CPE) of 5.5 TCD_{50} units (and lower doses) of ECHO virus 11. Figure 2c shows that generally activities are small in relation to vaccinia virus in agreement with previous findings with DNA viruses[13].

Zusammenfassung. 1-Allyl und 1-Crotyl-2-(-oxy-benzyl)-Benzimidazol hemmen die Vermehrung von Poliovirus 1, 2 und 3, sowie diejenige der Coxsackieviren A 9 und A 21 und diejenige des ECHO-Virus 11.

D. G. O'SULLIVAN and CAROLYN M. LUDLOW

Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, W1P 5PR (England),
13 January 1972.

[11] D. G. O'SULLIVAN, C. M. LUDLOW and A. K. WALLIS, Experientia *27*, 1025 (1971).
[12] Selective activity at a given fraction of MTC.
[13] D. G. O'SULLIVAN, D. PANTIC, D. S. DANE and M. BRIGGS, Lancet *1*, 446 (1969).