110 °C for 24 h at neutral pH. Under these conditions, more histidinoalanine was formed than lysinoalanine. The identity with histidinoalanine was demonstrated by high performance liquid chromatography in 2 different solvent systems and by 2-dimensional TLC on a microcrystalline cellulose plate. The time-course of the histidinoalanine formation in bovine serum albumin is shown in table 2. About 4 residues of histidinoalanine were formed in 1 molecule after heating at 110 °C for 96 h.

The results suggest that histidinoalanine cross-links are probably formed to some extent in food proteins during industrial processing and home cooking. The production of histidinoalanine uses up histidine, an essential amino acid for infants, and serine (or cysteine) and can reduce the digestibility of the food proteins. Thus, histidinoalanine cross-links together with other cross-links will cause the deterioration of food proteins. It has been reported that lysinoalanine induces renal lesions in rats[3]. By analogy, it may be possible that histidinoalanine is toxic, too. It is urgent to check the safety of this amino acid. Finally, the in vitro formation of histidinoalanine in proteins may serve as a model for the aging of proteins in vivo.

Table 2. Time-course of histidinoalanine formation in bovine serum albumin. The sample was heated in 0.1 M potassium phosphate, pH 7.4

Temperature (°C)	Time of heating (h)	Histidinoalanine formed (nmole/mg)
100	8	13.0
100	24	45.1
110	8	21.8
110	24	59.5
110	96	65.6
120	0.5	1.1
120	1	3.1
120	2	10.8
120	8	25.1

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Glucose phosphate isomerase heterophenotypes in the human filarial parasite *Brugia malayi* from peninsular Malaysia[1]

H.S. Yong and J.W. Mak

Department of Genetics and Cellular Biology, University of Malaya, Kuala Lumpur 22-11 (Malaysia), and Institute for Medical Research, Kuala Lumpur (Malaysia), 18 July 1983

Summary. Glucose phosphate isomerase of subperiodic *Brugia malayi* was studied by horizontal starch-gel electophoresis. Two heterophenotypes, each represented by 3 bands of enzyme activity, were found among 38 parasites studied. This finding is attributed to the occurrence of 2 *Gpi* gene loci.

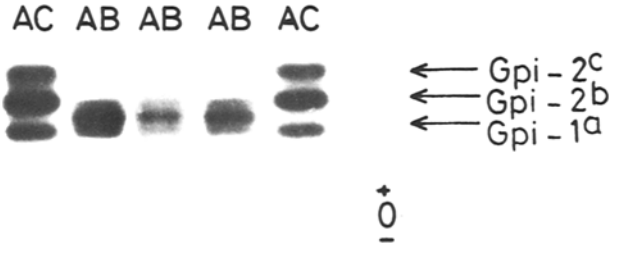
Human lymphatic filariasis affects about 250 million people in the world[2]. It is caused principally by the parasites *Brugia malayi* and *Wuchereria bancrofti*. These 2 parasites are endemic in Malaysia and other Southeast Asian countries. A 3rd species, *Brugia timori*, is confined to the islands of Timor, Rote, Alor and Flores in Indonesia.

In Malaysia, *Brugia malayi* occurs in 2 forms – nocturnally periodic and subperiodic. The periodic form is essentially a human parasite while the subperiodic form affects both humans and animals[3-5]. It has been postulated that zoonotic transmission of subperiodic *B. malayi* occurs from animals to humans and vice versa. Subperiodic *B. malayi* therefore poses unusual problems in the control strategies of the disease.

Because of its public health importance and its role in zoonotic transmission, we have undertaken to study the genetic profile of the subperiodic *B. malayi* in particular, and the other filarial parasites in general. We report here our finding of glucose phosphate isomerase (GPI, E.C.5.3.1.9) heterophenotypes in a subperiodic form of *B. malayi* from peninsular Malaysia.

Adult filarial worms, recovered from jirds in which the subperiodic *B. malayi* parasites were maintained, were washed with RPMI and stored in liquid nitrogen or a deep freezer until used for electrophoresis within 2 weeks. Individual worms were studied. This enables the present study to discriminate intraspecific genetic variation. Previous work has utilized as many as 15 adult worms per sample[7].

Of several gene-enzyme systems studied, the electrophoretic patterns of glucose phosphate isomerase are unique and noteworthy. Two electrophoretic phenotypes (GPI-AB and GPI-AC) are observed in the present material (figure). Each GPI electromorph is represented by 3 bands of enzyme activity, with the band of intermediate mobility being darker stained.



Electrophoretic phenotypes of glucose phosphate isomerase in a subperiodic form of *Brugia malayi* from peninsular Malaysia. Individual worms were electrophoresed as described by Yong et al.[10].

This kind of pattern is characteristic of a heterophenotype in which the enzyme involved is dimeric[8].

The simplest explanation of a heterophenotype is that it is governed by a heterozygous genotype. However, in the present material all the 38 parasitic worms studied are characterized by heterophenotypes (table). It indicates that 2 loci are involved. This is supported by the observation, under identical experimental conditions, of a single band of GPI enzyme activity, with distinctly slower mobility, in a related species *Brugia pahangi* which is an animal filarial parasite.

Assuming the presence of 2 *Gpi* loci, the present results indicate the occurrence of a single allele for the *Gpi-1* locus and 2 alleles for the *Gpi-2* locus. Further evidence for the presence of 2 *Gpi* loci in subperiodic *Brugia malayi* rests with the finding of the other expected heterophenotypes in future material.

The absence of the heterozygous *Gpi-2*^{b}/*Gpi-2*^{c} genotype could be due to the fact that the worms used in the present study were recovered from experimental animal hosts. As the parasites have been maintained in laboratory hosts, through passaging from one host to another, for about 20 years, it is reasonable to suggest that homozygosity within a single host could have been established. Experiments will be carried out in due course to infect a single jird with parasites of both GPI-phenotypes in the hope of recovering the heterozygous phenotype.

The GPI electrophoretic phenotypes in 3 species of cattle filarial parasites (*Onchocerca* spp.) have also been reported to possess multiple bands of enzyme activity[9]. The author, however, attributed the finding as 'probably due to oxidation during processing'.

Distribution of glucose phosphate isomerase (GPI) phenotypes in a subperiodic form of *Brugia malayi* from peninsular Malaysia

Sex of worm	GPI-AB	GPI-AC
Male	4	8
Female	13	13
Total	17	21

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Inhibition of pig kidney dopa decarboxylase by coenzyme-5-hydroxytryptophan adducts[1]

P. Dominici, M. Curini, A. Minelli and C. Borri Voltattorni

Institutes of Biological Chemistry and Organic Chemistry, Faculty of Pharmacy, University of Perugia, I-06100 Perugia (Italy), 14 October 1983

Summary. The effect of N-(5'-phosphopyridoxyl)-L-5-hydroxytryptophan, N-(5'-phosphopyridoxyl)-D-5-hydroxytryptophan and N-(5'-phosphopyridoxyl)-5-hydroxytryptamine on the reactivation of apoDopa decarboxylase to holoenzyme has been investigated. The different degree of inhibition exerted by these adducts has been interpreted on the basis of a different orientation of the 2 isomers of 5-HTP at the active of Dopa decarboxylase.

Phosphopyridoxyl-amino acids (PPxy-amino acids), products of reduction of Schiff bases formed between pyridoxal-P and amino acids, are adducts which combine both substrate and coenzyme into a single molecule and are structurally similar to the covalent intermediates in the reaction pathway of pyridoxal-P dependent enzymes. Their inhibitory effect on many pyridoxal-P dependent enzymes has been used to investigate aspects of the mechanism of catalysis[2-8].

Similar coenzyme-amino acids adducts behave as inhibitors of the recombination of apoDopa decarboxylase with pyridoxal-P[9]; moreover, kinetic studies on the nature of the inhibition caused by these adducts led to the assumption that in addition to a high-affinity coenzyme-binding active site, Dopa decarboxylase has at least 1 additional low-affinity pyridoxal-P binding site[10]. The adducts employed in these studies are derivatives of aromatic amino acids with a catechol-related structure.

Since 5-hydroxytryprophan (5-HTP) and its analogues also bind to the active site of the enzyme[11-13], we investigated the interaction of N-(5'-phosphopyridoxyl)-L-5-hydroxytryptophan (PPxy-L-5HTP), N-(5'-phosphopyridoxyl)-D-5-hydroxytryptophan (PPxy-D-5HTP) and N-(5'-phosphopyridoxyl)-5-hydroxytryptamine (PPxy-HT) with Dopa decarboxylase from pig kidney. The results allow the evaluation of the relative importance of the α-carboxylate group for the binding of the 2 enantiomers of 5-HTP to Dopa decarboxylase and reinforce the concept that these may orientate differently at the active site of the enzyme, as previously suggested[14].

Materials and methods. PPXy-L-5HTP, PPxy-D-5HTP and PPxy-HT were synthesized following the general procedure of Ikawa[15]. Dopa decarboxylase (EC 4.1.1.28) from pig kidney was highly purified according to Borri Voltattorni et al.[16]. Two procedures were used to prepare apoenzyme. The first was by treatment with hydroxylamine, as previously described[17]. The second was as follows: holoenzyme was inactivated by incubating D-5HTP in 0.1 M Hepes pH 8.4 for 2 h at 37 °C and then dialyzed against 0.1 M potassium phosphate, pH 6.8. The resulting enzyme preparation has a residual activity ranging