We have recently isolated the ^{14}C-folate-tagged brush borders from rat intestinal mucosal cells. After solubilization of these borders with 0.2% sodium dodecyl sulphate and fractionation on Sephadex G 150, a dingle symmetrical protein peak appeared, to which labelled folate was found to be bound[19]. The characteristics of the saturation binding and other properties of this protein indicate that it is a high affinity soluble folate binder similar to the one reported in milk[2], intestine[6], rat kidney[5] and serum[1].

It is suggested that FABP might function in the storage of excess dietary folate as it has an avid affinity for polyglutamyl folates; FABP might possibly control the level of folate absorption by sequestering the polyglutamyl folates from the action of folyl conjugase and releasing them as and when necessary. Alternatively, these bound polyglutamyl folates, having higher cofactor activity than the monoglutamyl counterparts, may have a physiological function regulating intestinal mucosal cell activity.

Monoglutamyl folyl derivatives which as such are more suitable for intestinal absorption have less affinity for the FABP, again suggesting that the FABP activity is not related to the absorption process.

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Formation of histidinoalanine cross-links in heated proteins

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Summary. Formation of the cross-linking amino acid, histidinoalanine, was demonstrated in various proteins when they were heated. This cross-link formation may be partly responsible for the deterioration of food proteins resulting from heat treatment.

It is known that heat treatment of proteins impairs their nutritional values. One of the significant heat-induced changes in proteins is the formation of cross-links between polypeptide chains of proteins[1]. The presence of cross-links reduces the digestibility of the proteins and renders some constituent amino acids biologically unavailable. So far, the formation of isopeptide cross-links[2] and of lysinoalanine cross-links[3] have been demonstrated.

Histidinoalanine or N^{τ}-(2-amino-2-carboxyethyl)-histidine is a bifunctional cross-linking amino acid. It was first found in human dentin collagen[4] and subsequently found in proteins of various human connective tissues[5]. Its content was found to increase with age[5,6]. It has been proposed that histidinoalanine crosslinks might be related to the aging of human connective tissues[5,6].

This paper reports the formation of histidinoalanine in various proteins when they were heated at neutral pH. This cross-link, like those mentioned above, may be responsible for the deterioration of food proteins on heat treatment.

Materials and methods. Bovine serum albumin, ovalbumin and porcine pepsin were obtained from Sigma. Bovine tendon collagen was obtained from the Millipore Corporation. Milk casein was obtained from Merck. Human gamma globulin was obtained from Calbiochem.

The protein sample (10 mg) was dissolved or suspended in 1 ml of potassium phosphate buffer, 0.1 M, pH 6.8 or 7.4, in a sealed tube and heated. After the heat treatment, 1 ml of concentrated HCl was added and acid hydrolysis was performed at 110 °C for 24 h. Cross-linking amino acids were determined as described in the previous paper[6].

Results and discussion. As shown in table 1, histidinoalanine was formed in various proteins when they were heated at

Table 1. Formation of histidinoalanine and lysinoalanine in various proteins on heating. The protein sample (10 mg) was heated at 110 °C for 24 h in 0.1 M potassium phosphate buffer, pH 6.8. These cross-linking amino acids were not detected in unheated controls

Protein	Histidinoalanine formed (nmole/mg)	Lysinoalanine formed (nmole/mg)
Bovine serum albumin	39.0	6.4
Casein	15.3	2.6
Ovalbumin	8.3	2.0
Human gamma globulin	17.7	3.3
Porcine pepsin	7.0	0.1
Bovine tendon collagen	0.7	0.1

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]

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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.

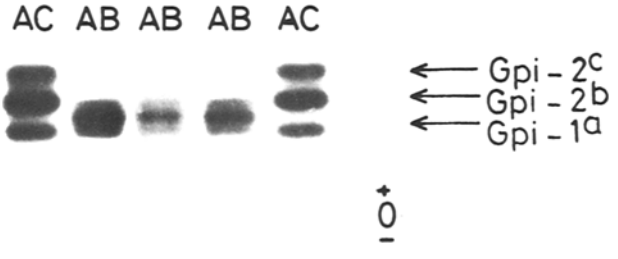
Human lymphatic filariasis affects about 250 million people in the world[2]. It is caused principally by the parasites *Brugia malayi* and *Wuchereria bancrofti*. These 2 parasites are endemic in Malaysia and other Southeast Asian countries. A 3rd species, *Brugia timori*, is confined to the islands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