Effect of diet on the occurrence of S-methylcysteine and the free amino acid pattern in insect blood

The remarkably high concentration of free amino acids found in the blood or haemolymph of various insects constitutes a biochemical characteristic of the Class[1-3]. Although this fact has been known for over 30 years[4], neither the origin nor the significance of this high titer has yet been explained. During the course of studies on the free amino acids of various biological fluids and tissue extracts we have re-examined the larval haemolymph of the Southern Armyworm, *Prodenia eridania*. In thus extending the earlier qualitative data of PRATT[5] we have demonstrated for the first time the occurrence of free S-methylcysteine in animal as opposed to plant[6] tissue. The experiments here reported also show that the occurrence of this amino acid is dependent upon the presence of a precursor in the diet.

Pooled haemolymph from 30–40 fully grown larvae raised entirely on kale (*Brassica oleracea*) was deproteinized with absolute ethanol to a final concentration of 80% (v/v). The precipitate obtained after centrifugation was washed in the centrifuge with 80% ethanol, and the combined supernatant and washings desalted by passage through a 1 × 4 cm column of Dowex 50, 50–100 mesh, in the H^+ form. The column was washed with deionized water until the pH of the effluent attained neutrality, following which the amino acids were eluted with 9 *N* NH_3. The excess NH_3 was removed *in vacuo* at 25° and the extract lyophilized. The residue was dissolved in a known volume of water, and suitable aliquots taken for determination of amino acid nitrogen[7] and for two-dimensional paper chromatography[8]. After spraying with ninhydrin an approximate quantitation of the amino acids was obtained by scanning each "spot" with a densitometer, employing the "maximum density" method[10]. The sum of the densities of the individual amino acid "spots" was equated to the total amino nitrogen applied to the paper, and the amount of each amino acid was then expressed as per cent of the total. Proline, which gives an atyptical ninhydrin color, was estimated by visual comparison with the most closely approximating ninhydrin "spot" of similar size.

A ninhydrin-reactive "spot" whose position on the chromatograms corresponded to that of free S-methylcysteine was conspicuous in the haemolymph of *P. eridania* larvae raised on kale. The material was characterized by co-chromatography with the known compound in two different solvents, and by its reaction with platinic iodide[10].

In order to determine whether the S-methylcysteine was formed *de novo* or was derived from the diet, 80%-ethanol extracts of kale were examined by procedures similar to those employed for the insect haemolymph. In addition, a batch of *P. eridania* was raised first on kale until about the second instar, by which stage the larvae were large enough to be transferred to, and to continue their development on potato slices. Haemolymph from these potato-fed larvae, and 80%-ethanol extracts of the potatoes were also exmined[11]. The data are presented in Table I.

The kale extracts did not contain any trace of S-methylcysteine, although its oxidation product S-methylcysteine sulfoxide[12,13] was present in high concentration. Since under our experimental conditions the sulfoxide was not artifically reduced to S-methylcysteine, it appears likely that the reduction is performed enzymically by the insect. Neither of the above S-containing amino acids was found in either the

TABLE I

FREE AMINO ACIDS IN *P. eridania* HAEMOLYMPH AND EXTRACTS OF FOOD PLANTS

A (—) indicates insufficient material to be detected on the chromatogram. Values in per cent of total.

Amino acid	P. eridania *raised on kale 1.45 mg amino N/ml haemolymph*	*Kale extract 0.891 mg amino N/g kale*	P. eridania *raised on potato 1.45 mg amino N/ml haemolymph*	*Potato extract 0.015 mg amino N/g potato*
Cystine and/or peptides	1.0	2.2	1.1	0.4
Histidine	8.7	0.4	5.7	1.8
Asparagine	0.7	2.1	2.5	4.4
Ornithine?	3.9	—	2.5	—
"Before lysine" unknown	—	1.0	—	—
Lysine	15.2	3.9	9.0	6.5
Arginine	0.8	2.8	3.2	7.2
"Below arginine" unknown	—	5.6	—	—
Aspartic acid	Trace	2.4	1.9	8.5
Glutamic acid	1.8	12.9	4.1	8.5
Glycine	6.7	0.9	3.7	1.4
S-methyl cysteine	2.3	—	—	—
S-methyl cysteine sulfoxide	1.4	12.5	—	—
Glutamine	8.1	15.1	12.8	16.6
Serine	11.1	7.3	12.8	4.1
Threonine	6.6	2.8	4.2	2.4
β-alanine	2.1	0.9	2.9	0.6
γ-aminobutyric acid	1.0	3.4	7.1	15.7
Alanine	4.6	5.4	6.5	1.8
Ethanolamine	—	3.7	—	0.9
Proline	10.0	4.0	6.4	1.8
α-aminobutyric acid	0.4	—	0.4	0.4
Valine	4.6	6.5	5.3	7.4
Methionine	—	—	1.9	1.8
Isoleucine	2.0	2.6	1.9	2.2
Leucine	2.4	0.5	1.3	1.4
Phenylalanine	1.3	0.3	1.1	1.3
Tryptophan	—	—	—	0.3
Tyrosine	2.6	—	1.9	1.4
Pipecolic acid	Trace	0.3	Trace	1.1

potato extracts or in the haemolymph of *P. eridania* raised on potatoes. We conclude, therefore, that the insect S-methylcysteine is derived from the corresponding sulfoxide contained in the diet.

Inspection of the Table reveals that although the proportions of individual free amino acids in the two types of haemolymph or in the two plant extracts may differ more or less widely, nevertheless a certain regularity exists. Thus by comparing the haemolymph amino acids with those of the corresponding diet on which the insects were raised, it appears that the insects can selectively accumulate particular amino acids (*e.g.* histidine, lysine, serine, proline, etc.) irrespective of their actual concentration in the diet. Conversely, the relative proportions of other haemolymph amino acids (*e.g.* asparagine, aspartic acid, arginine, etc.) are to a varying degree lower than that of the diet. This applies especially to those amino acids which are not normally constituents of proteins, viz. γ-aminobutyric acid, pipecolic acid, ethanolamine and the two unknown ninhydrin-positive "spots" from kale. This suggests that these latter amino acids are either metabolized and/or excreted or, in the extreme case where they are actually absent from the haemolymph, not absorbed. Their presence

or absence as compared with the diet is thus an indication of the extent to which the insect controls its precise pattern of haemolymph-free amino acids.

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A simplified procedure for the preparation of phosvitin and vitellin*

The two major phosphoproteins of egg yolk are phosvitin and vitellin, the latter being in the form of a lipoprotein complex[1]. MECHAM AND OLCOTT[2] described a procedure for the isolation of phosvitin, containing 10 % phosphorus and accounting for nearly 70 % of total yolk phosphorus. The lipovitellin, first isolated by ALDERTON AND FEVOLD[3], was subjected to a detailed electrophoretic and ultracentrifugal study[4,5] which revealed that this protein is a mixture of several components, involving the total phosvitin of egg yolk and the lipid-bound vitellin. The lipovitellin fraction of ALDERTON AND FEVOLD[3] thus promised to be good starting material for the preparation of both phosvitin and vitellin.

Butanol, which is being widely used to liberate the proteins bound to lipids[6], was employed in the present investigation to disrupt the lipid–protein complex of lipovitellin. This procedure, while rendering vitellin insoluble, incidentally released all the phosvitin into solution from which it could be recovered by isoelectric precipitation. The method adopted was as follows:

Yolks from 50 eggs were freed of adhering white and chalazae, and diluted with two volumes of distilled water. The emulsion was passed through a Sharples centrifuge[3] and the residue was taken up in 500 ml 10 % NaCl. Lipovitellin was precipitated by diluting the salt solution with 7–8 l water. The precipitate was collected by centrifuging, and dissolved in 500 ml 10 % NaCl. The saline extract was treated with 250 ml *n*-butanol and the solution was stirred at room temperature for 1 h after which it was kept in the cold for 24 h. After centrifugation, the top

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