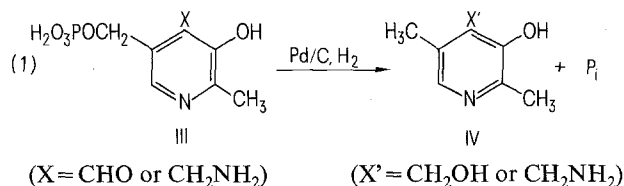


production of 5'-deoxypyridoxal derivatives, using as starting materials the readily available corresponding phosphorylated compounds. The method involves, hydrogenolysis using 10% palladium on charcoal as catalyst. The 5'-phosphate ester group in **III** becomes reduced to the methyl group as shown in eq. 1, accompanied by the release of inorganic phosphate.



Proof of structures was made by comparison with authentic samples of the products obtained after catalytic hydrogenolysis. In addition to the experiments described below, we also have been able to obtain by this method 5'-deoxypyridoxyl-amino acids from the corresponding 5'-phosphopyridoxyl derivatives.

Experimental. 5'-Deoxypyridoxamine (**IV**, $\text{X}' = \text{CH}_2\text{NH}_2$) dihydrochloride. Pyridoxamine phosphate monohydrochloride monohydrate (250 mg, 0.88 mmoles) was dissolved in 7 ml of water containing 150 mg of 10% palladium on charcoal. Hydrogen gas was continually passed through the well-stirred reaction mixture for 24 h at atmospheric pressure. The catalyst was filtered and the filtrate applied to a 1.8×20 cm column of Dowex 50×8 (50–100 mesh) in the hydrogen form. The column was washed with 500 ml of water, followed by 500 ml of 1 N HCl. The product was then eluted with 3.6 N HCl until the absorbancy at 295 nm dropped below 0.10; this required approximately 1.4 l. The solvent was removed on a rotary evaporator and the residual white solid was evaporated from ethanol and then ether. After drying to constant weight in a vacuum desiccator, the yield of 5'-deoxypyridoxamine $\cdot 2$ HCl was found to be 177 mg (90%). This product was identical to an authentic sample⁸ with respect to its NMR-, UV-, and mass spectra. On TLC, it migrated as a single spot having an R_f identical to that of an authentic marker sample in 2 different solvent systems.

5'-Deoxypyridoxine (**IV**, $\text{X}' = \text{CH}_2\text{OH}$) hydrochloride. Pyridoxal phosphate monohydrate (250 mg, 0.94 mmoles) was reduced to 5'-deoxypyridoxine as described above for the hydrogenolysis of pyridoxamine phosphate. After the cata-

lyst was filtered, the pH of the filtrate was adjusted to approximately 6.5 with dilute KOH solution. The solvent was removed on a rotary evaporator and the residue was exhaustively extracted with hot ethanol. Evaporation of the ethanol left a white solid that was converted to 5'-deoxypyridoxine \cdot HCl by evaporation from dilute HCl; the yield was 147 mg (82%). After recrystallization from ethanol/ether, the compound was shown to be identical to an authentic sample²³ of 5'-deoxypyridoxine \cdot HCl by UV-, IR-, NMR-, and mass spectra, as well as by melting point and TLC. This procedure works equally well using glacial acetic acid as the solvent instead of water. Reoxidation of the 4-hydroxymethyl group to a 4-carboxaldehyde group can be readily accomplished using published procedures^{19,23}.

- 1 Supported by PHS Grant No. 5429-16-9.
- 2 To whom inquiries may be addressed.
- 3 T.C. Bruice and R.M. Topping, J. Am. chem. Soc. 85, 1493 (1963).
- 4 T.C. French, D.S. Auld and T.C. Bruice, Biochemistry 4, 77 (1965).
- 5 J.W. Thanassi, A.R. Butler and T.C. Bruice, Biochemistry 4, 1463 (1965).
- 6 D.S. Auld and T.C. Bruice, J. Am. chem. Soc. 89, 2098 (1967).
- 7 J.W. Thanassi, Biochemistry 9, 525 (1970).
- 8 J.W. Thanassi, Biochemistry 11, 2909 (1972).
- 9 J.W. Thanassi, Biochemistry 12, 5109 (1973).
- 10 M. Blum, W.C. Cunningham and J.W. Thanassi, Bioorg. Chem. 5, 415 (1976).
- 11 M. Blum and J.W. Thanassi, Bioorg. Chem. 6, 31 (1977).
- 12 D. Heyl, E. Luz, S.A. Harris and K. Folkers, J. Am. chem. Soc. 73, 3430 (1951).
- 13 J.C. Rabinowitz and E.E. Snell, Archs Biochem. Biophys. 43, 408 (1953).
- 14 L.J. Arcement, W. Korytnyk and W.B. Dempsey, Bact. Proc. 68, 121 (1968).
- 15 W.A. Newton, Y. Morino and E.E. Snell, J. biol. Chem. 240, 1211 (1965).
- 16 J.E. Ayling and E.E. Snell, Biochemistry 7, 1626 (1968).
- 17 D. Heyl, S.A. Harris and K. Folkers, J. Am. chem. Soc. 75, 653 (1953).
- 18 T. Kuroda, Vitamin 29, 116 (1964); Chem. Abstr. 62, 515 (1965).
- 19 C. Iwata, Biochem. Prep. 12, 117 (1965).
- 20 D. Heinert and A.E. Martell, Tetrahedron 3, 49 (1958).
- 21 D. Heinert and A.E. Martell, J. Am. chem. Soc. 81, 3933 (1959).
- 22 M.H. O'Leary and J.R. Payne, J. med. Chem. 14, 773 (1971).
- 23 P.F. Mühlradt and E.E. Snell, J. med. Chem. 10, 129 (1966).

Free amino acids in the adult citrus brown mite, *Eutetranychus orientalis* (Klein)

I.Z. Bector and A.H. Rasmy

Laboratory of Plant Protection, National Research Centre, Dokki, Cairo (Egypt), 14 July 1978

Summary. Amino acids contained in extracts of adult *Eutetranychus orientalis* were separated and determined quantitatively by 2-dimensional paper chromatography. 14 amino acids were identified. Asparagine, ornithine, histidine, lysine, aspartic acid, serine and glycine were the major components of the free amino acid pool, comprising 83.94% of the total content.

Free amino acids in insect haemolymph and tissue have been frequently studied and reviewed¹⁻⁹. Studies on the free amino acids in mites have not received considerable attention. The aim of the present study was to determine the concentration of the free amino acids in female adult citrus brown mite, *Eutetranychus orientalis* (Klein), which is considered as a noxious pest on citrus trees in Egypt^{10,11}.

Materials and methods. Female adults of the citrus brown mite, *E. orientalis*, were collected from a culture maintained on 1-year-old seedlings of sour orange. The procedure of Pant and Agrawal¹² was used for the preparation of amino acid extracts from adult citrus brown mite (about 500 mites). Free amino acids in the tissue extract were separated and determined quantitatively by 2-dimensional paper

chromatography according to the method detailed in the preceding paper by Boctor⁸.

Results and discussion. The data available on qualitative and quantitative estimation of free amino acids in mites are still very scarce. Rodriguez and Hampton¹³ determined the essential amino acids in young adult *Tetranychus urticae* females. 18 protein amino acids and 3 nonprotein amino acids were detected. In the present investigation, analysis of whole body extract of adult mite by 2-dimensional paper chromatography revealed the presence of 12 amino acids and 2 amides. Asparagine, ornithine, histidine, aspartic acid, serine, lysine and glycine are the major components of the free amino acid pool, comprising 83.94% of the total amino acid content. As shown in the table, asparagine and glutamine generally predominate in insect haemolymph and certain tissues^{1,8,9,14}. The second most concentrated amino acid in mite extract is histidine. In most insects, histidine was also found at a high level^{1,8,9}.

Our results also show that glutamine, glutamic acid and cystine occurred in relatively smaller amounts. The concentrations of alanine, threonine and citrulline were almost the

same and were found in the smallest quantity. Proline was detected on the chromatogram but because of its low concentration, could not be measured quantitatively. The trace of proline in mite tissue contrasts with relatively high concentrations in insects, probably owing to its special role in insect flight metabolism¹⁵⁻¹⁷, and the difference between muscle energetics of walking mites and flying insects. Tyrosine was not detected in mite tissues. In insect haemolymph, tyrosine occurred in substantial amounts owing to its role in insect cuticle sclerotization^{18,19}.

Free amino acids of female *E. orientalis* adults

Amino acids	µmoles/100 g tissues	Percent of total content
Glycine	1065.944	10.94
Alanine	10.551	0.11
Serine	850.699	8.73
Threonine	15.782	0.16
Aspartic acid	742.449	7.62
Glutamic acid	415.754	4.27
Glutamine	515.223	5.29
Lysine	740.406	7.60
Histidine	1395.144	14.32
Citrulline	18.836	0.19
Ornithine	1353.060	13.89
Cystine	587.500	6.03
Asparagine	2029.970	20.84
Proline	Trace	Trace
Tyrosine	-	-
Totals	9741.318	99.99

- 1 M. Florkin, in: *Biochemistry of Insects*, p.63. Ed. L. Levenbook. Pergamon Press, London 1959.
- 2 D. Gilmour, *The Biochemistry of Insects*. Academic Press, New York 1961.
- 3 D. Gilmour, *The Metabolism of Insects*. Oliver and Boyd, London 1965.
- 4 G.R. Wyatt, *A. Rev. Ent.* 6, 75 (1961).
- 5 P.S. Chen, in: *Amino Acid Pools*, p.115. Ed. J.T. Holden. Elsevier, Amsterdam 1962.
- 6 P.S. Chen, in: *Advances in Insect Physiology*, vol.3, p.53. Ed. J.W.L. Beament, J.E. Treherne and V.B. Wigglesworth. Academic Press, New York 1966.
- 7 L. Levenbook and M.L. Dinamarca, *J. Insect Physiol.* 12, 1343 (1966).
- 8 I.Z. Boctor and S.I. Salem, *Comp. Biochem. Physiol.* 45B, 785 (1973).
- 9 I.Z. Boctor, *Zool. Jb. Physiol.* 79, 480 (1975).
- 10 A.H. Rasmy, *Can. Ent.* 101 (18), 1078 (1969).
- 11 A.H. Rasmy, *Acarologia* 19 (2), 222 (1977).
- 12 R. Pant and H.C. Agrawal, *J. Insect Physiol.* 10, 443 (1964).
- 13 J.G. Rodriguez and R.E. Hampton, *J. Insect Physiol.* 12, 1209 (1966).
- 14 L. Levenbook, *J. Insect Physiol.* 8, 559 (1962).
- 15 E. Bursell, *J. Insect Physiol.* 9, 439 (1963).
- 16 E. Kirsten, R. Kirsten and P. Arese, *Biochem. Z.* 337, 167 (1963).
- 17 B. Sacktor, in: *The Physiology of Insecta*, vol.2, p.483. Ed. M. Rockstein. Academic Press, New York 1965.
- 18 P.C.J. Brunet, *Ann. N.Y. Acad. Sci.* 100, 1020 (1963).
- 19 R.H. Hackman, in: *The Physiology of Insecta*, vol.3, p.471. Ed. M. Rockstein. Academic Press, New York 1964.

Alterations of β -adrenoceptor-density and cAMP-synthesis in rat-erythrocytes after stress erythropoiesis¹

G. Kaiser, J. Dietz, G. Wiemer and D. Palm

Department of Pharmacology, University of Frankfurt, Theodor-Stern-Kai 7, D-6000 Frankfurt a. M. 70 (Federal Republic of Germany), 20 July 1978

Summary. During the maturation of red blood cells from rats after stress erythropoiesis, adenylyl cyclase activity and β -adrenoceptor density (pmoles/mg protein) decrease at distinctly different rates suggesting a different turnover of these membrane units.

Previous investigations^{2,3} have shown that mature erythrocytes from rats contain very low adenylyl cyclase activity which can be stimulated specifically by isoprenaline (Ipn). In contrast, by means of ligand binding, e.g. with (-) (³H) dihydroalprenolol (DHAP), a relatively high density of β -adrenoceptors has been demonstrated in membrane preparations from mature red blood cells⁴. When stress erythropoiesis was induced in rats by treatment with acetylphenylhydrazide (APH), a pronounced increase of Ipn-stimulated adenylyl cyclase activity (about 100-fold) and also an increase of density of β -adrenoceptor sites (about 4-6-fold) occurred. These increments were linearly correlated to the respective reticulocyte counts, the latter depending on

the dose of APH used⁴. These results are in accordance with the hypothesis that in the cytoplasmic membrane, the enzyme adenylyl cyclase and the β -adrenoceptor are different entities⁵⁻⁷ which may have independent and different turnover rates during the maturation process of the red blood cell⁸.

It seemed of interest, therefore, to investigate the increase and decrease of the Ipn-stimulated adenylyl cyclase activity and the density of β -adrenoceptor sites during the time course of the reticulocyte crisis produced by treatment of the animals with a fixed dose of APH.

Methods and materials. Male Wistar rats weighing 150-200 g were injected with 40 mg/kg acetyl-phenylhydrazide