this derivative, which results from the cleavage of the C-7 and C-8 bond of the thiazole, showed that the methyl group contained 8.8% ^{2}H. Since the molecule contains only 1 oxygen atom and since previous labeling have shown that ^{2}H on C-3 of glycerol is incorporated only at C-6 and C-8 of the thiazole, then the distribution of label on C-8 of the thiazole can be calculated. This calculation shows that 4.1% of the C-8 carbons of the biosynthesized thiazole had 1 ^{2}H and no ^{18}O and 7.5% had both 1 ^{2}H and 1 ^{18}O. These results clearly indicate that the oxygen and both hydrogens of C-3 of glycerol are incorporated into the thiazole as a complete unit. The dilution of the label incorporated into the thiazole can be accounted for by the synthesis of nonlabeled precursor triose-P in the cell from the amino acids. In addition, the increase in the number of molecules containing only 1 deuterium can be accounted for by the resynthesis of the labeled precursor triose-P after passing through phosphoenolpyruvate which would lose the ^{18}O but retain the deuterium. This resynthesis was confirmed by the analysis of the isotope incorporation into the cellular glycerol-P which showed 5.1% with 1 ^{2}H and 11.7% with both 1 ^{2}H and 1 ^{18}O. From the change in the ratio of molecules containing 1 ^{2}H, and 1 ^{2}H and 1 ^{18}O between the fed glycerol and the isolated glycerol-P, we find that ~ 14% of the glycerol-P present in the cell was resynthesized through phosphoenolpyruvate. However, since the thiazole C-8 has a different isotopic distribution than the cellular glycerol-P this strongly suggests that the true precursor triose-P of the thiazole is not glycerol-P but a metabolically closely related triose-P i.e. glyceraldehyde-P[1].

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Free amino acids of the haemolymph of the cotton leaf-worm, *Spodoptera littoralis* Boisduval full-grown larvae, infected with nuclear-polyhedrosis virus

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Summary. Free amino acids in the haemolymph of *Spodoptera littoralis* full-grown larvae infected with a nuclear-polyhedrosis virus were compared with those in the haemolymph of normal insects. Amino acids were separated by 2-dimensional paper chromatography and quantified colorimetrically. Most of the amino acids in the haemolymph of diseased full-grown larvae decreased markedly in concentration but proline, lysine, aspartic acid and histidine occurred in greater concentration in the haemolymph of diseased full-grown larvae than in the haemolymph of healthy insects.

Nucleopolyhedrosis is characterized by formation of polyhedral shaped inclusion bodies within the nuclei of susceptible cells. During the course of nuclear-polyhedrosis, large amounts of protein are synthesized for the formation of the virus particles and inclusion bodies. The host organism, therefore, will be affected in its protein and amino acid metabolism. Hypoaminoacidemia and hypoproteinemia during polyhedrosis were reported[1-6]. Hyperaminoacidemia generally occurred prior to inclusion body formation, whereas hypoaminoacidemia occurred after inclusion body formation.

The present investigation was undertaken to see if quantitative changes of the free amino acids in the haemolymph take place in full-grown larvae of the cotton leaf-worm *Spodoptera* during the course of a nuclear-polyhedrosis.

Materials and methods. Spodoptera littoralis was reared on castor-oil leaves in the laboratory according to El-Ibrashy and Chenouda[7]. Haemolymph of healthy and virus-infected full grown larvae (1 day before the prepupal stage i.e. wandering larvae) was obtained by cutting 1 or 2 of the abdominal legs. Haemolymph wells up at the wound site and was collected by microcapillary tubes. The pooled haemolymph (0.5–1 ml) of healthy and virus-infected full grown larvae was kept immersed in a freezing mixture to prevent clotting and darkening. The procedure of Pant and Agrawal[8] was used for the preparation of amino acid extracts from the haemolymph. Free amino acids in the haemolymph extracts were separated and determined quantitatively by 2-dimensional paper chromatography according to the method detailed in the earlier paper by Boctor and Salem[9]. For each sample 5–7 chromatographic separations were carried out, and the average and experimental error were calculated (table).

Results and discussion. The concentrations of free amino acids in the haemolymph of healthy and diseased full-grown larvae are given in the table. The amount of most of the amino acids in the haemolymph of diseased full-grown larvae decreased markedly, but the proline, lysine, aspartic

Free amino acids of the haemolymph of healthy and diseased full grown larvae of *S. littoralis*

Amino acids	Healthy full grown larvae	Diseased full grown larvae
Glycine	430.4 ± 32.9	289.7 ± 18.7
Alanine	82.2 ± 4.6	86.0 ± 3.3
Serine	465.0 ± 26.3	239.5 ± 9.4
Threonine	635.1 ± 37.1	138.0 ± 6.3
Valine	343.7 ± 19.2	167.9 ± 10.8
Leucine	179.9 ± 4.5	107.6 ± 5.9
Aspartic acid	38.2 ± 1.5	48.2 ± 1.4
Asparagine	1800.3 ± 82.8	1323.3 ± 68.5
Glutamic acid	30.1 ± 2.0	3.7 ± 0.3
Glutamine	860.9 ± 25.3	587.3 ± 23.2
Proline	242.3 ± 12.1	411.9 ± 14.6
Lysine	68.7 ± 2.2	95.8 ± 3.4
Arginine	55.0 ± 2.7	40.2 ± 2.9
Histidine	380.6 ± 18.4	438.2 ± 22.5
Tyrosine	221.8 ± 11.2	117.4 ± 6.6
Citrulline	49.9 ± 1.8	52.3 ± 4.0
Ornithine	1035.5 ± 51.3	157.0 ± 9.8
Cystine	358.1 ± 14.6	301.0 ± 16.7
γ-Amino butyric acid	87.4 ± 2.4	57.6 ± 4.1
Methionine	231.7 ± 12.1	65.0 ± 3.6
Phenylalanine	229.2 ± 13.9	60.4 ± 3.7
Totals	7826.0	4788.0

The values are given as µmoles amino acids/100 ml of haemolymph.

acid and histidine contents of the haemolymph of diseased full grown larvae were higher than those in the normal haemolymph. Those amino acids which were extremely reduced in amount by the disease were leucine, tyrosine, serine, valine, methionine, phenylalanine, threonine, ornithine and glutamic acid. These amino acids decreased by about 40, 47, 48, 51, 71, 73, 78, 84 and 87% respectively. Alanine and citrulline stayed at relatively constant levels in the haemolymph of healthy and diseased full-grown larvae. The total amount of free amino acids in the haemolymph declined from a high level of 7826 µmoles/100 ml of haemolymph in healthy full grown larvae to a low level of 4788 µmoles/100 ml of haemolymph in diseased larvae.

The results of the present investigation agree with the findings of several investigators that a marked decrease of most amino acids in the haemolymph is observed during nucleopolyhedrosis[5,6] and cytoplasmic polyhedrosis[4]. This decrease of most of the free amino acids in the haemolymph of infected full-grown larvae of *Spodoptera* could indicate that free amino acids are being used to make viral and/or inclusion body protein. These are, of course, only indications; injection of labeled amino acids and determination of the incorporation rate of these amino acids in the virus particles and inclusion bodies could prove this hypothesis.

In the present study the concentrations of lysine and histidine were higher in diseased full grown larvae than in healthy ones. Also, histidine[1,10] and lysine[4,10] accumulated during virosis. The present data differ from those obtained for *Heliothis zea* larvae[6] and for *Peridroma saucia*[5] which showed an increase of glycine during polyhedrosis.

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Occurrence of unconjugated pterins in a higher plant, *Stizolobium hassjoo*

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Summary. 3 unconjugated pterins, D-erythro-neopterin, 6-hydroxymethylpterin and isoxanthopterin, were isolated from the pericarps of a leguminous plant, *Stizolobium hassjoo*.

A pyrazine derivative, stizolamine (1-methyl-3-guanidino-6-hydroxymethylpyrazin-2-one), was isolated from *S. hassjoo* (Leguminosae)[1,2]. The compound is widely distributed in leguminous seeds[3] and is biosynthesized from U-^{14}C-GTP[4]. The structural resemblance of stizolamine to pterins, and the commonness of the precursor, led to the inference that stizolamine might originate from a pterin(s) in the plant[4]. As a 1st approach to check the theory we have surveyed unconjugated pterins in the plant and found them in an extract from pericarps of the plant. The present paper describes isolation and characterization of 3 major pterins in the extract.

The pericarps of *S. hassjoo* (8 weeks old) were powdered in liquid nitrogen. The powder (50 g) was put into 10 vol of 5 mM 2-mercaptoethanol at 80 °C and kept at 60 °C for 1 h with occasional stirring. The suspension was then squeezed through a silk cloth and centrifuged at 6000 × g for 10 min. The precipitate was re-extracted as above. The combined supernatant was concentrated to about 50 ml at 40 °C under reduced pressure. After centrifugation, the concentrate was applied to a Dowex 1 X8 column (formate, 3 × 30 cm). On elution with distilled water, the blue fluorescent band was resolved into 3 fluorescent zones. The 1st and 2nd blue fluorescent zones were eluted with distilled water (fractions I and II) and the 3rd purple fluorescent zone with 2 N formic acid (fraction III). The main pterin in each fraction was purified by column chromatography and/or TLC. In all cases, the columns were developed with distilled water. The concentrated fraction I was applied to a QAE-Sephadex A-25 column (formate, 3 × 18 cm). The blue fluorescent eluate was concentrated and subjected to TLC (Avicel SF. ethanol/acetic acid/H_2O, 15:1:34, v/v). The band corresponding to D-erythro-neopterin (R_f, 0.49) was eluted with 5 mM 2-mercaptoethanol, concentrated and applied to a Sephadex G-25 column (3.5 × 43 cm). On evaporation to dryness, the eluate gave a pale yellow powder (2.7 mg). The material (compound 1) was converted to 6-carboxypterin by alkaline permanganate oxidation. Compound 1 was identified as D-*erythro*-neopterin by TLC (table) and spectral analyses: UV. λ_{max}, 236, 274, 344 nm (H_2O); fluorescence: excited, 284, 362 nm, emission, 440 nm (H_2O); GC-MS (TMS): M^+, m/e, 613 (pentakis-TMS-neopterin, 8%). The configuration of the side chain was confirmed with CD by comparing with authentic D-erythro-neopterin (0.1 N HCl); $[\theta]_{312}^{max} = +3.00 \times 10^3$, $[\theta]_{270}^{min} = -1.25 \times 10^3$ and $[\theta]_{248}^{max} = +6.25 \times 10^3$. The main pterin in

R_f-values of isolated pterins on TLC

Compound	Solvent system I	II	III	IV
Compound 1	0.61	0.25	0.37	0.41
Compound 2	0.48	0.33	0.46	0.46
Compound 3	0.33	0.26	0.34	0.34
$KMnO_4$ oxidation product of 1	0.60	0.28	0.24	0.24
$KMnO_4$ oxidation product of 2	0.60	0.28	0.24	0.24
D and L-erythro-neopterin	0.61	0.25	0.37	0.41
D and L-threo-neopterin	0.61	0.23	0.19	0.28
6-Hydroxymethylpterin	0.48	0.33	0.46	0.46
Isoxanthopterin	0.33	0.26	0.34	0.34
6-Carboxypterin	0.60	0.28	0.24	0.24
7-Carboxypterin	0.60	0.28	0.32	0.28

Solvent systems: I; 0.1 M Na-phosphate buffer (pH 6.0); II, *n*-propanol/ethyl acetate/H_2O (7:1:2); III, ethanol/5% ammonium borate/3% NH_4Cl (2:1:1); IV, *n*-propanol/1% NH_4OH (2:1). Avicel SF plates (Asahi Chemical Industries).