old rat contained essentially 1 radioactive product (figure 2, A) while a considerable mixture of products occurred in the adult (figure 2, B). In order to determine whether the product obtained from the 2-week-old rats was an intermediate in the metabolic sequence found in the adult, it was purified and injected into an adult rat. The resulting urine contained tritium-labelled products identical to those observed from $PGF_{2\alpha}$ indicating that the urinary metabolite from the 2-week-old rat is a normal intermediate in the adult pathway and that its accumulation in the neonate is a result of the absence of certain enzymes in the newborn rat or enzymes which have not yet become active at 2–4 weeks of age.

Our findings reveal that some enzyme pathways in the prostaglandin catabolic sequence are age-dependant in vivo and that although certain enzymes are active at an early age (fetal or early neonatal) others in the catabolic sequence (likely the ω-oxidative system) are induced at a later stage. In the rat these latter enzymes become active around 4–6 weeks postnatally.

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Synthesis of L-[3-^{2}H,^{18}O]glycerol and its incorporation into the 4-methyl-5-hydroxyethyl thiazole moiety of thiamine by *Escherichia coli*

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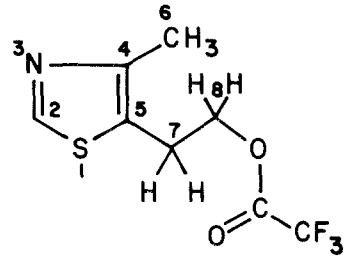
Summary. L-[3-^{2}H,^{18}O]glycerol was prepared and fed to *Escherichia coli* in order to determine the origin of the oxygen atom in the biosynthesized thiazole moiety of thiamine. Measurement by GC-MS of the isotope incorporation into the thiazole from this substrate confirmed that the 2 hydrogens and the oxygen on the C-3 carbon of glycerol are incorporated directly into the thiazole.

Recent work has established that the 5 contiguous carbon atoms in the 4-methyl-5-hydroxyethyl thiazole moiety of thiamine are derived in *E. coli* from pyruvate and a 3-carbon unit originating from glucose[1]. Pyruvate was shown to provide the C-4 and 6 carbons of the thiazole and the C-4, 5 and 6 carbons of glucose were shown to provide the C-5, 7, and 8 carbons of the thiazole, respectively. It was proposed that the 3-carbon unit was incorporated via a triose-P during the biosynthesis. If this theory is correct, then the oxygen atom of the thiazole would also have its origin from a triose-P. This paper reports the further confirmation of this theory by the observation of the incorporation of ^{18}O from stereospecifically labeled glycerol into the thiazole. Furthermore, by using glycerol labeled at C-3 with both ^{2}H and ^{18}O, it was shown that both hydrogens and the oxygen on the C-3 of the glycerol are incorporated into the thiazole with no exchange.

Materials and methods. E. coli B was grown on 100 ml of a defined medium containing 400 mg of labeled glycerol and casamino acids as previously described[2]. The labeled glycerol was prepared as follows: 1,2-O-isopropylidene-D-glyceraldehyde[3] (340 mg) was dissolved in 3.2 ml of tetrahydrofuran containing 200 µl of $H_2{}^{18}O$ (90 atom% ^{18}O) and 120 µl of piperidine. [Under these conditions the aldehyde oxygen was found to exchange with the labeled water with a half life of ~2.3 min at 24 °C. In contrast to this rapid oxygen exchange, no change was observed in the optical rotation of control solutions (using nonlabeled water) for periods up to 6 h, the longest tested. This indicates that epimerization at C-2 of the glyceraldehyde did not occur under these reaction conditions.] After 2 h at 24 °C, 60 mg of sodium borodeuteride (98 atom% ^{2}H) was added to the reaction mixture with constant stirring. After 3 h, 3 ml of saturated sodium bicarbonate solution was added and the resulting solution extracted 2 times with methylene chloride. Evaporation of the combined extracts gave 300 mg of crude 1,2-O-isopropylidene-D-[3-^{2}H,^{18}O]glycerol which was purified by column chromatography to give a pure oil. Mass spectral analysis of this product showed 1.3% of the molecules had no label, 22% had 1 ^{2}H, 3.2% had 1 ^{18}O and 73.3% had both an ^{18}O and ^{2}H. This material was subsequently deprotected with dilute acid and the resulting free glycerol was mixed with nonlabeled glycerol. The final glycerol had 4.1% of its molecules with 1 ^{2}H, 0.61% with 1 ^{18}O and 14.1% with both an ^{18}O and ^{2}H.

This labeled glycerol was subsequently fed to *E. coli* and the isotopes' incorporation into the thiazole and the glycerol-P in the cells were measured by GC-MS[1].

Results and discussion. The isolated thiazole, as the trifluoroacetate ester, showed the following isotopic distribution as measured from the molecular ion at m/e 239: 79.2% no label, 11.4% 1 ^{2}H, 1.4% 2 ^{2}H or 1 ^{18}O, 6.9% 1 ^{2}H and 1 ^{18}O and 0.95% 2 ^{2}H and 1 ^{18}O. The m/e 112 ion in the MS of



4-methyl-5-hydroxymethyl thiazole trifluoroacetate.

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.