AMINOACETONE FORMATION AND DECOMPOSITION IN LIVER *

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In a study of the chemicals that mimic acute porphyria, the metabolism of δ-aminolevulinic acid by guinea pig liver was examined. When a guinea pig liver homogenate or liver cell suspension is incubated with glycine and members of the citric acid cycle the aminoketone formed is aminoacetone not δ-aminolevulinic acid.

The synthetic activity for aminoacetone resides primarily, if not solely, in the mitochondria. Maximum aminoacetone formation results from pyruvate and glycine as substrates (Table I). The presence of an aminoketone with chromatographic properties similar to those of aminoacetone was suggested by studies of normal human urine with a chromatographic and colorimetric method developed by Mauzerall and Granick (1956). The formation of aminoacetone from pyruvate (via acetyl-CoA) and glycine was proposed by Shemin in 1955 and has now been demonstrated in particulates of chicken erythrocytes by Gibson, Laver and Neuberger (1958) and independently, in a preparation from Rhodopseudomonas spheroides, by Kikuchi, Kumar, Talmage and Shemin (1958). The formation of aminoacetone from threonine was first reported by

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Table 1

Aminoacetone Formation By Guinea Pig Liver Mitochondria From Glycine Together With The Substrates Noted.

Substrate	Concentration	Glycine	Aminoacetone formed per 1.0 ml packed mitochondria **
none	-	-	00 mμ M
none	-	0.1 M	10
Pyruvic acid	0.0025 M	0.1	100
	0.05	0.1	1520
	0.10	0.1	4200
	0.20	0.1	3750
	0.10	-	10
Citric acid	0.01	0.1	250
	0.10	0.1	270
Succinic acid	0.01	0.1	30
	0.10	0.1	30
α-Keto-glutaric acid	0.01	0.1	40
	0.05	0.1	90
α-Keto-butyric acid *	0.10	0.1	240
α-Keto-valeric acid *	0.10	0.1	340
α-Hydroxy-butyric acid *	0.10	0.1	140
Threonine	0.10	-	2360
Phenyl pyruvic acid	0.10	0.1	0

The mitochondria were isolated by the method of Hogeboom (1955) and suspended in Earle salt medium (1943) containing 0.3 M sucrose in place of glucose. To 1 ml mitochondrial suspension were added the substrates and buffer to make the final concentration 0.05 M in phosphate, pH 7.0, in a total volume of 2.0 ml. This mixture was incubated aerobically at 38° on a

shaker for 2 hours. After incubation the mixture was treated with an equal volume of 0.3 M trichoroacetic acid and centrifuged. An aliquot of the supernatant solution was chromatographed on Amberlite IRC 50 (Elliott, 1960) to separate aminoacetone from δ-aminolevulinic acid. The aminoketones were condensed with acetylacetone (Mauzerall and Granick, 1956) and the resulting pyrroles determined colorimetrically with a modified Ehrlich reagent. The aminoketone from the chromatographic column was characterized as aminoacetone by way of the pyrrole. This pyrrole had the same Rf on paper chromatography in a butanol-ammonia-water solvent as that prepared from aminoacetone. In addition the pyrrole was extractable into ether from solutions of pH 2 and pH 7.

* The aminoketones formed from these compounds have not been identified.

** One ml packed volume of mitochondria is equivalent to 29.4 mg Kjeldahl N.

Elliott (1959) in washed suspensions of Staphylococcus aureus. Neuberger and Tait (1960) obtained a soluble preparation from Rhodopseudomonas spheroides which contained a DPN enzyme that oxidized threonine to α-amino-β-ketobutyric acid, which decarboxylated spontaneously to aminoacetone. We have also observed active conversion of threonine to aminoacetone by guinea pig liver mitochondria (Table I).

With the liver mitochondria the rate of aminoacetone formation from pyruvate and glycine diminished with time. This is due primarily to a decomposition of aminoacetone. Elliott (1960) found an enzyme in ox plasma which oxidizes aminoacetone to methylglyoxal. In the blood plasma or the red blood cells of the guinea pig we have found only very slight decomposition of aminoacetone. In the guinea pig liver the activity for decomposition of aminoacetone resides in the particulate portions of the cell, primarily the mitochondria and the nuclei. With

aminoacetone at 0.0001 M, 1 ml of packed mitochondria can decompose about 450 mμ moles aminoacetone in 1 hour at 38°C, whereas under optimum conditions it can form about 2500 mμ moles aminoacetone from pyruvate and glycine.

Typical amine oxidase inhibitors as D-amphetamine, aminoguanidine, benzylamine and phenylethylamine at 0.005 M inhibit aminoacetone (0.0001 M) decomposition by about 70 percent. Isoniazid is also similarly effective but not percaine or benadryl.

Elliott (1960) has shown that aminoacetone undergoes oxidation to methyl glyoxal and ammonia, and has proposed that methyl glyoxal would then be readily converted to pyruvic acid by glyoxylase. Nemeth, Russel and Shemin (1957) have observed that in the rat the labelled C atom in CH_3-CO-$C^{14}H_2NH_2$ is oxidized to CO_2 more readily than is the α-carbon of glycine.

These observations suggest a cyclic pathway for glycine oxidation in liver mitochondria via aminoacetone, regenerating pyruvate, i.e. a Shemin cycle involving aminoacetone rather than δ-aminolevulinic acid. The importance of this cycle in liver metabolism and the functions of aminoacetone remain to be determined.

Summary: The formation of aminoacetone by guinea pig liver mitochondria from pyruvate and glycine has been shown. Aminoacetone is decomposed by liver mitochondria and nuclei. A cycle for glycine oxidation via aminoacetone is suggested.

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