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Oxidation of L-Glutamic Acid by Rat Heart.* (20361)

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Glutamic acid is known to be oxidized in tissues other than the liver and kidney and the enzymes responsible for its oxidation are known in a number of instances. Weil-Malherbe(1) for example observed that brain was able to oxidize glutamic acid. Dewan(2) isolated the enzyme and established its requirements. Independently from Dewan, von Euler and associates(3-5) made similar observations and reported an exhaustive study on glutamic dehydrogenase. In a large number of tissues, however, glutamic dehydrogenase exists in very low concentrations and could not account for the rapid utilization of glutamic acid. In heart muscle the glutamic dehydrogenase activity is very low or nonexistent(6), but glutamic acid is well utilized by homogenates of this organ, when fortified with ATP and coenzyme I. The results reported here clearly indicate that glutamic acid is oxidized only after transaminating with oxalacetic acid; the resulting α-ketoglutaric acid is then oxidized by way of the Krebs' cycle.

Methods. Male rats of the Sprague-Dawley strain and weighing approximately 250 g were used in all the experiments. The animals were sacrificed by decapitation, blood was drained off as much as possible, the heart removed and placed on a Petri dish resting over cracked ice. The muscle was freed of connective tissue and blood, then weighed and homogenized in a Potter homogenizer with ice cold isotonic KCl solution. The final concentration of the homogenate was 10%. In all experiments, 0.5 ml of the 1:10 homogenate was used in a final volume of 3 ml. The composition of the medium was identical to that given by Potter, Pardee, and Lyle(7) for the oxalacetic acid system, except that 500 γ of DPN were added in our experiments. The substrate concentration was 20 μM per flask unless stated otherwise. Oxygen uptake was measured by conventional Warburg methods at 38°C with air as the gas phase. *Ammonia* was determined in the deproteinized medium by distillation and nesslerization of the distillate. Aspartic acid and glutamic acid were determined by quanti-

TABLE I. Oxidations in Heart Homogenates.

Substrate (20/flask)	O_2 uptake 15m	30m	60m
No substrate	42	42	43
Glutamate	100	208	400
Succinate	60	120	219
α-Ketoglutarate	91	195	369
Oxalacetate	150	296	395
Citrate	42	46	54
Fumarate	80	160	273

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TABLE II. Oxidation of L-Glutamic Acid by Heart Homogenates.

Substrate	Added (final conc.) ATP	DPN	O_2 uptake in 1 hr
No substrate	.001 M		30 (26- 36) 4*
Glutamate	"		80 (36- 90) 4
No substrate		300	58 (50- 64) 4
Glutamate		"	56 (48- 62) 12
No substrate	"	"	45 (40- 46) 4
Glutamate	"	"	385 (350-420) 8

* No. of determinations.

TABLE III. Formation of Aspartic Acid from Glutamic Acid in Heart Homogenate. 5 experiments.

O_2 uptake, μM	Aspartic acid formed, μM Theory	Observed
8.4	5.6	6.0
9.5	6.3	6.6
9.6	6.4	5.3
8.5	5.7	7.0
6.7	4.1	5.2

tative paper chromatography(8). The *L-glutamic* acid was obtained commercially and it was used as such.

Results. The ability of the heart homogenate to oxidize various intermediates of the Krebs' cycle was first established. The results are shown in Table I. With the notable exception of citrate all other components are utilized actively, including glutamic acid. Ammonia did not increase after the oxidation of glutamate, thus ruling out the possibility that glutamic dehydrogenase was involved. Furthermore, the addition of semicarbazide inhibited the oxidation of glutamate completely. In Table II are shown data obtained from several experiments. Glutamate was oxidized only when both ATP and DPN were added. The exclusion of either component resulted in a complete failure of the homogenate to oxidize glutamic acid. The concentration of ammonia was found to remain unchanged in all the experiments. Analyses of the reaction products by paper chromatography revealed that whenever glutamic acid was oxidized a substantial amount of aspartic acid was formed. The aspartic acid formed after the oxidation of glutamic acid was determined quantitatively and the results of several experiments are shown in Table III.

Few experiments were carried out to determine the effect of sodium monofluoroacetate on the oxidation of glutamate. Fluoroacetate is assumed to react with oxalacetic acid to form fluorocitrate. In the presence of fluoroacetate, the oxidation of glutamate was inhibited nearly completely. The inhibition, however, could be lifted by the addition of fumarate or malate.

Discussion. As far back as 1930, Needham(9) had observed that glutamate was oxidized by minced frog muscle to yield succinate without a concomitant reduction of amino groups in the system. Undoubtedly she was dealing with a transamination reaction. A similar type of oxidation has been described for aspartic acid by Nakada and Weinhouse(10). They used washed liver particles and observed that rapid utilization occurred. In the present study, a transamination is also responsible for the deamination of glutamic acid. There is probably sufficient oxalacetic acid present in the tissue at the start, which can and does transaminate with the added glutamate. The resulting α-ketoglutaric acid can be oxidized and yield more oxalacetic acid. This is probably the case since aspartic acid can be found in quantities which correspond closely to the theoretical amounts as calculated from the oxygen uptake. The inhibition by fluoroacetate could be interpreted as a removal of the oxalacetic acid by the inhibitor. In a recent paper, the author demonstrated that the injection of nonlethal doses of fluoroacetate resulted in a diminution of the concentration of free glutamic and aspartic acids in the heart(11). The present results and those obtained *in vivo,* however, are not comparable. The animal has other sources of keto-acids and there is always the added complication that blood can remove excess metabolites.

Summary. Glutamic acid was found to be oxidized by heart homogenates, by a mechanism involving transamination with oxalacetic acid and subsequent oxidation of the resulting α-ketoglutaric acid by way of the Krebs' cycle.

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Persistence of Amethopterin in Normal Mouse Tissues.* (20362)

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In a previous report(1) it was shown that following the intravenous administration of amethopterin (4-amino-N^{10}-methyl-pteroylglutamic acid) to normal mice in a single dose of 0.5 mg/kg, the greater part of the compound was rapidly excreted or metabolized within approximately 4 hours. The remainder was found to be concentrated mainly in those tissues (liver and kidneys) which have been reported(2) as containing relatively high concentrations of folic acid and citrovorum factor.

As a consequence of this observation it was of interest to determine the persistence of the antimetabolite in various tissues after the administration of amethopterin, by various assay schemes, and furthermore to establish the identity of the anti-metabolite.

Materials and methods. The mice used in this experiment were of the inbred AkR stock, weighing approximately 20 g. All were young mice of approximately the same age. Groups of mice of different sexes were used. Previous to and throughout the experiment the mice were fed the standard Purina Laboratory Chow diet. For convenience of description the mice used in this study are divided into 6 groups based on the administration of amethopterin. *Group I* (31 mice) received amethopterin intraperitoneally at a dose of 3 mg/kg on alternate days for a total of 3 injections. *Group II* (18 mice) received 1 mg/kg on the above schedule. *Group III* (10 mice) received a single intraperitoneal injection of 3 mg/kg, and *Group IV* (12 mice) was given 1 mg/kg intravenously on alternate days for 3 injections. *Group V* (18 mice) was given amethopterin as for Group I. Seventy-two hours after the last injection 2 mice were sacrificed, and thereafter at daily intervals all the remaining mice were given citrovorum factor (CF)§ intraperitoneally at doses ranging from 0.25 to 30.0 mg/kg. Mice were sacrificed daily 24 hours after last injection of CF. The procedures employed in obtaining and preparing tissues for assay were those previously reported(1) and the assay procedure was that of Burchenal *et al.*(3).

Group VI. For purposes of identification

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