# CCXXXIII. METABOLISM OF α-KETOGLUTARIC ACID IN ANIMAL TISSUES

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DEWAN [1938] and Adler *et al.* [1939] reported that certain enzyme preparations obtained from heart muscle bring about a reductive amination of α-ketoglutaric acid. The hydrogen required in this reaction may be provided from other dehydrogenase systems via coenzyme I or II, especially from the β-hydroxybutyric and *iso*citric dehydrogenases, according to the schemes:

(1) α-ketoglutaric acid + $NH_3$ + *iso*citric acid → glutamic acid + oxalosuccinic acid, or

(2) α-ketoglutaric acid + $NH_3$ + β-hydroxybutyric acid → glutamic acid + acetoacetic acid.

We have studied the occurrence of these reactions (so far observed only in artificially combined enzyme systems) in surviving tissues. We find that a reductive amination of α-ketoglutaric acid does in fact take place in kidney and heart muscle, but this reduction proves independent of the presence of *iso*citric or β-hydroxybutyric acids. On the other hand it is found to be accompanied by a formation of succinic acid and $CO_2$, and the quantities of these substances formed show that the following reaction takes place:

(3) 2α-ketoglutaric acid + $NH_3$ = glutamic acid + succinic acid + $CO_2$.

## EXPERIMENTAL

Pigeon breast muscle and heart muscle were minced in the Latapie mill and suspended in 5 to 10 parts of 0·1 *M* phosphate buffer of *p*H 7·1 or 7·4. Other tissues were sliced and suspended in the balanced salt solution of Krebs & Henseleit [1932]. The experiments were carried out in conical manometer flasks provided with sidearms and centre cups. The latter contained yellow P in all anaerobic experiments. The temperature of the bath was 40°.

Glutamic and succinic acids were determined manometrically as previously described [Cohen, 1939]. The $CO_2$ production was also measured manometrically. The figures given in the tables for the $CO_2$ formation represent the total $CO_2$, i.e. $CO_2$ formed from α-ketoglutaric acid and liberated from the $NaHCO_3$ of the medium by acids.

### *α-Ketoglutaric acid and ammonia in kidney cortex*

In Table I two experiments are recorded in which kidney cortex was incubated anaerobically with α-ketoglutarate and $NH_4Cl$. It will be seen that small quantities of glutamic acid are formed when α-ketoglutarate alone is added. Addition of $NH_4Cl$ increases the glutamic acid formation and at the same time approximately equivalent quantities of succinic acid and $CO_2$

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Table I. *α-Ketoglutaric acid and $NH_3$ in kidney cortex*

| Species | Substrate added (final conc.) | Mg. tissue | Period of incubation min. | μl. $CO_2$ evolved | μl. succinic acid formed | μl. glutamic acid formed |
|---|---|---|---|---|---|---|
| Guinea-pig | 0·02 *M* α-ketoglutarate | 28·8 | 100 | — | — | 122 |
| | 0·02 *M* α-ketoglutarate, 0·02 *M* $NH_4Cl$ | 39·7 | 100 | — | — | 518 |
| Rat | — | 13·5 | 140 | 74 | 0 | 0 |
| | 0·02 *M* α-ketoglutarate | 17·1 | 140 | 136 | 80 | 81 |
| | 0·02 *M* α-ketoglutarate, 0·02 *M* $NH_4Cl$ | 17·9 | 140 | 327 | 322 | 234 |

appear. In the experiment on rat kidney the ratio of glutamic acid : succinic acid : $CO_2$ is 234 : 222 : 229, or 1 : 0·95 : 0·98. These data are in agreement with equation (3).

Table II. *Anaerobic disappearance of $NH_3$ in guinea-pig kidney cortex in the presence of α-ketoglutarate*

(Total volume of medium per flask 4·6–4·9 ml.; incubation 195 min.; initial concentration of α-ketoglutarate 0·026 *M*)

| μl. $NH_3$ added (as $NH_4Cl$) | mg. tissue | μl. $NH_3$ found after incubation | μl. $NH_3$ used per mg. tissue | μl. $CO_2$ formed per mg. tissue | "Extra $CO_2$" (due to addition of $NH_3$) | "Extra $CO_2$" formed / $NH_3$ used |
|---|---|---|---|---|---|---|
| — | 78·5 | — | — | 5·68 | — | — |
| 1320 | 70·2 | 382 | 13·38 | 16·62 | 10·94 | 0·82 |
| 660 | 130·0 | 120 | 4·16 | 8·65 | 2·97 | 0·71 |
| 330 | 104·2 | 86·5 | 2·34 | 7·89 | 2·21 | 0·95 |

Table II shows a disappearance of $NH_3$ of the expected magnitude. At a low $NH_3$ concentration the "extra $CO_2$" (i.e. $CO_2$ formed on addition of $NH_3$) is very nearly equivalent to the quantity of $NH_3$ absorbed. With rising concentrations of $NH_3$ the calculated "extra $CO_2$" becomes smaller than the $NH_3$ absorbed. This may be explained on the assumption that a "simple" dismutation of α-ketoglutaric acid (reaction (4)

(4) 2α-ketoglutaric acid + $H_2O$ = α-hydroxyglutaric acid + succinic acid + $CO_2$,

which accounts for most of the $CO_2$ formation from α-ketoglutarate in the absence of $NH_4Cl$ [see Krebs & Johnson, 1937, 1; Weil-Malherbe, 1937] competes with reaction (3). As the $NH_3$ concentration rises, the rate of (4) decreases owing to the conversion of α-ketoglutaric into α-iminoglutaric acid. Thus our method of calculation would yield too low values for the extra $CO_2$.

As regards the glutamic acid formation in the absence of added $NH_3$, this may be due to transamination or amination from endogenous amino-compounds or $NH_3$.

*α-Ketoglutaric acid and $NH_3$ in heart muscle*

Similar experiments on heart muscle are recorded in Table III. The increases in succinic acid, glutamic acid and $CO_2$ formation after addition of $NH_4Cl$ and α-ketoglutarate are distinct, but smaller than in kidney cortex. It is noteworthy that the succinic acid formation is considerably smaller than the glutamic acid formation in the experiment on sheep heart. This suggests that there are other reactions, in addition to (3), in which glutamic acid is synthesized.

Table III. *α-Ketoglutaric acid and $NH_3$ in minced heart muscle*

Incubation period 120 min.

| Species | Quantity of tissue | Substrates added (final conc.) | μl. $CO_2$ | μl. succinic acid | μl. glutamic acid |
|---|---|---|---|---|---|
| Sheep | 500 mg. fresh muscle in 3·6 ml. | — | 22 | 50 | 35 |
| | 0·1 *M* phosphate buffer, | 0·02 *M* α-ketoglutarate | 160 | 90 | 149 |
| | *p*H 7·1 | 0·02 *M* α-ketoglutarate, 0·02 *M* $NH_4Cl$ | 277 | 236 | 420 |
| | | 0·02 *M* α-ketoglutarate, 0·02 *M* $NH_4Cl$, 0·02 *M* citrate | 270 | 139 | 402 |
| Pig | 370 mg. fresh muscle in 3·4 ml. | — | — | 31 | 3 |
| | 0·1 *M* phosphate buffer, | 0·02 *M* α-ketoglutarate | — | 76 | 70 |
| | *p*H 7·1 | 0·02 *M* α-ketoglutarate, 0·006 *M* $NH_4Cl$ | — | 146 | 110 |

Table IV. *Total $CO_2$ production in the presence of α-ketoglutarate and $NH_4Cl$ in various tissues*

| Tissue | Substrates added (final conc.) | $Q_{CO_2}$ |
|---|---|---|
| Guinea-pig kidney | 0·02 *M* α-ketoglutarate | 2·9 |
| | 0·02 *M* α-ketoglutarate, 0·02 *M* $NH_4Cl$ | 8·2 |
| Guinea-pig kidney | — | 2·04 |
| | 0·02 *M* α-ketoglutarate | 3·07 |
| | 0·02 *M* α-ketoglutarate, 0·02 *M* $NH_4Cl$ | 7·9 |
| | 0·04 *M* α-ketoglutarate, 0·04 *M* $NH_4Cl$ | 9·4 |
| | 0·02 *M* pyruvate | 4·0 |
| | 0·02 *M* pyruvate, 0·02 *M* $NH_4Cl$ | 4·6 |
| | 0·02 *M* oxaloacetate | 7·5 |
| | 0·02 *M* oxaloacetate, 0·02 *M* $NH_4Cl$ | 7·9 |
| Rat kidney | — | 2·35 |
| | 0·02 *M* α-ketoglutarate | 3·42 |
| | 0·02 *M* α-ketoglutarate, 0·02 *M* $NH_4Cl$ | 7·84 |
| | 0·02 *M* pyruvate | 4·68 |
| | 0·02 *M* pyruvate, 0·02 *M* $NH_4Cl$ | 5·29 |
| Rat liver | — | 6·4 |
| | 0·02 *M* α-ketoglutarate | 7·5 |
| | 0·02 *M* α-ketoglutarate, 0·02 *M* $NH_4Cl$ | 6·2 |
| Pigeon liver | 0·02 *M* α-ketoglutarate | 4·30 |
| | 0·02 *M* α-ketoglutarate, 0·02 *M* $NH_4Cl$ | 5·85 |
| Pigeon brain | 0·02 *M* α-ketoglutarate | 1·82 |
| | 0·02 *M* α-ketoglutarate, 0·02 *M* $NH_4Cl$ | 2·08 |
| | 0·02 *M* α-ketoglutarate, 0·02 *M* pyruvate | 3·74 |
| | 0·02 *M* α-ketoglutarate, 0·02 *M* pyruvate, 0·02 *M* $NH_4Cl$ | 3·92 |
| Guinea-pig brain | — | 0·78 |
| | 0·04 *M* α-ketoglutarate | 1·66 |
| | 0·04 *M* α-ketoglutarate, 0·02 *M* $NH_4Cl$ | 1·52 |
| Rat intestine | — | 2·18 |
| | 0·02 *M* α-ketoglutarate | 3·22 |
| | 0·02 *M* α-ketoglutarate, 0·02 *M* $NH_4Cl$ | 3·00 |
| Rat testis | — | 1·18 |
| | 0·02 *M* α-ketoglutarate | 1·33 |
| | 0·02 *M* α-ketoglutarate, 0·02 *M* $NH_4Cl$ | 1·43 |
| Rat spleen | — | 1·98 |
| | 0·02 *M* α-ketoglutarate | 2·09 |
| | 0·02 *M* α-ketoglutarate, 0·02 *M* $NH_4Cl$ | 1·80 |
| Pigeon breast muscle | — | 0·10 |
| | 0·02 *M* α-ketoglutarate | 1·64 |
| | 0·02 *M* α-ketoglutarate, 0·04 *M* $NH_4Cl$ | 1·65 |
| | 0·02 *M* α-ketoglutarate, 0·04 *M* $NH_4Cl$, 0·02 *M* citrate | 1·57 |

*Anaerobic $CO_2$ production in the presence of α-ketoglutaric acid and $NH_3$ in various tissues*

Of the products of reaction (3), $CO_2$ is the easiest to determine. We have used the determination of the $CO_2$ production in the presence of α-ketoglutarate and $NH_4Cl$ in order to test a series of tissues for reaction (3). Brain, intestine, spleen, liver, testis and pigeon breast muscle show no significant increase in $CO_2$ production (Table IV). Kidney and heart thus appear to be the only tissues in which reaction (3) is of major importance.

*Effects of β-hydroxybutyrate and citrate on the synthesis of glutamic acid.* In order to see whether reaction (2) occurs in surviving tissues a series of tissues (rat liver, rat and guinea-pig kidney cortex, guinea-pig, pigeon and sheep brain, sheep heart muscle and pigeon breast muscle) were incubated anaerobically with α-ketoglutarate, $NH_4Cl$ and *dl*-β-hydroxybutyrate. After incubation, acetoacetic acid was determined by the aniline citrate method [Edson, 1935]. No significant amounts of acetoacetic acid were found and it must therefore be concluded that reaction (2) does not occur to an appreciable extent in the tissues tested, although the tissues appear to contain all the catalysts required for this reaction.

To test for reaction (1) citrate was added together with α-ketoglutarate and $NH_4Cl$ to various tissues. Citrate rapidly forms *iso*citrate under the conditions of the experiment [Johnson, 1939] and if reaction (1) occurred an increased glutamic acid formation would be expected; no increase was found, however (Table III).

*Reactions analogous to (3).* Guinea-pig kidney cortex, liver and brain and pigeon breast muscle were incubated anaerobically with ammonium pyruvate and ammonium oxaloacetate, but no significant increase in $CO_2$ production was observed (see Table IV). There is therefore no reason to assume that other α-ketonic acids react in the same way as α-ketoglutaric acid.

## Discussion

*α-Iminoglutaric acid.* Knoop & Oesterlin [1925] found that solutions containing α-ketoglutaric acid and $NH_3$ yield glutamic acid on catalytic dehydrogenation. This fact may be taken as conclusive evidence of the existence of α-iminoglutaric acid in these solutions and reaction (3) may therefore be written in the following form:

(5) α-ketoglutaric acid + α-iminoglutaric acid + $H_2O$ = glutamic acid + succinic acid + $CO_2$.

The analogy between (5) and the simple dismutation of α-ketonic acids (4) is obvious. Reaction (5) may be considered as a special form of dismutation in which the α-imino-acid replaces one of the α-ketonic acids.

*Glutamic acid as a hydrogen carrier.* If reaction (3) is followed by the reoxidation of glutamic to α-iminoglutaric acid, the system glutamic acid $\rightleftharpoons$ iminoglutaric acid acts as a hydrogen-transporting system, a conception which was put forward some time ago, in a general way, by Knoop & Oesterlin [1925]. This would explain previous observations [Krebs, 1932; Edson, 1935] which showed that addition of $NH_4Cl$ catalytically increases the respiration in kidney cortex in the presence of α-ketoglutarate and of those substances which may give rise to the formation of α-ketoglutarate, such as lactate, pyruvate or glucose [see Krebs & Johnson, 1937, 2]. The fact that the effect of $NH_4Cl$ is

not observed in other tissues in which reaction (3) does not occur supports this explanation.

It is also suggestive to explain the widespread occurrence of glutamic dehydrogenase in animal tissues by its specific function as a hydrogen carrier in cellular respiration. In connexion with this hypothesis arises the question of the nature of the hydrogen donators for iminoglutaric acid. Our attempts to find donators other than α-ketoglutaric acid were so far without positive results. Glucose, lactate, pyruvate, α-glycerophosphate, *dl*-glyceric aldehyde had no effect on the reduction of iminoglutaric acid in sliced brain cortex.

*Oxidative breakdown of α-ketoglutaric acid.* Since reaction (3) does not occur in all the tissues which oxidize α-ketoglutaric acid there must be other mechanisms, e.g. in pigeon breast muscle, or in brain, whereby α-ketoglutaric acid is broken down. In kidney cortex, however, reaction (3) appears to be the chief pathway of the breakdown of α-ketoglutaric acid.

## SUMMARY

The following reaction was found to take place when α-ketoglutarate and $NH_4Cl$ were added to sliced kidney cortex or minced heart muscle:

$$2\alpha\text{-ketoglutaric acid} + NH_3 = \text{glutamic acid} + \text{succinic acid} + CO_2.$$

This reaction is probably a step in the normal oxidative breakdown of α-ketoglutarate and of those substances which give rise to α-ketoglutarate. This is borne out by the fact that $NH_4$ salts catalytically increase the rate of oxidation of α-ketoglutarate.

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