* Criticism addressed on page 6 (....."believe it to be an unimportant side reaction....")

93. THE CITRIC ACID CYCLE AND THE SZENT-GYÖRGYI CYCLE IN PIGEON BREAST MUSCLE

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(*Received 1 April 1940*)

IT is known from Szent-Györgyi's work [1935; 1936] that oxaloacetate can be reduced by muscle tissue to form a mixture of fumarate and *l*(−)-malate. Szent-Györgyi formulates this reaction as follows:

(1) Oxaloacetate $\xrightarrow{+\text{"hydrogen of the substrates"}}$ {fumarate + *l*(−)-malate

So far two reactions which amplify the term "hydrogen of the substrates" have been described, viz.:

(2) "Triose" + oxaloacetate → pyruvate + {fumarate + *l*(−)-malate [Szent-Györgyi, 1936; Parnas & Szankowski, 1937]

and

(3) {Oxaloacetate + pyruvate} + oxaloacetate → citrate + CO_2 + {fumarate + *l*(−)-malate [Krebs & Johnson, 1937].

In this paper is described a third reaction in which oxaloacetate is reduced:

(4) Citrate + oxaloacetate → α-ketoglutarate + CO_2 + {fumarate + *l*(−)-malate

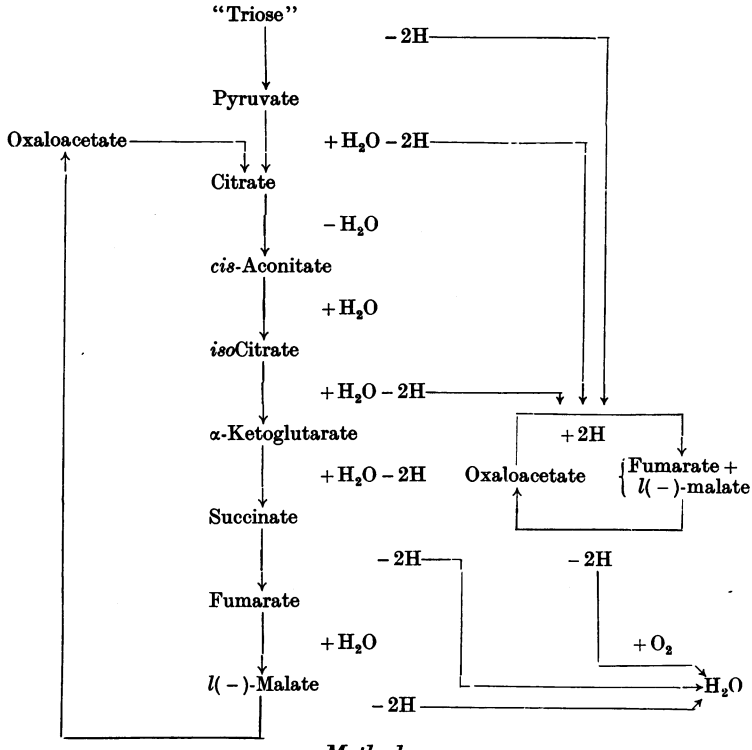
The reactions 2, 3 and 4 define the relations between the "citric acid cycle" and the "Szent-Györgyi cycle" in pigeon breast muscle. When one triose equivalent undergoes complete oxidation, 6 pairs of H atoms are released, in the course of the series of reactions shown in the right-hand section of the scheme on p. 776.

According to reactions 2, 3 and 4 the first 3 pairs of H atoms are transferred to oxaloacetate. They react later in the same way as the sixth pair (see below). The pathway of the fourth pair, arising from the dehydrogenation of α-ketoglutarate, is not yet clear. The fifth pair, arising from succinate, is known to react more or less directly with activated oxygen. The sixth and last pair, arising from *l*(−)-malate, reacts with activated oxygen through the intermediation of coenzymes. Thus the Szent-Györgyi cycle is concerned with the transport of (at least) 6 of the 12 H atoms released during the oxidation of one triose equivalent, as indicated by the scheme below. Since malate and oxaloacetate are not only catalysts, but also intermediates, the reaction malate $\xrightarrow{-2H}$ oxaloacetate occurs (at least) 4 times, and the reaction oxaloacetate $\xrightarrow{+2H}$ malate (at least) 3 times during the oxidation of a triose equivalent.

No attempt is made in this paper to describe the role of coenzymes in the aforementioned reactions, but it should be pointed out that reaction 4 presents a coenzyme problem of special interest. Adler *et al.* [1939] reported that *iso*citric dehydrogenase (which is responsible for the oxidation of citrate) requires the triphosphopyridine nucleotide (coenzyme II). Oxaloacetate on the other hand accepts H only from the diphosphopyridine nucleotide (coenzyme I). Neither of the two coenzymes therefore can act alone as a H carrier in reaction 4. One must assume that either both coenzymes together with an intermediate carrier

take part, or else that the transport of H by the coenzymes is coupled with their phosphorylation and dephosphorylation, resulting in an interconversion of the triphospho- into the diphospho-pyridine nucleotide. The possibility of such a reaction has been demonstrated by Euler & Bauer [1938] and by Adler *et al.* [1940].

Scheme of the oxidative breakdown of carbohydrate in pigeon breast muscle



Methods

The technique was in general the same as that used previously [Krebs & Eggleston, 1940]. Minced pigeon breast muscle was suspended in phosphate saline and shaken at 40° under varying conditions.

Citrate was determined according to Pucher *et al.* [1936]. The values obtained for citrate were multiplied with the factor 1·25 in order to include *iso*citrate and *cis*-aconitate with which citrate forms an equilibrium [Martius, 1938; Johnson, 1939]. The factor 1·25 follows from Johnson's data. Succinate was determined manometrically according to Szent-Györgyi & Göszy [1935] and Krebs [1937]. α-Ketoglutarate was treated at room temperature with an excess of permanganate, in the presence of $MnSO_4$ and H_2SO_4, and determined as succinate. Fumarate was determined by a new method worked out with the collaboration of Dr E. A. Evans and Dr D. H. Smyth. Fumarate is reduced to succinate in the presence of Zn and acid and the succinate is determined manometrically. The details of this method will be published later. The ratio fumarate/malate in the presence of fumarase was found to be 3·8 when equilibrium is established under the conditions of these experiments (40°, *p*H 7·4) [see Jacobsohn, 1934]. The concentration of malate was calculated on the basis of this figure.

Most experiments were carried out in triplicate or quadruplicate and the contents of one manometer cup (4 ml. muscle suspension) were used for determination of citrate, succinate, α-ketoglutarate and fumarate + malate respectively.

Oxidation of citrate in the presence of oxaloacetate

It will be seen from Table 1 that no appreciable quantities of citrate disappear when citrate alone is added anaerobically to muscle suspensions. When oxaloacetate and citrate are added together, however, citrate is removed and an

Table 1. *Oxidation of citrate in the presence of oxaloacetate*

Minced pigeon breast muscle; anaerobic conditions; 40°; muscle suspensions in 4 ml. phosphate-saline.

Exp.	Wet wt. muscle in 4 ml. mg.	Substrates added	Period of incubation min.	Changes in citrate μl.	α-Ketoglutarate formed μl.	Succinate formed μl.	Fumarate + malate formed μl.
1	266	1. 448 μl. citrate	50	+ 34	73	—	—
		2. 448 μl. citrate; 448 μl. oxaloacetate	50	−180	252	—	—
		3. 448 μl. citrate; 896 μl. oxaloacetate	50	−242	288	—	—
		4. 448 μl. oxaloacetate	50	—	52	—	—
		5. 896 μl. oxaloacetate	50	+ 25	100	—	—
		6. 448 μl. citrate; 448 μl. fumarate	50	+ 44	—	—	—
2	400	1. —	60	0	96	51	—
		2. 1344 μl. citrate	60	−274	195	25	—
		3. 1344 μl. oxaloacetate	60	—	128	114	—
		4. 1344 μl. citrate; 1344 μl. oxaloacetate	60	−780	697	89	—
3	400	1. 1344 μl. oxaloacetate; 1344 μl. citrate	10	−371	554	0	—
		2. 1344 μl. oxaloacetate; 1344 μl. citrate	20	−441	665	9	—
		3. 1344 μl. oxaloacetate; 1344 μl. citrate	40	−525	775	17	—
4	400	1. 1344 μl. oxaloacetate	15	+ 35	231	21	696
		2. 1344 μl. oxaloacetate; 1344 μl. citrate	15	−420	540	30	755
		3. —	15	+ 17	90	42	0
5	400	1. 1344 μl. oxaloacetate	40	+ 20	223	24	840
		2. 1344 μl. oxaloacetate; 1344 μl. citrate	40	−574	658	0	730

approximate equivalent of α-ketoglutarate is formed. The reaction occurs at relatively low concentrations of citrate and oxaloacetate (0·005 *M*). The rate of the reaction is very rapid: 400 mg. muscle (80 mg. dry wt.) remove 371 μl. citrate in 10 min. (exp. 3). If Q_{O_2} is 50 (the highest value observed in muscle) and if the total respiration passes through the citric acid cycle, the tissue should be capable of oxidizing at least 50/6 = 8·3 μl. citrate per mg. per hr. The observed figure (28 μl. per mg. dry wt. per hr.) is much higher than the postulated minimum.

The oxidation of citrate to α-ketoglutarate must be associated with an equivalent reduction. The only reductive process of sufficient magnitude accompanying the oxidation of citrate is the conversion of oxaloacetate into fumarate and malate. About 50–60 % of the added oxaloacetate is reduced to fumarate and malate. This figure is not essentially affected by the addition of citrate, especially if the period of incubation is comparatively long (40 min.). The formation of α-ketoglutarate, via reactions 3 and 4, is the oxidative equivalent of the reduction of oxaloacetate in the absence of added citrate.

Discussion of side issues

1. *Oxidation of α-ketoglutarate.* Attempts to demonstrate a dismutation between α-ketoglutarate and oxaloacetate

(5) α-Ketoglutarate + oxaloacetate + H_2O → succinate + CO_2 + $\left\{\begin{matrix}\text{fumarate} + \\ l(-)\text{-malate}\end{matrix}\right.$

have so far been unsuccessful, but the possibility that this reaction occurs in pigeon breast muscle cannot be excluded. If it occurs, two further H atoms of the triose equivalent pass through the Szent-Györgyi cycle.

In kidney cortex α-ketoglutarate is (chiefly) oxidized through the reaction [Krebs & Cohen, 1939]:

$$2\ \alpha\text{-Ketoglutarate} + NH_3 \rightarrow \text{glutamate} + \text{succinate} + CO_2.$$

2. *Reduction of fumarate to succinate.* The scheme of carbohydrate breakdown on p. 776 does not include the reaction fumarate → succinate. It is at present uncertain whether this reaction takes place and whether the system succinate⇌ fumarate acts as a H-transporting system under physiological conditions. The reaction seems to occur when high concentrations of fumarate are present and when the conditions are anaerobic. Succinate is also found when oxaloacetate is added anaerobically to muscle tissue [see Krebs & Johnson, 1937; Krebs, 1940], but it is possible that it is not formed by reduction but oxidatively through the citric acid cycle, including reaction 5.

3. *α-Ketoglutarate formation from oxaloacetate.* The control experiments in Table 1 show that oxaloacetate yields anaerobically considerable quantities of α-ketoglutarate, a fact which is explained by the reactions 3 and 4. In previous papers [Krebs & Johnson, 1937; Krebs & Eggleston, 1940; Krebs, 1940], the formation of α-ketoglutarate was overlooked since the solution was treated with permanganate to remove malonate. The ketoglutarate therefore appeared as succinate in the results. As was previously pointed out the conclusions drawn from those experiments are not affected by the fact that part of the succinate found was derived from α-ketoglutarate.

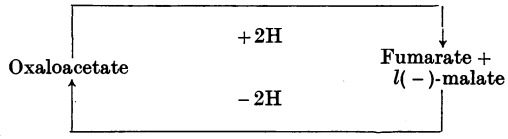
The oxidation of citrate by oxaloacetate accounts for the relatively low yields of citrate when tissues are incubated with oxaloacetate, i.e. when reaction 3 occurs [see Breusch, 1939; Thomas, 1939; Krebs, 1940].

Summary

It is shown that citrate and oxaloacetate react as follows in pigeon breast muscle:

$$(4)\quad \text{Citrate} + \text{oxaloacetate} = \alpha\text{-ketoglutarate} + CO_2 + \left\{\begin{array}{l}\text{fumarate} + \\ l(-)\text{-malate}\end{array}\right.$$

Thus the Szent-Györgyi cycle



acts as a hydrogen carrier in the oxidation of citrate (reaction 4), in the formation of citrate [reaction 3; Krebs & Johnson, 1937] and in the oxidation of "triose" to pyruvate [reaction 2; Szent-Györgyi, 1936; Parnas & Szankowski, 1937]. The Szent-Györgyi cycle is therefore concerned with the transport of (at least) 6 of the 12 hydrogen atoms released during the oxidation of one triose equivalent, as shown in the scheme of carbohydrate oxidation on p. 776.

In other words, the oxidation of a triose equivalent involves one complete citric acid cycle and three repetitions of the Szent-Györgyi cycle.

This work was aided by grants from the Rockefeller Foundation and the Medical Research Council.

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