127. THE OXIDATION OF PYRUVATE BY HEART MUSCLE

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BRAUN-MENENDEZ *et al.* [1939], working with heart-lung and isolated heart preparations, have shown that pyruvate can be utilized by the mammalian heart. Minced heart muscle, as will be seen later, oxidizes pyruvate at about the same rate as the intact heart, and it was used in the present work for a study of the mechanism of the oxidation of pyruvate. The object of the investigation was to determine whether oxidation occurs by way of the citric acid cycle as in pigeon breast muscle [Krebs & Eggleston, 1940].

The main evidence for the occurrence of the citric acid cycle in pigeon breast muscle is as follows:

- (1) The oxidation of pyruvate is inhibited by malonate.
- (2) The malonate inhibition is partially overcome by the addition of fumarate, and the extra amount of pyruvate used in these conditions is in the proportion of 1 mol. of pyruvate to each mol. of added fumarate. In the presence of malonate succinate is formed in amounts equivalent to the fumarate added, the reaction being

- (3) Under certain conditions fumarate and pyruvate can be shown to form citrate and α -ketoglutarate.

The general method of investigation in this work was to examine if these same reactions can be demonstrated in heart muscle, and it will be shown that the process of pyruvate oxidation in mammalian heart muscle is fundamentally the same as in avian skeletal muscle.

Methods

In general the procedure followed closely that described by Krebs & Eggleston [1940], and the same methods were used for the various estimations.

The tissue used was sheep's heart, obtained from the abattoir. As soon as possible after killing the animal the heart was removed, cooled to 0° and maintained at this temperature until the experiment was begun. The rapid cooling after death was found to be very important, and if not carried out within a few min. the rates of O₂ consumption and of pyruvate utilization were found to be greatly reduced. The muscle of the left ventricle was minced in the Latapie mill, and suspended in ice-cold phosphate-saline. Except where otherwise stated a suspension consisting of 1 g. of muscle and 7.5 ml. saline were used; 3 ml. of this were measured into a Warburg cup, and the volume made up to 4 ml. with added substrates, inhibitors and distilled water. The respiration was measured for periods varying from 30 min. to 2 hr. at 40° in the presence of various substrates. The usage of the added substrates was measured, and also the formation of succinate, α -ketoglutarate and citrate under various conditions.

In expressing the results the amounts of all substances are expressed as $\mu\text{l.}$ of gas, 1 millimol being taken as equivalent to 22,400 $\mu\text{l.}$

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1. *Oxidative removal of pyruvate*

Pyruvate usage. Pyruvate added to suspensions of heart muscle in the presence of O_2 is readily oxidized. The amounts used and also the time course of the pyruvate usage are shown in Table 1. In the first experiment quoted there

Table 1. *Pyruvate utilization and O_2 consumption in heart muscle*

(4 ml. suspension)						
Heart muscle mg.	Initial conc. of pyruvate <i>M</i>	Duration of experiment min.	Pyruvate used μ l.	O_2 used μ l.	O_2 used Pyruvate	
375	0.02	30	293	559	1.91	
		60	573	1134	1.97	
		120	1113	2569	2.31	
353	0.02	120	882	1868	2.11	
353	0.02	120	1333	2461	1.84	
353	0.02	125	884	1979	2.24	
353	0.02	120	944	2000	2.12	

is no falling off in the O_2 consumption or in the pyruvate usage for the 2 hr. period. In most experiments however there is a progressive decrease in the oxidative processes as measured by the O_2 consumption (Table 2), so that the

Table 2. *Effect of pyruvate on the respiration of heart*

(4 ml. suspension)				
Heart muscle mg.	Initial conc. of pyruvate <i>M</i>	O_2 consumption (μ l.)		
		30 min.	60 min.	120 min.
375	0	456	711	1001
	0.02	603	1215	2579
353	0	500	770	880
	0.02	650	1260	1870
353	0	430	701	1059
	0.02	630	1249	2461
353	0	396	574	640
	0.02	700	1367	2100
400	0	300	500	659
	0.02	653	1273	2141

initial rate of pyruvate usage is probably greater than the values shown, which are the average rates over the whole 2 hr. period. At a concentration of pyruvate of 0.02 *M* the amounts used vary from 474 to 744 mg. pyruvic acid/100 g. heart muscle/hr. Similar figures were reported for the intact heart by Braun-Menendez *et al.* [1939], who obtained values varying from a maximum of 870 mg./100 g./hr. to very low values depending among other factors on the concentration of pyruvate. The fact that minced heart uses pyruvate at about the same rate as the intact heart may be taken as evidence that the processes occurring in the minced tissue are of a similar nature to those in the intact beating heart.

The values for pyruvate given here include the total pyruvate which disappeared during the experiment. Anaerobic controls showed that while there was a definite anaerobic disappearance of pyruvate also taking place this did not amount to more than 20% of the total pyruvate used.

O_2 usage. The figures given in Tables 1 and 2 show the rate of O_2 consumption. In the presence of pyruvate the Q_{O_2} varies from 16 to 20, which is equivalent to a usage of 320 to 400 ml./100 g. fresh tissue/hr. The higher values are approaching

the values recorded for the intact beating dog's heart doing the maximal amount of work. Evans *et al.* [1935], from estimation of the O_2 content of the coronary arterial and venous blood in the heart-lung preparation, found values of 221 ml./100 g./hr. Higher values have been found in other experiments. With varying conditions of activity Evans [1912] found O_2 consumptions of 250 to 500 ml./100 g./hr., and other results fall within this range [Starling & Evans, 1914; Cruickshanks & Startup, 1934; Gremels, 1933; Cohn & Steele, 1935]. Few corresponding data are available for the *in vitro* respiration of the heart. Tsuneyoshi [1927] reported a Q_{O_2} of about 20 for the rabbit's heart and for the same tissue Stare & Baumann [1939] found a Q_{O_2} of 12–17. It is seen again that the oxidative processes in minced heart and in active intact heart are of the same order of magnitude.

The complete oxidation of 1 mol. of pyruvate requires $2\frac{1}{2}$ mol. of O_2 . The figures given show that this ratio of O_2 to pyruvate was approached but never attained. This is possibly explained by the simultaneous anaerobic disappearance of a certain amount of pyruvate, or possibly by incomplete oxidation of the pyruvate. As will be shown later this is known definitely to occur under certain conditions.

The addition of pyruvate in a concentration of 0.02*M* to heart muscle has a marked effect in increasing the respiration (Table 2). The effect as noted by Krebs & Eggleston in pigeon breast muscle is more marked in the later stages of the experiment. In the case of heart muscle the magnitude of the effect of pyruvate is much greater and results in an increase of about 150% in the 2 hr. period as compared with an increase of 30% in pigeon breast muscle. The increase in O_2 consumption on addition of pyruvate is not however sufficient to account for the pyruvate which disappears, so that the added pyruvate is presumably oxidized in preference to other substances already present in the heart.

Effect of the concentration of pyruvate. The increase in O_2 consumption on the addition of pyruvate depends on the concentration of pyruvate used. Maximal respiration and pyruvate usage over 2 hr. periods were obtained with about 0.02*M* initial concentration. Experiments carried out for shorter periods showed

Table 3. *Effect of concentration of pyruvate on the O_2 consumption and pyruvate utilization*

(400 mg. heart muscle in 4 ml.)

Initial conc. of pyruvate <i>M</i>	O_2 consumption (μ l.)		Pyruvate used μ l. 60 min.	O_2 used Pyruvate used
	30 min.	60 min.		
0	547	1006	—	—
0.01	628	1273	585	2.18
0.02	588	1157	639	1.81
0.03	432	772	635	1.21
0.04	331	492	400	1.22
0.05	261	338	—	—
0	523	913	—	—
0.0075	624	1278	—	—
0.025	559	1189	—	—
0.04	516	1084	—	—
0.0075	535	1145	438	2.61
0.015	562	1118	524	2.13
0.03	598	1186	726	1.63
0	414	514	—	—
0.0075	676	1295	—	—
0.025	521	847	—	—

that lower concentrations (0.0075 *M*) usually caused a slightly higher rate of respiration, but that higher concentrations always caused a diminution of O_2 consumption, which in the case of the highest concentration tested, 0.05 *M*, reached 70% inhibition. This is illustrated in Table 3, which also includes some figures for the pyruvate disappearance with different initial concentrations of pyruvate. While the amount of pyruvate used does not always diminish with the rising concentration of pyruvate, the ratio of O_2 used to pyruvate does so in every case, so that only with the lowest concentrations is the pyruvate which disappears oxidized completely. This inhibition of the oxidative processes by large amounts of pyruvate is used later to demonstrate some of the intermediate products of pyruvate metabolism.

2. Malonate inhibition

Malonate inhibits both the O_2 consumption and the pyruvate usage of heart muscle. Table 4 gives the figures for pyruvate utilization. At the highest concentration of malonate used, 0.025 *M*, there is an inhibition of pyruvate usage up

Table 4. *Effect of malonate on the usage of pyruvate*

(353 mg. heart muscle in 4 ml.)

Conc. of malonate <i>M</i>	Initial conc. of pyruvate <i>M</i>	Pyruvate used in 120 min. μ l.
0	0.0075	882
0.001	0.0075	697
0	0.02	944
0.01	0.02	254
0	0.02	1333
0.025	0.02	238
0	0.0075	504
0.025	0.0075	206

Table 5. *Effect of malonate on the O_2 consumption of heart muscle*

(353 mg. heart muscle in 4 ml.)

Conc. of malonate <i>M</i>	Initial conc. of pyruvate <i>M</i>	O_2 consumption (μ l.)		
		30 min.	60 min.	120 min.
0	0	456	711	1001
0.001	0	290	485	570
0	0.02	690	1260	1868
0.001	0.02	383	755	979
0	0	430	701	1059
0.025	0	89	117	141
0	0.02	630	1249	2461
0.025	0.02	133	180	208
0	0.0075	601	1020	1151
0.025	0.0075	59	85	109
0	0.02	572	1138	2000
0.01	0.02	183	267	351
0	0.02	492	758	908
0.01	0.02	69	98	128

to about 80%. Since a part of the residual pyruvate disappearance is probably due to an anaerobic reaction, the inhibition of the oxidative removal of pyruvate must be regarded as higher than this value and possibly nearly complete.

Table 5 shows the very marked inhibition of O_2 consumption by various concentrations of malonate. The O_2 consumption is also greatly reduced by malonate when no pyruvate is added.

Table 6. *Effect of fumarate on the removal of pyruvate in the presence of malonate*

(4 ml. suspension)

Heart muscle mg.	Initial conc. of pyruvate <i>M</i>	Malonate <i>M</i>	Fumarate added μ l.	Pyruvate used μ l.	Extra pyruvate μ l.
353	0.0075	0.001	0	697	—
353	0.0075	0.001	448	1027	330
353	0.02	0.025	0	238	—
353	0.02	0.025	448	683	445
353	0.0075	0.025	0	429	—
353	0.0075	0.025	56	536	107
353	0.0075	0.025	112	560	131
353	0.02	0.01	0	254	—
353	0.02	0.01	224	524	270
353	0.02	0.01	448	574	320
353	0.0125	0.025	0	65	—
353	0.0125	0.025	224	230	165
353	0.0125	0.025	448	388	323
400	0.01	0.025	0	109	—
400	0.01	0.025	56	177	68
400	0.01	0.025	112	211	102
400	0.01	0.025	224	330	221
400	0.01	0.025	448	586	477
353	0.02	0.001	0	312	—
353	0.02	0.001	56	545	233
353	0.02	0.001	112	590	278

Table 7. *Effect of fumarate on O_2 consumption in the presence of malonate*

(4 ml. suspension)

Heart muscle mg.	Duration of exp. min.	Initial pyruvate conc. <i>M</i>	Malonate conc. <i>M</i>	Fumarate added μ l.	O_2 used μ l.	Extra O_2 μ l.
353	120	0.0075	0.001	0	979	—
353	120	0.0075	0.001	448	2325	1346
353	120	0.02	0.025	0	208	—
353	120	0.02	0.025	448	1098	890
353	120	0.0075	0.025	0	383	—
353	120	0.0075	0.025	56	575	192
353	120	0.0075	0.025	112	716	333
353	120	0.0075	0.025	224	999	616
353	120	0.0075	0.025	448	1474	1091
353	120	0.0125	0.025	0	194	—
353	120	0.0125	0.025	224	625	431
353	120	0.0125	0.025	448	902	708
353	120	0.02	0.01	0	210	—
353	120	0.02	0.01	56	452	242
353	120	0.02	0.01	112	607	397
353	120	0.02	0.01	224	789	579
353	120	0.02	0.01	448	941	731
400	90	0.01	0.025	0	187	—
400	90	0.01	0.025	56	312	125
400	90	0.01	0.025	112	434	247
400	90	0.01	0.025	224	603	416
400	90	0.01	0.025	448	1052	865

Effect of fumarate. The effect of fumarate in removing the malonate inhibition of both pyruvate usage and O_2 consumption is shown in Tables 6 and 7. The extra amount of pyruvate which disappears in the presence of malonate is roughly proportional to the amount of fumarate added, the relation being 1 mol. of pyruvate to 1 mol. of added fumarate. These results are explained by the hypothesis of the citric acid cycle, and so far no alternative explanation is available. In the case of lower concentrations of malonate the fumarate may produce a catalytic action, as illustrated in the last experiment quoted in Table 6, where in presence of a concentration of 0.001 *M* malonate the addition of 56 and 112 μ l. of fumarate led to the removal of 233 and 278 μ l. of pyruvate. In this case the malonate inhibition, owing to its competitive nature, is overcome by the succinate formed during oxidation of the pyruvate.

The O_2 consumption is also increased by the addition of fumarate and the increase bears a fairly definite relationship to the amount of fumarate added. According to equation (1) the formation of succinate from fumarate and pyruvate in the presence of malonate requires 2 mol. of O_2 for each mol. of fumarate and from Table 7 it is seen that the extra O_2 used is of this order.

Effect of citrate and α -ketoglutarate. Since citrate and α -ketoglutarate, according to the citric acid cycle, are intermediate products in the formation of succinate from pyruvate and fumarate, they should be capable of increasing the respiration in the presence of malonate. With α -ketoglutarate this is certainly true and in some cases the extra O_2 on addition of ketoglutarate is of the order of $\frac{1}{2}$ mol. of O_2 to 1 mol. of ketoglutarate, i.e. the amount necessary to convert the ketoglutarate into succinate (Table 8). In the case of citrate there is some variation

Table 8. *Effect of citrate and α -ketoglutarate on the O_2 consumption in presence of malonate*

(4 ml. suspension)					
Heart muscle mg.	Duration of exp. min.	Malonate conc. <i>M</i>	Citrate added μ l.	α -ketoglutarate added μ l.	O_2 used μ l.
353	90	0.025	—	—	155
353	90	0.025	448	—	186
353	90	0.025	—	448	232
353	120	0.01	—	—	187
353	120	0.01	448	—	53
353	120	0.01	—	448	226
353	60	0.005	—	—	157
353	60	0.005	448	—	56
353	60	0.00125	—	—	301
353	60	0.00125	448	—	49
353	120	0.01	—	—	316
353	120	0.01	224	—	407
353	120	0.01	448	—	503
353	115	0.005	—	—	249
353	115	0.005	896	—	393
353	115	0.005	—	896	590

in the results, as in some cases the addition of citrate in the presence of malonate increases the respiration, while in others the inhibition of respiration is more marked on the addition of citrate. Somewhat similar observations were made by Stare & Baumann [1939]. Even where there is an increase in respiration this is not so great as that produced by addition of ketoglutarate, although the amount of O_2 required to convert citrate into succinate is twice that required to convert the corresponding amount of α -ketoglutarate.

There are several possible explanations for the citrate effects.

(1) The malonate may cause an inhibition of the citric dehydrogenase as suggested by Stare & Baumann [1939].

(2) The succinic and other dehydrogenases may be inhibited by citrate.

(3) Since the oxidation of citrate is associated with the reduction of oxaloacetate the amounts of this substance present will influence the effect of citrate on the malonate inhibition.

These possibilities are being further investigated.

Formation of succinate. According to equation (1) the fumarate added in the presence of malonate is converted into succinate, the intermediate products of reaction being citrate and α -ketoglutarate. That this occurs in heart muscle is shown by the fact that succinate can be recovered on addition of these substances in the presence of malonate (Table 9). It has already been pointed out that under

Table 9. *Formation of succinate in the presence of malonate*

(4 ml. suspension)						
(a) From fumarate						
Heart muscle mg.	Duration of exp. min.	Initial conc. of pyruvate <i>M</i>	Conc. of malonate <i>M</i>	Fumarate added μ l.	Succinate formed (μ l.)	
					Total	Extra
353	140	0.0125	0.01	—	114	—
353	140	0.0125	0.01	224	332	218
353	140	0.0125	0.01	448	402	288
353	120	0.02	0.01	—	60	—
353	120	0.02	0.01	448	290	230
400	60	0.01	0.025	—	104	—
400	60	0.01	0.025	112	180	76
400	60	0.01	0.025	224	254	150
353	140	—	0.01	—	92	—
353	140	—	0.01	224	296	204
400	60	—	0.025	—	96	—
400	60	—	0.025	112	140	44
400	60	—	0.025	224	182	86
(b) From citrate and oxaloacetate						
Heart muscle mg.	Duration of exp. min.	Substrate and amount added		Conc. of malonate <i>M</i>	Succinate formed μ l.	Extra succinate μ l.
353	120	None		0.01	60	—
353	120	224 μ l. citrate		0.01	161	101
353	120	448 μ l. citrate		0.01	258	198
353	120	448 μ l. oxaloacetate		0.01	167	107
353	120	448 μ l. oxaloacetate + 448 pyruvate }		0.01	322	262
400	90	872 μ l. citrate		0.01	40	—
		1344 μ l. citrate		0.01	260	—
		2688 μ l. citrate		0.01	232	—

these conditions conversion of citrate into succinate is incomplete, and this is in agreement with the smaller amounts of succinate found on addition of citrate as compared with fumarate. It is also seen that even in the absence of added pyruvate a certain amount of succinate is formed. If it is assumed that also in the absence of added pyruvate succinate is formed by reaction (1), it follows that the heart can form pyruvate from substances already present, and hence that pyruvate is normally an intermediate product in the metabolism of the heart muscle.

3. *Formation of citrate and α -ketoglutarate*

High concentrations of pyruvate have been shown to cause an inhibition of the respiration and an incomplete oxidation of the pyruvate which disappears. This inhibition was used by Krebs & Eggleston [1940] to demonstrate the formation of the intermediate products of reaction, citrate and α -ketoglutarate. Table 10 shows that formation of these substances also takes place in the heart. While small amounts of citrate are formed in the absence of added fumarate the amounts formed are greatly increased by the addition of this substance.

Table 10. *Formation of citrate and α -ketoglutarate*

Heart muscle mg.	Initial conc. of pyruvate <i>M</i>	Fumarate added μ l.	Ketoglutarate formed μ l.	Citrate formed μ l.
353	0	—	84	20
353	0.02	—	163	20
353	0.04	—	166	—
353	0.04	448	308	—
353	0.04	—	138	9
353	0.04	448	412	30
400	0.02	—	—	17
400	0.02	224	—	51
400	0.04	—	—	17
400	0.04	224	—	38

4. *Usage of citrate*

Since Breusch [1939] has questioned the usage of citrate by the heart, figures are given to show that citrate added to minced heart disappears (Table 11). The amount of citrate used is of the order of magnitude postulated by the citric acid cycle.

Table 11. *Aerobic and anaerobic usage of citrate*

(4 ml. suspension)

(a) Aerobic

Heart muscle mg.	Period of exp. min.	Initial conc. of citrate <i>M</i>	Citrate used μ l.
400	90	0.0075	288
400	90	0.015	474
400	90	0.03	326
353	90	0.01	269

(b) Anaerobic

Heart muscle mg.	Period of exp. min.	Initial conc. of citrate <i>M</i>	Initial conc. of oxaloacetate <i>M</i>	Change in citrate μ l.	Ketoglutarate formed μ l.
666	60	0.02	0	- 11	0
666	60	0.02	0.02	- 449	782
666	60	0	0.02	+ 231	340

Citrate can also be used anaerobically in the presence of oxaloacetate in the same way as in pigeon breast muscle. [Krebs, 1940]. From the figures in Table 11 it may be concluded that the reaction described by Krebs also occurs in heart muscle

5. *Maintenance of the respiration by substrates other than pyruvate*

Stare & Baumann [1939] have shown that citrate and fumarate can each cause increase in the respiration of the heart. The effect of these and other substances was investigated. Table 12 shows the effects of a number of substances

Table 12. *Rate of respiration by heart muscle in the presence of various substances*

Heart muscle mg.	Substrate added	Conc. of added substrate <i>M</i>	O ₂ consumption		
			30 min.	60 min.	120 min.
353	—	—	391	533	590
353	Citrate	0.01	557	824	905
353	α -Ketoglutarate	0.01	747	1493	1945
400	—	—	300	500	659
400	Pyruvate	0.01	653	1273	2141
400	Fumarate	0.01	486	959	1745
400	Citrate	0.01	530	980	1604
400	Succinate	0.01	729	1208	1852
400	Aspartate	0.01	319	560	783
400	Glutamate	0.01	645	1285	1953
400	Fumarate	0.0025	715	1482	2480
	Pyruvate	0.01			
353	—	—	368	441	493
353	Citrate	0.005	423	834	1089
353	Citrate	0.01	543	883	1069
353	Citrate	0.02	623	1078	1440
353	Pyruvate	0.02	570	1175	1860
353	—	—	317	461	—
353	Citrate	0.01	383	864	—
(90 min.)					
400	—	—	456	821	1213
400	Citrate	0.0075	624	1220	1825
400	Citrate	0.015	558	1116	1676
400	Citrate	0.03	513	989	1415

on the O₂ consumption of the heart. These include most of the substances of the citric acid cycle, and also the related substances aspartate and glutamate. All of the substances tested with the exception of aspartate increase and maintain the respiration of the heart. Citrate would appear to have a smaller effect than the other substances tested, and also the effect of citrate may decrease with increasing concentrations.

DISCUSSION

It has been shown that the main reactions on which the citric acid cycle is based occur in minced heart muscle. These reactions are the malonate inhibition of O₂ consumption and pyruvate usage, the specific effect of fumarate in combining with pyruvate to form succinate in the presence of malonate, the formation of citrate and α -ketoglutarate, and the effect of the substances involved in the citric acid cycle in maintaining respiration.

This view that the citric acid cycle plays a part in the metabolism of heart muscle is supported by the work of Hallman & Simola [1939], who showed that the synthesis of citric acid in heart muscle can occur in the presence of pyruvate and fumarate or oxaloacetate, and also that the citric acid can break down to α -ketoglutarate, succinate, fumarate, malate and oxaloacetate.

Heart muscle also provides an opportunity not given by skeletal muscle of comparing the relative rates of the oxidative processes in minced tissue and in

physiologically active tissue. The pyruvate consumption found is of the same order as that in the intact beating heart, while the O_2 consumption approaches the highest values found for the heart working at maximal effort. While the data from which these comparisons are made are obtained from different species, observations by Van Heyningen [1936] indicate a uniformity in the rate of metabolism in the same tissue in different species irrespective of the size of the animal.

In the intact heart Braun-Menendez *et al.* [1939] found that high concentrations of pyruvate gave rise to extra-systoles and other cardiac irregularities. It is of interest that the concentration of pyruvate (above $0.02 M$) which produced these effects caused in the minced heart a decrease in the stimulating effect of pyruvate on respiration and an incomplete oxidation of the pyruvate used.

Certain observations made here point to pyruvate as a normal intermediate substance in the metabolism of the heart. These are:

- (1) The effect of pyruvate in maintaining the respiration of the heart in the absence of other added substrates.
- (2) The inhibition of respiration by malonate even in the absence of added pyruvate.
- (3) The ability of fumarate in these conditions to restore partially the respiration and to form succinate.

SUMMARY

1. Minced heart muscle of the sheep used O_2 and pyruvate at about the same rate as the intact working heart of the dog.

2. Both the O_2 consumption and the pyruvate utilization are inhibited by malonate.

3. This inhibition is partially removed by the addition of fumarate. Each mol. of fumarate added causes the removal of one extra mol. of pyruvate, and an extra consumption of 2 to 3 mol. of O_2 . Succinate is formed in amounts approximately equivalent to the added fumarate.

4. Addition of pyruvate in concentrations up to $0.02 M$ maintains the respiration of the minced heart. Higher concentrations have an inhibitory effect on O_2 consumption, and the pyruvate which disappears is incompletely oxidized.

5. The inhibitory action of high concentrations of pyruvate results in the appearance of citrate and α -ketoglutarate. The amounts of these formed are increased by the addition of fumarate.

6. Citrate added to minced heart muscle disappears in presence of molecular O_2 and also anaerobically in presence of oxaloacetate.

7. The substances involved in the citric acid cycle, pyruvate, fumarate, succinate, citrate and α -ketoglutarate, are all capable of increasing the respiration of the heart.

8. These observations support the view that pyruvate is oxidized in the heart by way of the citric acid cycle and they cannot be satisfactorily explained by any other hypothesis.

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