CCXIV. THE INTENSITY OF SUCCINATE OXIDATION IN SURVIVING LIVER TISSUE

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IN previous studies [Rosenthal, 1935] on the metabolism of surviving rat liver the question arose as to the extent to which the great differences in the respiration of liver tissues of individual rats are due to differences in the amount of "respiration ferment" (indophenol oxidase-cytochrome system).

Measurement of the speed of oxidation of *p*-phenylenediamine—the direct determination of the amount of indophenol oxidase—does not give satisfactory results in the case of tissue slices. The autoxidation of the base interferes with the accuracy of the measurement; furthermore, the precipitate of dyestuff which is formed on the surface of the slices seems to injure the tissue. On the other hand, the measurement of the oxidation of succinate by tissue slices provides a suitable method for the determination of the minimum amount of indophenol oxidase in the organ, since succinate is oxidized by molecular O_2 through the indophenol oxidase-cytochrome system.

The speed of succinate oxidation by slices of rat liver is extremely high, much higher than one might expect from the results of Krebs [1933], Edson [1936] and Califano [1937]. The speed of this oxidation exceeds the speed of the oxidation of other substrates by several hundred % and remains at this level for a long period of time. There is relatively little fluctuation in the values obtained from different livers.

In the present paper the conditions under which the maximum speed of succinate oxidation can be measured in slices of rat liver will be shown. The values for the amount of "respiration ferment" in different livers obtained by this method will be compared with the values of the respiration with and without addition of other substrates.

EXPERIMENTAL

(a) *The manometric method*

Oxygen consumption was measured by the manometric method of Warburg [1930]. The measurement started 12 min. after placing the manometers in the thermostat (37.5°). $(Q_{O_2})_a$ indicates the O_2 uptake during the first 20 min. or during that part of the first hour of the experiment in which the respiration is constant. $(Q_{O_2})_b$ indicates the average O_2 uptake during the second hour. In the tables the $(Q_{O_2})_b$ values are not included but the quotient $(b - a/a) \times 100$ is given in order to show the percentage change in the respiration between the initial period and the second hour of the experiment.

Respiration measurements were carried out by both the indirect and the direct methods of Warburg. For the indirect method, box-shaped vessels of about 10 ml. volume, containing 1.5 and 3.5 ml. of suspension medium, were used; the gas space was filled with O_2 containing 5% CO_2 . For the direct method, sometimes similar vessels with side bulb were used, and sometimes conical vessels of about 18 ml. volume with inner tube and side bulb. 2 ml. of suspension medium were used, the vessel was filled with pure O_2 and the side bulb or the inner tube contained 0.2 ml. KOH solution for absorption of CO_2 . Sugar-free Ringer solution was used as suspension medium. For the indirect method the medium contained $2.6 \times 10^{-2} M$ bicarbonate (Warburg's "Ringer solution for glycolysis"), and for the direct method $3 \times 10^{-3} M$ bicarbonate ("Ringer solution for respiration"). Other media used in the direct method are mentioned in section (c). The results given below for experiments with added succinate were all obtained by the direct method. The values calculated from the indirect method fluctuated irregularly and a very large apparent acid disappearance was found, which was evidently due to some unsuitability in the method, since succinate is oxidized only as far as the fumarate-malate step [Elliott *et al.* 1937]. Probably the requirements for a correct calculation of the respiration by the indirect method—equal conditions of substrate concentration and O_2 provision in the two vessels with different fluid volumes—could not be fulfilled, although the amounts of tissue used were as low as possible and increase in the rate of shaking did not increase the O_2 uptake. However, to measure the succinate oxidation in the higher bicarbonate medium, it was sufficient to use only one manometer with the smaller amount of medium, since all other metabolic processes which could produce pressure changes are negligible compared with the succinate oxidation. The pressure changes observed in such a medium without CO_2 absorption by KOH are practically exactly the same as in a parallel experiment with the ordinary direct method, with CO_2 adsorption, for the same time and with the same weight of tissue.

(b) *Concentration of succinate and thickness of slices*

Even in the presence of $M/50$ succinate the rate of O_2 uptake is so high (see Table I) that an optimum oxygen supply is uncertain in the case of slices of the usual thickness. In fact a considerable variation of the Q_{O_2} values was found among different samples of the same liver.

Table I. *Influence of succinate concentration on the rate of succinate oxidation*

Succinate molarity $\times 10^{-2}$		0	0.1	2	4
$-(Q_{O_2}) a$	No. 1	6.2	14.1	52.0	—
	No. 2	5.0	—	59.0	91.0

From Warburg's formula for the limiting permissible thickness of slices (d'), the following relationship between Q_{O_2} and d' values can be calculated: $Q_{O_2} = -50$, $d' = 0.2$ mm.; $Q_{O_2} = -100$, $d' = 0.15$ mm.; $Q_{O_2} = -200$, $d' = 0.105$ mm. The thickness (d) of the slices usually varied between 0.15 and 0.20 mm. This means that d was not below the limiting value for Q_{O_2} values higher than 50.

The d' and d values indicated here are calculated for a wet weight/dry weight ratio of 3.3, although this ratio, valid for fresh liver, is higher for slices in saline solution because of swelling and loss of weight. However, the value 3.3 was

preferred since it is practically a constant in healthy animals, and since Krogh's diffusion coefficient of oxygen, the basis of Warburg's formula, is calculated for fresh and not for swollen tissue. Moreover, most of the error in the calculation of the d values is caused by the loss of weight during the manometric measurement and will be compensated for since the corresponding Q_{O_2} values are calculated for the same dry weight which may be too low by about 30 % [cf. Elliott *et al.* 1937].

For the determination of the optimum succinate concentration slices thinner than 0.15 mm. were chosen. A representative experiment can be seen in Table I, no. 2. The thickness of the slices was 0.12 mm. Small variations of succinate concentration in the neighbourhood of $M/25$ did not affect the rate of O_2 uptake and, since it was not possible to have reasonably isotonic salt solutions of much greater succinate concentration than this, $M/25$ succinate solution was used in the following investigations.

Decreasing the thickness of the slices below 0.12 mm. does not increase the rate of oxidation to a significant extent. Examples are given in Table II. As

Table II. *Influence of the thickness of slices on the rate of succinate oxidation*

Group	No. of exp.	d (mm.)	$-(Q_{O_2})a$	$\frac{\Delta d}{\%}$	$\frac{\Delta Q_{O_2}}{\%}$	Remarks
$d > 0.106$	9	0.122	85.2	—	—	Mean values
$d < 0.106$	8	0.089	96.3	-27	+13	
Exp. 9 of Table III	—	0.110	103	—	—	
	—	0.067	119	-40	+15	

it was impossible to choose slices of the same liver in such a manner that a large range of thickness below 0.15 mm. could be covered, the average values for the respiration of thin and thick slices in 17 experiments were calculated. The mean values for all the experiments were: $d = 0.106$; $Q_{O_2} = -90$. In 9 experiments d was higher and in 8 experiments lower than 0.106. On an average, a decrease in thickness of 27 % below 0.12 increases the rate of oxidation by only 13 %. The single experiment of Table II in which a great variation of the thickness occurred confirms this conclusion. Thus a thickness of slice of about 0.12 mm. and a succinate concentration of $M/25$ are suitable for the determination of the maximum speed of succinate oxidation.

(c) Buffering

Ringer solution "for respiration", which contains a small amount of bicarbonate, can be used for the measurement of the rate of succinate oxidation by the direct method over short experimental periods. The absorption of CO_2 by the KOH causes a steady conversion of the bicarbonate in the medium into carbonate. With larger amounts of tissue slices the carbonate is neutralized by small amounts of acid produced by the tissue. With the small amounts of tissue which have to be used for estimating succinate oxidation on account of the very rapid O_2 uptake, not enough acid bodies are formed and the reaction of the medium becomes alkaline. As a result the succinate oxidation rate falls off rapidly in the second hour even though such high concentrations of succinate are present that the succinate concentration is inappreciably affected by the amount used up. In a bicarbonate-free, $M/25$ succinate Ringer solution neutralized with NaOH to 7.3–7.4, the respiration remains constant for 2 hr. In most of the experiments quoted in Table III this medium proved to be a sufficient

Table III. *Comparison of the rates of spontaneous respiration and succinate oxidation in rat liver*

No.	Diet*	O		S			$\frac{Q_{O_2}}{S - \frac{Q_{O_2}}{O}}$ %	Remarks‡
		No addition		+ M/25 succinate				
		$-(Q_{O_2})a$	$b - a/a^\dagger$ %	d mm.	$-(Q_{O_2})a$	$b - a/a^\dagger$ %		
Group I								
1	f.	10.1	-21	0.104	84.0	+ 5	+ 731	—
2	f.	8.36	-22	0.096	71.0	+18	+ 749	—
3	f.	10.4	-25	0.114	75.5	- 0.5	+ 626	P
4	st. 12	9.25	-19	0.078	129.0	-30	+1292	—
5	st. 12	7.41	-18	0.123	72.0	(-36)	+ 875	—
6	st. 24	8.05	-29	0.146	72.5	-10	+ 801	—
7	st. 24	7.63	-40	0.109	78.9	- 4	+ 936	—
8	st. 24	10.9	-10	0.116	110.0	- 3	+ 910	—
9	st. 23	10.3	-32	0.110	103.0	(-45)	+ 900	—
Average		9.13	-24	0.111	88.4	- 3§	+ 869	
Group II								
10	f.	6.71	-16	0.084	79.6	-10	+1080	P
11	st. 12	3.63	-43	0.137	84.5	- 5	+2220	—
12	st. 20	4.35	-30	0.080	100.0	-11	+2180	P
13	st. 46	5.91	-61	0.081	104.0	(-54)	+1660	—
14	st. 60	6.41	-58	0.123	74.3	- 2	+1060	—
15	st. 48	5.04	-67	0.120	91.0	-17	+1705	—
16	st. 53	3.96	-47	0.086	97.5	-13	+2100	P
17	st. 60	2.43	-26	0.106	105.0	(-52)	+4220	—
Average		4.58	-44	0.101	92.0	-10§	+2080	

* f.=well-fed animals; st.=starved animals. The figures indicate the period of starvation in hours.

† $b - a/a$ =percentage difference between the O_2 uptake rate in the second hour and the initial period.

‡ In experiments marked P, the medium was Ringer-phosphate solution. See section (c).

§ In calculating averages the figures in brackets were neglected since in these cases the pH had changed considerably. See section (c).

buffer system. In some experiments, however, the speed decreased during the second hour of experiment and the pH was found to be increased. Possibly, the NaOH solution used for the neutralization of the succinic acid was not free from bicarbonate. The $b - a/a$ values of these experiments are enclosed in brackets. In the experiments marked by a P, Ringer-phosphate solution of pH 7.4 [Dickens & Šimer, 1930], was used. The values are the same as in the bicarbonate-free Ringer solution. The respiration is also constant in the high bicarbonate- CO_2 buffer measured without CO_2 absorption, and its rate is the same as with the media used for the direct method. This shows that neither phosphate nor bicarbonate, as such, influences the succinate oxidation, and it is only the maintenance of the optimum pH, about 7.4, which is of importance.

(d) *Stability of succinic oxidase*

If the O_2 uptake is measured on a slice which has been respiring for 30–60 min. without substrate before succinate is tipped into the vessel from a side bulb, the rate of succinate oxidation is always about 30% lower than in control slices which were kept in succinate solution from the beginning. Obviously, succinate protects the succinic oxidase against spontaneous inactivation. Analogous phenomena are known for many enzyme systems. Therefore only experiments in which succinate was present from the start are quoted in Table III.

RESULTS

If the rates of succinate oxidation of different livers are measured under the conditions described, the fluctuation of the values is considerably less than in the case of the oxidation of other substrates.

In Table III the rate of succinate oxidation is compared with the rate of respiration without addition of substrate (spontaneous respiration). In 9 experiments the Q_{O_2} values without added substrate were greater than 7 (group I). The mean value is 9.13. The decrease of the respiration rate $(b-a)/a$ amounts to 24% on an average. In 8 experiments the Q_{O_2} values without added substrate were lower than 7 (group II). In the latter group the average value for respiration is half as high as in the former group and the average decrease of the respiration twice as high. However, the average rate of O_2 uptake in the presence of succinate is the same in both groups, and there is little decrease in the rate with either group.

Most of the animals of group II were starved for different lengths of time. Therefore low values of the spontaneous respiration could be due to a deficiency of substrates, and it might be expected that the variations in the liver respiration would always disappear after addition of other substrates, as was the case after the addition of succinate. However, Meyerhof & Lohmann [1926] have shown that the oxidation of lactate and pyruvate by liver tissue is diminished after rather long periods of starvation. Mongiu [1934] found an analogous phenomenon in the case of acetate oxidation.

In order to demonstrate the particular behaviour of succinate, the respiration of livers of well-fed and starved rats was also studied in the presence of other

Table IV. *Influence of nutrition on respiration processes in rat liver. Mean values*

Date	No. of exp.	O		Substrate	S		Q_{O_2} $S-O/O^*$ %	Experiments in which $S-O/O > 20\%$		
		No addition			With addition			No. of exp.	% of exp.	$S-O/O \dagger$ %
		$-(Q_{O_2})a$	$b-a/a$ %		$-(Q_{O_2})a$	$b-a/a$ %				
f.	16	10.1	-21	—	—	—	—	—	—	
st. 12-27	10	7.19	-28	—	—	—	—	—	—	
st. 36-60	24	5.70	-39	—	—	—	—	—	—	
f.	5	9.89	-32	Lactate	10.1	-34	+ 10	0	0	+ 0
st. 12-26	5	8.05	-23		11.8	-39	+ 46	3	60	+ 86
st. 36-60	10	6.16	-36		7.35	-41	+ 16	4	40	+ 47
f.	2	9.23	-21	Pyruvate	12.0	-5	+ 30	2	100	+ 30
st. 12-24	4	8.60	-25		9.68	-45	+ 13	3	75	+ 30
st. 48-60	5	5.99	-50		6.32	-50	+ 6	1	20	+ 65
f.	2	10.0	-25	Glycero- phosphate	14.5	-37	+ 45	1	50	+ 81
st. 12-24	5	7.52	-33		13.4	-47	+ 78	5	100	+ 201
st. 48-60	5	4.42	-52		9.06	-49	+ 105	5	100	+ 112
f.	4	8.89	-21	Succinate	77.5	+ 3	+ 772	4	100	+ 796
st. 12-24	8	7.69	-28		92.7	-11	+ 1110	8	100	+ 1264
st. 48-60	5	4.75	-52		94.5	-11	+ 1890	5	100	+ 2149

* Calculated from the mean Q_{O_2} values in O and S given in the table.

† Average of the $S-O/O$ values of those experiments in which $S-O/O$ was greater than 20%.

substrates. These experiments are shown in Table IV. In the case of the spontaneous respiration and of pyruvate and lactate oxidation, some of the measurements were carried out with the indirect method and some with the direct

method. The results of both methods are the same. For glycerophosphate¹ only results obtained by the indirect method are given, since in experiments carried out by the direct method in a bicarbonate-free medium the values were lower. In addition to the mean value for the increase of the respiration by substrates ($S-O/O \times 100$), Table IV also shows the number of experiments in which the increase exceeds 20 % and the average increase in these experiments. This arrangement allows one to judge whether the mean values for all experiments may be considered as typical; for differences of the Q_{O_2} values below 20 % may be found occasionally among samples of the same liver.

From the figures in Table IV it can be seen that the speed of succinate oxidation is to some extent higher in starved liver than in the liver of well-fed animals, possibly because of a lessening of substrate competition by starvation. The oxidation of the other substrates shows a similar behaviour. However, with substrates other than succinate the increase in the speed of oxidation of starved liver is usually less regular showing a maximum when the starvation period is short and a lessening in magnitude or regularity after longer periods of starvation. Furthermore, only succinate is able to prevent the decrease in respiration which normally occurs over the 2 hr. experimental period. The following details about the action of the various substrates may be mentioned.

Lactate ($M/50$) does not increase the respiration of the liver of well-fed animals, but it may be metabolized. This is indicated by the fact that in experiments carried out by the indirect method the quotient Q_S/Q_{O_2} ² is always decreased. Bach & Holmes [1937] found that respiration increases more frequently if $M/33$ lactate is used. However, in starvation liver no significant differences in the effects of $M/50$ and $M/33$ lactate were found. After rather long periods of starvation, the increase of the respiration is smaller and less regular and the quotient Q_S/Q_{O_2} is diminished only during the initial period of the experiment. The latter fact may suggest that the metabolizing of lactate stops very soon in starved liver. That not the fat content of the liver but the time of starvation is the decisive factor has been shown in a previous paper [Rosenthal, 1936].

Pyruvate ($M/100$ – $M/50$), in general, increases respiration in the livers of well-fed animals or animals starved for short periods. After longer periods of starvation, the respiration is sometimes diminished, and the quotient Q_S/Q_{O_2} increased. Elliott *et al.* [1937] have also observed occasional lowering of respiration in the presence of pyruvate and, at the same time, an increase in r.q. They suggest that pyruvate may act as a hydrogen acceptor in place of oxygen. It seems to me that a lowering of respiration will occur under these conditions if the oxidation of lactate, formed by the reduction of pyruvate, decreases as in the case of starved liver.

β -*Glycerophosphate* ($M/100$ – $M/50$) is the substrate most similar to succinate in so far as it increases respiration quite regularly and exerts a relatively strong action even after rather long periods of starvation. It differs from succinate in that it produces the maximum increases of respiration after short periods of starvation and lacks the ability of succinate to maintain the speed of oxidation for 2 hr.

In summary it can be said that while the speed of the oxidation of some substances by liver tissue can be changed by starving the animals, the speed of

¹ Sodium glycerophosphate, crystalline, Fraenkel and Landau.

² Respiration CO_2 plus CO_2 liberated by acid formation/ O_2 uptake. A diminution of the quotient indicates a removal of acids (or an inhibition of the formation of acids) provided that the r.q. and the ammonia production do not change considerably

succinate oxidation is not influenced by starvation. This fact proves that starvation does not affect the oxidase-cytochrome system of the cell, and it suggests that determination of the rate of succinate oxidation, when measured under optimum conditions, would be a useful method for detecting whether changes in the respiration of surviving tissues are due to changes in the oxygen-activating system.

DISCUSSION

(1) Measurement of the maximum rate of succinate oxidation by tissue slices should provide an estimate of the respiratory ferment activity of which the cells are capable under the conditions under which studies of the metabolism of surviving tissue and the oxidation of metabolites are commonly made. It is necessary, however, to deviate from these conditions to some extent, since, on account of the high rate of succinate oxidation, the slices used have to be of a thinness at which, according to Quastel & Wheatley [1934] and Druckrey [1935], only incomplete oxidation of other metabolites occurs and the respiration ceases after a short time. This disadvantage of extra thin slices is presumably due to the more rapid washing out of diffusible constituents of enzyme systems, such as coenzymes. The succinic oxidase system, however, contains no such diffusible components and its activity remains constant and indeed it shows its fullest activity in the very thin slices since the competition of other activated substrates for the respiratory ferment is lessened. It might be expected that greater damage to the cell structure would render the thin slices unsuitable as material for comparison with the slices of usual thickness used in metabolism studies. Quastel [1932] first showed that the succinate oxidation of muscle is much decreased after grinding with sand. Preliminary experiments have shown that the same applies to liver. The O_2 uptake rate of a thin slice of liver with succinate was measured. The slice was then ground with quartz sand, cooled in ice, resuspended in succinate-Ringer solution and the O_2 uptake measured. The rate was only about 10 % of that of the intact slice. The O_2 uptake rates of portions of the same sample of tissue, ground with varying thoroughness, were found to be smaller the fewer cells could be recognized microscopically; but even a cell-rich suspension was less active than a thin slice.

Presumably destruction of the cell structure leads to a separation of the various components of the succinic oxidase system and so to a "dilution effect" such as Krebs [1935] has described and discussed for reactions of higher order.¹ However, since the succinate oxidation is actually more constant with the thin slices than is ever found with other enzyme-containing extracts or suspensions, it is evident that washing-out effects are not disturbing the measurements, and the activity is so high that the cell destruction effects must be comparatively small. It seems therefore justifiable to assume that results obtained with the extra thin slices represent the approximate maximum succinate-oxidizing ability of the tissue under nearly physiological conditions.

(2) Comparison of the oxidations of succinate and other substrates has led to the conclusion that the modifications of tissue respiration in starved animals are not caused by alterations of the oxidase-cytochrome system. It is necessary therefore to seek alterations in other enzymes or their components, and the

¹ According to the figures given by Potter & Elvehjem [1936] this appears to be true also for liver preparations which were "homogenized" in a way which would cause less cell destruction. However, Elliott (private communication) has found that liver, homogenized by the method of Potter & Elvehjem, shows just as high succinate-oxidizing intensity as do thin slices, provided that the conditions are made favourable.

possibility of changes in the coenzyme content of the tissue suggests itself. The oxidations of lactate and pyruvate, which were particularly influenced by starvation, are dependent on the presence of coenzymes. The necessity for the participation of cozymase in the oxidation of lactate has been shown by the investigations of Ogston & Green [1935] as well as by those of Euler & Alder [1936]. The metabolism of pyruvate leads, in addition to lactate, to substances such as fumarate and malate,¹ all of which need coenzymes for their oxidation [Green & Brosteaux, 1936; Banga & Szent-Györgi, 1937]. The oxidation of β -glycerophosphate was less influenced by starvation than was that of lactate and pyruvate. According to Meyerhof [1919], β -glycerophosphate, like succinate, can be oxidized by washed muscle preparations and liver granules. Obviously, then, this oxidation² does not need a coenzyme. However, as Meyerhof showed, the oxidation is incomplete. On the other hand, according to Euler *et al.* [1937], the carrying of hydrogen from α -glycerophosphate to pyruvate by muscle extract requires the aid of cozymase. This reaction could initiate a further breakdown of the substance. Perhaps, in liver tissue an analogous way of metabolizing β -glycerophosphate may be feasible in addition to its oxidation without the aid of coenzymes. Such a way is suggested by the fact that in livers in which lactate strongly increases respiration, the intensity and the course of lactate and glycerophosphate oxidation are identical. These two ways of oxidation would explain the intermediate position of the oxidation of glycerophosphate.

The succinate oxidation is distinguished not only by its independence of the state of nutrition of the animal but also by the constancy of its speed. Perhaps the falling off of other oxidations is due to the instability of their coenzymes. Thus, Euler & Günther [1936] have reported that cozymase is destroyed by liver slices. In the case of glycerophosphate oxidation an inhibition by derivatives may also be responsible to some extent. For instance, 3-phosphoglyceric acid inhibits the respiration of starved liver (unpublished experiments), and Green [1936] found an inhibition of α -glycerophosphate dehydrogenase by phosphoglyceric acid.

(3) The estimation of succinate oxidation is here suggested as a method for obtaining a minimum estimate of the oxidase-cytochrome system. But, as far as can be judged from estimations of indophenol oxidase (*p*-phenylenediamine oxidation) and succinic oxidase activity in heart and liver preparations [Ogston & Green, 1935], the amounts of the two do not differ greatly. If this is true, the estimation of the rate of succinate oxidation is to be preferred to that of *p*-phenylenediamine since succinate is a physiological substance which can be used with slices, and the use of slices is to be preferred for a direct comparison of cell respiration and oxidase content. Ogston & Green found that the O_2 uptake of sand-ground liver with *p*-phenylenediamine corresponded to about $Q_{O_2} = -10$, and concluded that the oxidase content of liver was sufficient to activate all the O_2 necessary for the total respiration. But the figures for succinate oxidation rates by slices, given in this paper, show that liver tissue contains a considerable excess of the oxidase-cytochrome system, so that the respiration rate of the liver must be limited not by a scarcity of "respiratory ferment" but by the amount of activated substrates. How far this situation applies to other tissues and whether the rate of succinate oxidation can be used generally as a measure of the "respiratory enzyme" content of cells remain to be studied.

¹ See e.g. Elliott & Schroeder [1934], Krebs [1936].

² Like the oxidation of α -glycerophosphate, according to Ogston & Green [1935].

SUMMARY

The determination of the intensity of succinate oxidation by liver slices is suggested as a method for estimating the amount of the oxidase-cytochrome system (respiration ferment) in the cell in order to elucidate the factors which cause the great differences in the respiration of the liver tissues of individual rats. The conditions for the measurement of the maximum speed of succinate oxidation are described and the following results obtained.

(1) The intensity of succinate oxidation by liver slices when measured under optimum conditions exceeds the intensity of other respiration processes by nearly 1000 %. The speed is constant for about 2 hr. The fluctuation of the values obtained from different livers is relatively small.

(2) Although the intensity of spontaneous respiration and the oxidation of lactate and pyruvate by liver slices are decreased in starved animals, the intensity of succinate oxidation is not influenced by starvation. The oxidation of glycerophosphate appears to be quite similar to that of succinate. It is inferred from this that starvation does not affect oxidation processes by decreasing the amount of respiration ferment in the cell. It is suggested that a decrease in the concentration of coenzymes in the cell may be responsible since the oxidations of lactate and pyruvate, in contrast to the oxidations of succinate and glycerophosphate, are known to require coenzymes.

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