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The Inhibition of Pyruvate and L$_s$(+)-Isocitrate Oxidation by Succinate Oxidation in Rat Liver Mitochondria

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1. The effects of succinate oxidation on pyruvate and also isocitrate oxidation by rat liver mitochondria were studied. 2. Succinate oxidation was without effect on pyruvate and isocitrate oxidation when respiration was maximally activated with ADP. 3. When respiration was partially inhibited by atractylate, succinate oxidation severely inhibited the oxidation of pyruvate and isocitrate. 4. This inhibitory effect of succinate was associated with a two- to three-fold increase in the reduction of mitochondrial NAD^+ but no change in the reduction of cytochrome *b*. 5. It is concluded that, in the partially energy-controlled state, respiration is more severely inhibited at the first phosphorylating site than at the other two. 6. The effects of succinate oxidation are compared with those of palmitoylcarnitine oxidation. It is concluded that a rapid flow of electrons directly into the respiratory chain at the level of cytochrome *b* is in itself inadequate to inhibit the oxidation of intramitochondrial NADH. 7. The effects of succinate oxidation on pyruvate oxidation were similar in rat heart and liver mitochondria.

When rat liver mitochondria are presented with two oxidizable substrates simultaneously, it is commonly observed that the rate of oxygen uptake with the two substrates together is less than the sum of that obtained from either alone. This effect has been observed with the following substrate pairs: pyruvate and 2-oxoglutarate (Haslam & Krebs, 1963), pyruvate and fumarate (König, Marosvari & Lipcsey, 1964), pyruvate and succinate (König & Szabados, 1966, 1967; Haslam, 1966), pyruvate and palmitoylcarnitine (Bremer, 1966; Nicholls, Shepherd & Garland, 1967) and pyruvate and octanoate (Walter, Paetkau & Lardy, 1966; Nicholls *et al.* 1967). In at least some instances the non-additive oxidation of substrate pairs is converted into additive oxidation by the addition of reagents that uncouple oxidative phosphorylation (König & Szabados, 1966; Nicholls *et al.* 1967).

The mechanisms that bring about non-additive oxidation of substrate pairs are not fully understood, and there is no reason to suppose that the same mechanism is responsible in all cases. The purpose of this paper is to describe the effects of succinate oxidation on pyruvate and isocitrate oxidation by rat liver mitochondria, with particular reference to the mitochondrial 'energy state' and also the comparable effects of palmitoylcarnitine oxidation.

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The terminology introduced by Chance & Williams (1956) to describe mitochondrial energy states is briefly described in Table 1, and is used throughout this paper. Whether the extreme states 3 and 4 have counterparts *in vivo* is not clearly known. Scholz & Bücher (1965) have concluded from the effect of 2,4-dinitrophenol on the isolated perfused rat liver that the intracellular mitochondrial state is somewhere between states 3 and 4. It seems reasonable that any tissue not functioning at its maximal energy-utilizing capacity (e.g. resting muscle) would also be in a state between 3 and 4. This condition, in which mitochondrial respiration is partially inhibited by the suboptimum availability of ADP, is referred to in this paper as state $3\frac{1}{2}$.

A mitochondrial suspension cannot be maintained in state $3\frac{1}{2}$ for more than a few seconds merely by setting the initial concentration of ADP at a low value; the apparent K_m of the mitochondrial adenine nucleotide transferase for ADP is low [about 20–30 μM (Chance & Williams 1956); 2–3 μM (Klingenberg & Pfaff, 1968)], and the phosphorylation of ADP rapidly brings the system into state 4. However, the K_m for ADP is increased by atractylate, a competitive inhibitor of the adenine nucleotide transferase (Klingenberg & Pfaff, 1966), and linear but partially inhibited rates of mitochondrial respiration can be obtained for several minutes in the presence of appropriate concentrations of ADP and atractylate.

Table 1. *Definitions of respiratory states of mitochondria*

The terminology is based on that of Chance & Williams (1956). In all the states listed oxygen and substrate supply are not rate-limiting for respiration.

State	Extra-mitochondrial ADP	Extra-mitochondrial phosphate	Inhibitor	Respiration	Intra-mitochondrial NAD	Energy state
3 (uncoupled)	Present	Present	Uncoupling agent	Activated	Oxidized	Minimal
3 (coupled)	Present	Present	None	Activated	Oxidized	Low
4	Absent	Present	None	Inhibited	Reduced	High
3½	Present	Present	Atractylate	Partially inhibited	Intermediate	Intermediate

MATERIALS AND METHODS

The preparation of rat liver mitochondria (Garland, Shepherd & Yates, 1965), the measurement of mitochondrial oxygen uptake and carbon dioxide output (Nicholls *et al.* 1967) and the sources of reagents have been described previously. Potassium atractylate was a kind gift from Dr S. Luciani. Mitochondrial NAD^+, NADH, $NADP^+$ and NADPH were assayed either fluorimetrically or with a dual-wavelength spectrophotometer in mitochondrial extracts as described by Klingenberg & Slenczka (1959). Rat heart mitochondria were prepared by the procedure of Chance & Hagihara (1961). Difference spectra of rat heart mitochondria were obtained with a wavelength-scanning spectrophotometer constructed in this laboratory. Changes in the reduction of cytochrome *b* in rat liver mitochondria were measured with a dual-wavelength spectrophotometer at 564nm. minus 575nm. (Chance & Williams, 1956).

RESULTS

Pyruvate oxidation by rat liver mitochondria. Fig. 1 shows continuously recorded measurements of mitochondrial oxygen uptake and carbon dioxide output, and the manner in which pyruvate oxidation (measured as carbon dioxide output) was inhibited on the addition of succinate.

The results of experiments performed in a similar manner to that of Fig. 1 are summarized in Table 2, from which it is apparent that: (i) succinate oxidation did not inhibit pyruvate oxidation when atractylate was absent, i.e. in state 3, whereas palmitoylcarnitine oxidation did; (ii) the presence of the uncoupling agent pentachlorophenol (Weinbach, Sheffield & Garbus, 1963) abolished the inhibitory effect of succinate oxidation on pyruvate oxidation; (iii) the oxidation of ascorbate and tetramethyl-*p*-phenylenediamine also inhibited pyruvate oxidation in the presence of atractylate. Further experiments demonstrated that this effect was abolished by the addition of pentachlorophenol (5 μM) or the deletion of atractylate. The calculation and significance of the electron flow rates shown in Table 2 are discussed below.

Isocitrate oxidation by rat liver mitochondria. In a previous paper (Nicholls & Garland, 1969) we concluded that, except in the presence of uncoupling agents, the oxidation of isocitrate by the

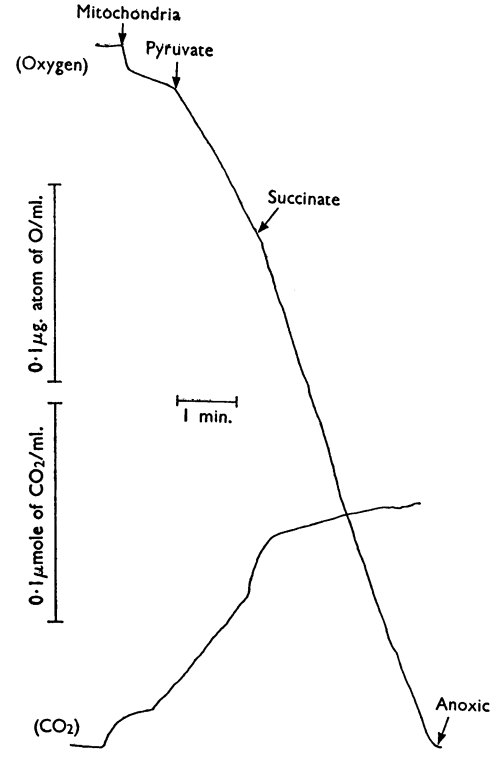


Fig. 1. Electrode measurements of rat liver mitochondrial oxygen uptake and carbon dioxide output. Initially 6·0ml. of air-saturated incubation medium at 37° contained KCl (80mM), tris–chloride buffer, pH7·2 (20mM), EDTA (1mM), potassium phosphate buffer, pH7·2 (3mM), *trans*-aconitate (8mM), ADP (2mM), atractylate (7 μM) and L-malate (2mM). Further additions shown in the figure were rat liver mitochondria (6·5mg. of protein), pyruvate (2mM) and succinate (3mM). The vertical bars indicate the electrode sensitivities. The apparent burst of CO_2 production on adding succinate is due to CO_2 in the succinate solution.

Table 2. *Inhibition of rat liver mitochondrial pyruvate oxidation by other substrates*

The experiments were performed in the manner of that shown in Fig. 1, except that atractylate (7 μM) was not present in all cases. Pentachlorophenol, when present, was 5 μM. The order of additions and observations was: (i) rat liver mitochondria (endogenous rates); (ii) pyruvate (increased rates due to pyruvate oxidation); (iii) the second substrate (carbon dioxide rate due to pyruvate oxidation, oxygen rate due to second substrate plus pyruvate oxidation). The second substrates were succinate (5 mM) or palmitoyl-DL-carnitine (14 μM) or tetramethyl-*p*-phenylenediamine (0·2 mM) with L-ascorbate (2 mM). The electron flow from succinate or electron-transferring flavoprotein to the respiratory chain was calculated as described in the legend of Scheme 1 and the text. Percentage reduction of NAD was calculated as 100[NADH]/[NADH + NAD^+]. Results are expressed per mg. of mitochondrial protein. Abbreviations: ETF, electron-transferring flavoprotein; PCP, pentachlorophenol; TMPD, tetramethyl-*p*-phenylenediamine.

Expt. no.	Inhibitor(s)	Substrate(s)	Carbon dioxide output (nmoles/min./mg.)	Oxygen uptake (ng.atoms/min./mg.)	Electron flow from succinate or ETF to respiratory chain (2 electron n-equivalents/min./mg.)	% reduction of NAD
1	None	Pyruvate	20	30	0	11
	None	Pyruvate + succinate	22	91	61	13
	None	Pyruvate + palmitoylcarnitine	2	79	37	24
2	Atractylate	Pyruvate	17	20	0	26
	Atractylate	Pyruvate + succinate	4	55	50	51
3	Atractylate + PCP	Pyruvate	22	30	0	12
	Atractylate + PCP	Pyruvate + succinate	25	105	80	13
4	None	Pyruvate	20	33	0	—
	None	Pyruvate + TMPD + ascorbate	21	92	0	—
5	Atractylate	Pyruvate	15	20	0	—
	Atractylate	Pyruvate + TMPD + ascorbate	6	80	0	—

respiratory chain of rat liver mitochondria proceeds almost exclusively through the NAD-specific isocitrate dehydrogenase (EC 1.1.1.41). The comments that follow therefore concern this enzyme rather than the NADP-specific isocitrate dehydrogenase (EC 1.1.1.42). Table 3 summarizes the results of experiments carried out in a similar manner to that of Fig. 1, except that isocitrate replaced pyruvate and arsenite replaced *trans*-aconitate. The effects of succinate oxidation on isocitrate oxidation are similar to those just described on pyruvate oxidation, in that inhibitory effects were observed in state $3\frac{1}{2}$ but not in state 3. By contrast, palmitoylcarnitine oxidation inhibited isocitrate oxidation in state 3.

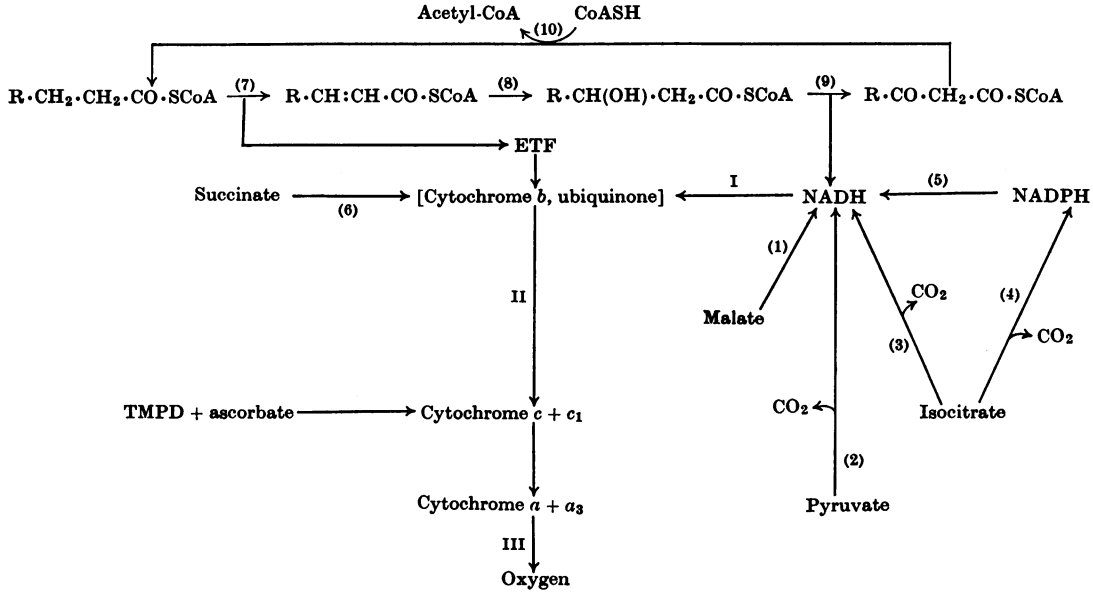
Calculated flow rates in different segments of the respiratory chain. The rates of oxygen uptake and carbon dioxide output reported in Tables 2 and 3 can be used to calculate the rate of two-electron transfer from electron-transferring flavoprotein or succinate to the respiratory chain at the level of cytochrome *b*. The basis for these calculations is shown in Scheme 1, which relates the various dehydrogenases to the respiratory chain. In addition the following assumptions are made: (1) that the oxidation of palmitoylcarnitine proceeds exclusively to acetoacetate rather than citrate; this is certainly not the case (Garland, Shepherd, Nicholls & Ontko, 1968), but the assumption permits a calculation of an upper limit for the flow from electron-transferring flavoprotein into the respiratory chain (Scheme 1); (ii) that exclusion of the endogenous rate from the measurements (Tables 2 and 3) does not seriously affect the calculated flow rates; this is so for succinate or palmitoylcarnitine oxidation where the oxidation rates were five to seven times the endogenous rate (e.g. Fig. 1); (iii) that pyruvate and isocitrate oxidation proceed with the ratio 1:1 between oxygen atoms consumed and carbon dioxide formed; this is so for isocitrate oxidation in the presence of arsenite (Nicholls *et al.* 1967), but for pyruvate oxidation the ratio would be 2:1 if citrate were the end product rather than acetoacetate, as the oxaloacetate used would be regenerated from malate.

Despite these limitations, the calculated flow

Table 3. *Inhibition of rat liver mitochondrial isocitrate oxidation by other substrates*

The experimental conditions and calculations were as described in Table 2, except that L-(+)-isocitrate (2mM) replaced pyruvate and sodium arsenite (0·2mM) replaced *trans*-aconitate. The rates of carbon dioxide output and oxygen uptake do not include the endogenous rates that occurred before the addition of substrate. Results are expressed per mg. of mitochondrial protein. Abbreviation: ETF, electron-transferring flavoprotein.

Expt. no.	Inhibitor	Substrate(s)	Carbon dioxide output (nmoles/min./mg.)	Oxygen uptake (ng.atoms/min./mg.)	Electron flow from succinate or ETF to respiratory chain (2 electron equivalents/min./mg.)	% reduction of NAD
1	None	Isocitrate	28	30	0	14
	None	Isocitrate+ succinate	31	94	63	20
2	Atractylate	Isocitrate	23	26	0	27
	Atractylate	Isocitrate+ succinate	12	87	75	50
3	None	Isocitrate	34	35	0	14
	None	Isocitrate+ palmitoylcarnitine	9	90	41	29



Scheme 1. Relationship between the respiratory chain and enzymes of fatty acid oxidation and the tricarboxylic acid cycle. Numbered enzymes are: (1) malate dehydrogenase (EC 1.1.1.37); (2) pyruvate dehydrogenase (EC 1.2.4.1); (3) NAD-specific isocitrate dehydrogenase (EC 1.1.1.41); (4) NADP-specific isocitrate dehydrogenase (EC 1.1.1.42); (5) a transhydrogenase (EC 1.6.1.1); (6) succinate dehydrogenase (EC 1.3.99.1); (7) acyl-CoA dehydrogenase (EC 1.3.2.2); (8) enoyl-CoA hydratase (EC 4.2.1.17); (9) L-3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35); (10) 3-oxoacyl-CoA thiolase (EC 2.3.1.16). The three phosphorylating sites are labelled I, II and III. The oxidation of tetramethyl-*p*-phenylenediamine and ascorbate is also shown. Abbreviations: ETF, electron-transferring flavoprotein; TMPD, tetramethyl-*p*-phenylenediamine.

rates in the respiratory chain (Tables 2 and 3) clearly demonstrate that a rapid flow of reducing equivalents from a substrate directly into the respiratory chain at the region of cytochrome *b* and ubiquinone is in itself insufficient to inhibit the oxidation of NAD-linked substrates such as pyruvate and isocitrate. This is evident not only from the effects of pentachlorophenol but also from

the failure of succinate oxidation to inhibit pyruvate and isocitrate oxidation in state 3. By contrast, palmitoylcarnitine oxidation inhibited the oxidation of pyruvate and isocitrate in state 3 when the flow of electrons from electron-transferring flavoprotein to the respiratory chain was not more than 60% of that succinate to the chain in the same state.

Reduction of mitochondrial NAD+. The mechanism whereby pyruvate and isocitrate oxidation are inhibited by succinate oxidation could involve one or more of the following: (i) inhibition of the penetration of pyruvate and isocitrate into mitochondria; (ii) inhibition of pyruvate dehydrogenase and isocitrate dehydrogenase as a consequence of increased reduction of intramitochondrial NAD^+; (iii) changes in the mitochondrial concentration of any other activators or inhibitors of pyruvate dehydrogenase and isocitrate dehydrogenase. Of these, the last possibility is clearly incapable of exhaustive investigation, and the first is technically difficult to explore under experimental conditions identical with those under which the regulatory phenomena were observed. However, the second possibility, involving changes in the reduction of mitochondrial NAD^+, is not only experimentally simple (Klingenberg & Slenczka, 1959) but also attractive in view of the inhibitory effects of NADH on pyruvate dehydrogenase (Garland & Randle, 1964; Bremer, 1969) and isocitrate dehydrogenase (Plaut & Aogaichi, 1967; Nicholls & Garland, 1969). Measurements of the percentage reduction of mitochondrial NAD^+ are shown in Tables 2 and 3.

An extensive reduction of intramitochondrial NAD^+ occurred when succinate was added to mitochondria oxidizing either isocitrate or pyruvate in the presence of atractylate. Associated with this reduction of NAD^+ was a fall in the carbon dioxide output to a lower but nevertheless still significant value. It follows that if carbon dioxide output is equated with the oxidative decarboxylation of pyruvate or isocitrate, and if the pathway of oxidation is via the respiratory chain to oxygen, then there is still an electron flow from NADH to the cytochromes. If this is the case, then the increased reduction of NAD^+ observed on the addition of succinate (Expt. 2 of Table 2 and Expt. 2 of Table 3) is more readily attributed to an inhibition of electron flow from NADH to the cytochromes rather than to a flow of electrons to NAD^+ from succinate (Chance & Hollunger, 1957).

When Expt. 1 of Table 2 was repeated in the absence of added pyruvate, it was observed that palmitoylcarnitine oxidation in state 3 caused a 25% reduction of NAD^+, a value that was greater than the 15% reduction observed with succinate. The amount of NAD^+ that remained oxidized in each case did not differ greatly, 75% for palmitoylcarnitine oxidation and 85% for succinate oxidation, and it cannot be concluded from these values that the large differences in the effects of succinate and palmitoylcarnitine oxidation are due to differences in the mitochondrial concentration of NAD^+. The inhibitory effects of palmitoylcarnitine oxidation on pyruvate oxidation may involve acetyl-CoA (Garland & Randle, 1964; Nicholls *et al.* 1967), but no similar involvement is known for isocitrate dehydrogenase.

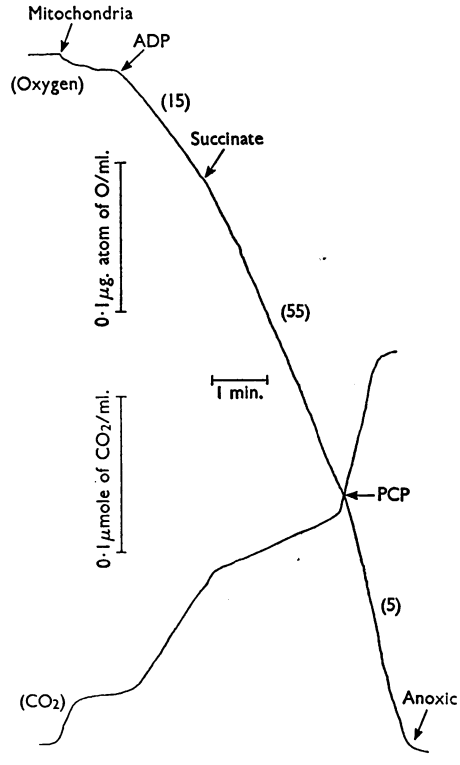


Fig. 2. Effect of succinate oxidation on pyruvate oxidation of rat heart mitochondria. Initially 6·0ml. of air-saturated incubation medium at 23° contained KCl (80mM), tris–chloride buffer, pH7·2 (20mM), EDTA (1mM), potassium phosphate buffer, pH7·2 (3mM), atractylate (7μM), L-malate (2mM) and pyruvate (2mM). Further additions shown in the figure were rat heart mitochondria (5·9mg. of protein), ADP (2mM), succinate (2mM) and pentachlorophenol (PCP) (5μM). The vertical bars indicate the electrode sensitivities. The artifact in the CO_2 trace on adding succinate (Fig. 1) has been excluded from the drawing. Numbers in parentheses are the percentage reduction of NAD.

Reduction of cytochrome b *in rat liver mitochondria.* Repetition of Expt. 2 of Tables 2 and 3 in the dual wavelength spectrophotometer failed to detect any significant change in the reduction of cytochrome *b* on the addition of succinate to mitochondria in state 3½ oxidizing pyruvate or isocitrate. In each

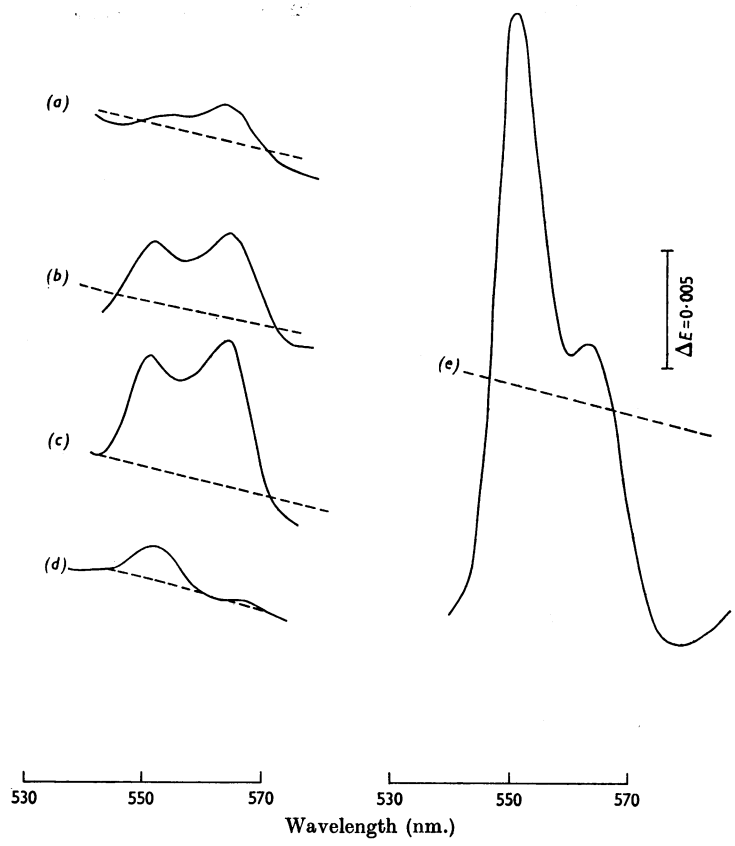


Fig. 3. Difference spectra in the cytochrome *b* and *c* α-band region for rat heart mitochondria under various metabolic states. Spectra are presented as the difference in extinction between a test and a reference cuvette. Spectra (*a*)–(*d*) are steady-state spectra, and were followed by the changes characteristic of anoxia. The spectral band width was 1·3nm., the light-path 1cm. and the scanning speed approx. 5nm./sec. The time-constant for the measuring apparatus was 0·1sec. The suspensions for spectroscopy were prepared by mixing 0·2ml. of mitochondrial suspension (60mg. of protein/ml.) into 5·0ml. of air-saturated medium at 23° containing KCl (80mM), tris–chloride buffer, pH7·2 (20mM), EDTA (1mM), potassium phosphate buffer, pH7·2 (3mM), and any further additions as indicated below. The suspension was then divided between the test and reference cuvettes, and a base line was recorded (broken line). The appropriate addition was then made to the test or reference cuvette, and the spectrum recorded (continuous line). Spectrum (*a*): state 3 minus 2: ADP (2mM) and rotenone (0·2μM) to each cuvette, succinate (3mM) to the test cuvette. Spectrum (*b*): state 3½ minus 3: pyruvate (2mM), L-malate (2mM) and ADP (2mM) to each cuvette, atractylate (7μM) to the test cuvette. Spectrum (*c*): state 4 minus 3: pyruvate (2mM) and L-malate (2mM) to both cuvettes, ADP (2mM) to the reference cuvette. Spectrum (*d*): state 3½ (+succinate) minus 3½: ADP (2mM), pyruvate (2mM), L-malate (2mM) and atractylate (7μM) to each cuvette, succinate (3mM) to the test cuvette. Spectrum (*e*): state 5 minus 2, obtained from the experiment of spectrum (*a*) after exhaustion of oxygen. In all spectra changes in the reduction of cytochrome $c+c_1$ are given by the difference 551nm. minus 540nm. and changes in the reduction of cytochrome *b* by the difference 564nm. minus 575nm. (Chance & Williams, 1955).

instance the percentage reduction of cytochrome *b* remained at about 30%. It therefore seems unlikely that the reduction of NAD^+ during succinate oxidation was due to a fall in the concentration of oxidized cytochrome *b*.

Rat heart mitochondria. The experiment of Fig. 2 demonstrated that pyruvate oxidation in rat heart mitochondria was inhibited by succinate oxidation in state 3½. Inhibition did not occur in state 3. Also shown in Fig. 2 is the percentage reduction of NAD^+, which behaves similarly under these conditions to the NAD^+ of liver mitochondria. Isocitrate oxidation was not investigated.

The appropriate difference spectra of the α-band region of cytochromes c_1, *c* and *b* (540–590nm.; Fig. 3) showed that, although the transitions from

state 2 to state 3 and from state 3 to state $3\frac{1}{2}$ caused significant changes in the reduction of cytochromes *b* and $c+c_1$ (Figs. 3*a* and 3*b*), the further addition of succinate in state $3\frac{1}{2}$ (pyruvate and malate already present) caused a small further reduction of cytochrome $c+c_1$ (550nm. peak) but not of cytochrome *b* (α-band peak at 564nm.) (Fig. 3*d*).

DISCUSSION

A major role of the tricarboxylic acid cycle is the oxidation of pyruvate to produce biologically utilizable forms of energy, either as well-characterized molecules such as NADH and ATP, or as more hypothetical entities such as 'X~I' (Slater, 1953) or membrane potential (Mitchell, 1966). We find it reasonable to assume that control of the tricarboxylic acid cycle would involve recognition of the fulfilment or otherwise of this role, with negative-feedback mechanism(s) from the end product(s) or state(s) on to certain rate-limiting enzymes. The energy-dependence of the regulation of the tricarboxylic acid cycle by fatty acid oxidation has already been stressed (Garland *et al.* 1968), and similar considerations apply to the regulation of pyruvate and isocitrate oxidation by succinate oxidation. König & Szabados (1967) proposed that succinate oxidation played a regulatory role in the utilization of pyruvate *in vivo*, and to this can now be added the utilization of isocitrate. The presence of two regulatory sites, one on the side of citrate synthesis and one on the side of citrate utilization, can be understood in the context of the important inhibitory effect of citrate on phosphofructokinase (EC 2.7.1.11) (Passoneau & Lowry, 1963; Garland, Randle & Newsholme, 1963). The two regulatory sites could allow control not only of the flow through citrate but also of the steady-state concentration of citrate independently of the flow rate.

The implication of NADH as the inhibitor of pyruvate dehydrogenase and isocitrate dehydrogenase during succinate oxidation in state $3\frac{1}{2}$ is consistent with the results presented in Tables 2 and 3. The extensive reduction of NAD^+ during succinate oxidation in state $3\frac{1}{2}$ can be attributed to inhibition of electron flow from NADH to the cytochromes. The inhibitory mechanism is presumably similar to that underlying respiratory control in state 4 (Klingenberg, 1964). One interesting aspect of this conclusion is that respiratory control in state $3\frac{1}{2}$ is more severely applied at the first phosphorylating site (between NADH and the region of cytochrome *b*) than at the other two phosphorylating sites lying between cytochrome *b* and oxygen. Alternatively, it could be said that there is a negative-feedback mechanism operating from the second and third phosphorylating sites to control the first.

The reduction of mitochondrial NAD^+ in state $3\frac{1}{2}$ is inevitably related to other aspects of this state, such as the phosphorylation of mitochondrial adenine nucleotides (Klingenberg, 1964), and the possibility that regulatory effects attributed to and consistent with an increased reduction of NAD^+ are in fact due to parallel phenomena is not easily discounted. One solution to this problem is possible with mitochondria from *Torulopsis utilis*, where phenotypic alterations can abolish the mechanism of energy conservation but not electron transport in the respiratory chain between NADH and the cytochromes (Light, Ragan, Clegg & Garland, 1968).

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REFERENCES

Bremer, J. (1966). *Biochim. biophys. Acta*, **116**, 1.
Bremer, J. (1969). *Europ. J. Biochem.* **8**, 535.
Chance, B. & Hagihara, B. (1961). *Proc. 5th int. Congr. Biochem., Moscow*, vol. 5, p. 3.
Chance, B. & Hollunger, G. (1957). *Fed. Proc.* **16**, 703.
Chance, B. & Williams, G. R. (1955). *J. biol. Chem.* **217**, 395.
Chance, B. & Williams, G. R. (1956). *Advanc. Enzymol.* **17**, 65.
Garland, P. B. & Randle, P. J. (1964). *Biochem. J.* **91**, 60c.
Garland, P. B., Randle, P. J. & Newsholme, E. A. (1963). *Nature, Lond.*, **195**, 381.
Garland, P. B., Shepherd, D., Nicholls, D. G. & Ontko, J. (1968). In *Advances in Enzyme Regulation*, vol. 6, p. 3. Ed. by Weber, G. Oxford: Pergamon Press Ltd.
Garland, P. B., Shepherd, D. & Yates, D. W. (1965). *Biochem. J.* **97**, 587.
Haslam, R. J. (1966). In *Biochim. biophys. Acta Library Volume* 7: *Regulation of Metabolic Processes in Mitochondria*, p. 108. Ed. by Tager, J. M., Papa, S., Quagliariello, E. & Slater, E. C. Amsterdam: Elsevier Publishing Co.
Haslam, R. J. & Krebs, H. A. (1963). *Biochem. J.* **86**, 432.
Klingenberg, M. (1964). *Angew. Chem. (int. Ed.)*, **3**, 54.
Klingenberg, M. & Pfaff, E. (1966). In *Biochim. biophys. Acta Library Volume* 7: *Regulation of Metabolic Processes in Mitochondria*, p. 180. Ed. by Tager, J. M., Papa, S., Quagliariello, E. & Slater, E. C. Amsterdam: Elsevier Publishing Co.
Klingenberg, M. & Pfaff, E. (1968). *Symp. biochem. Soc.* **27**, 105.
Klingenberg, M. & Slenczka, W. (1959). *Biochem. J.* **331**, 486.
König, T., Marosvari, I. & Lipcsey, A. (1964). *Acta physiol. hung.* **24**, 391.
König, T. & Szabados, G. Y. (1966). *Abstr. 3rd Meet. Fed. Europ. biochem. Soc., Warsaw*, p. 143.
König, T. & Szabados, G. Y. (1967). *Acta biochim. biophys. Acad. sci. hung.* **2**, 253.

Light, P. A., Ragan, C. I., Clegg, R. A. & Garland, P. B. (1968). *FEBS Lett.* **1**, 4.

Mitchell, P. (1966). *Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation.* Bodmin: Glynn Research Ltd.

Nicholls, D. G. & Garland, P. B. (1969). *Biochem. J.* **114**, 215.

Nicholls, D. G., Shepherd, D. & Garland, P. B. (1967). *Biochem. J.* **97**, 587.

Passoneau, J. V. & Lowry, O. H. (1963). *Biochem. biophys. Res. Commun.* **13**, 372.

Plaut, G. W. E. & Aogaichi, T. (1967). *Biochem. biophys. Res. Commun.* **28**, 628.

Scholz, R. & Bücher, Th. (1965). In *Control of Energy Metabolism*, p. 393. Ed. by Chance, B., Estabrook, R. W. & Williamson, J. R. New York and London: Academic Press Inc.

Slater, E. C. (1953). *Nature, Lond.*, **172**, 975.

Walter, P. A., Paetkau, V. & Lardy, H. A. (1966). *J. biol. Chem.* **241**, 2523.

Weinbach, E. C., Sheffield, J. & Garbus, J. (1963). *Proc. nat. Acad. Sci., Wash.*, **49**, 561.