CXXVII. FUMARATE AND TISSUE RESPIRATION.

I. EFFECT OF DICARBOXYLIC ACIDS ON THE OXYGEN CONSUMPTION.

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MALONATE strongly inhibits the enzymic dehydrogenation of succinate [Quastel and Wooldridge, 1928; Quastel and Wheatley, 1931]. Gözsy and Szent-Györgyi [1934] found that it also strongly inhibits the respiration of minced pigeon breast muscle. They considered that in this tissue a water-soluble substance acted as hydrogen carrier between activated substrate and activated oxygen, and that this substance was succinate or fumarate. As a result of further work on the same tissue by Szent-Györgyi and his colleagues this idea was elaborated [Annau *et al.*, 1935]. The hydrogen carrier is fumarate, which, activated by a dehydrogenase, is oxidised to oxaloacetate by the "Warburg-Keilin system", *i.e.* by cytochrome respiratory enzyme and oxygen. The oxaloacetate is immediately reduced back to fumarate (or malate) by the activated substrates. The inhibition of respiration by malonate was explained as follows.

During respiration a small fraction of the oxaloacetate formed is "over-reduced" to succinate. This would represent a loss of the fumarate which is acting as a hydrogen carrier were not the succinate promptly oxidised to fumarate by succinoxidase. In the presence of malonate the succinoxidase is inhibited, so that succinate accumulates, fumarate disappears, hydrogen transport fails, and the respiration falls.

The experiments described in this paper represent part of a critical study of Szent-Györgyi's theory which will attempt:

(1) to confirm the experimental basis of the theory;

(2) to find out whether fumarate can act as hydrogen carrier in tissues other than pigeon breast muscle; and

(3) to determine the nature of the activated substrates which can transfer their hydrogen to oxaloacetate, *i.e.* to see whether the theory applies to the oxidation of fat, protein or carbohydrate.

This paper deals with the effects of dicarboxylic acids on the respiration. In considering whether these effects accord with Szent-Györgyi's views the following must be borne in mind.

Firstly, it is not essential that added fumarate should accelerate the respiration of the tissue. This should only occur when the tissue contains less than the optimum amount of fumarate, either normally or as a result of loss by diffusion into the suspension fluid. Secondly, it is not essential that malonate should inhibit the respiration; this should only take place when the reduction intensity of the tissue is high enough for "over-reduction" of oxaloacetate to succinate to occur. Thirdly, if malonate does inhibit the respiration, this inhibition should be prevented by the simultaneous addition of fumarate.

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Experimental methods and results.

Measurements of oxygen consumption were made in the Haldane-Barcroft-Warburg apparatus. The suspension medium was buffered by sodium phosphates, p_H 7·3.

Preparation of tissue. Immediately after the death of the pigeon, the breast muscle was cut into several pieces, which were quickly cooled on distilled-water ice. After quick drying on filter-paper, the pieces were passed through an ice-cold mincer into an ice-cooled dish. The mincer forced the tissue by a plunger through a sieve plate and cut it on the far side by revolving knives, as specified by Annau *et al.* [1935]. In some experiments the minced muscle was weighed and added to 3 vols. ice-cold phosphate buffer; the suspension was then pipetted into the Warburg vessels. In other experiments, equal portions of tissue were quickly weighed on a torsion balance and transferred to the Warburg vessels. The vessels described by Dickens and Šimer [1930, 1] were found to be suitable, provided that the oxygen uptake did not exceed 500 μl. per hour. 100 mg. tissue (moist weight) were used in all experiments with pigeon breast muscle. The vessels were filled with air.

In experiments with tissue slices, the tissue was prepared in solutions on ice or at 38°, according to the circumstances; the vessels were filled with oxygen, sometimes while inside the thermostat, sometimes while outside.

Effects of malonate and fumarate on the respiration of minced pigeon breast muscle.

Banga [Annau *et al.*, 1935] found that with fumarate addition the muscle showed a large constant oxygen uptake; without addition, the respiration was either almost the same as that with fumarate, or, more usually, it progressively decreased. She concluded that "das Fumarat die Atmung eigentlich nicht 'steigert', sondern bloss 'konserviert'; vom Abfall bewahrt". Malonate caused a strong inhibition; with fumarate and malonate the respiration was the same as with fumarate alone. These observations have been confirmed.

The respiration of the tissue without fumarate or malonate addition depends on the osmotic pressure of the suspension fluid (Figs. 1 and 2). In the experiment of Fig. 1 the muscle was placed in a hypotonic solution. The solutions in the vessels were as follows:

	F ml.	*S* ml.	*N* ml.
Phosphate (0·11 *M*)	0·5	0·5	0·5
Fumarate (0·1 *M*)	0·4	—	—
NaCl (0·9 %)	—	0·4	—
Water	1·1	1·1	1·5

The solutions corresponding to the curves of Fig. 2 were very slightly hypertonic, and were as follows:

	F ml.	*N* ml.	*M* ml.
Phosphate (0·177 *M*)	0·3	0·3	0·3
Fumarate (0·1 *M*)	0·4	—	—
Malonate (0·1 *M*)	—	—	0·2
NaCl (0·9 %)	1·2	1·6	1·4

In the second experiment the respiration did not fall off in the absence of added fumarate. Malonate (0·01 *M*) inhibited strongly. In the first experiment (hypotonic solutions) the respiration was low and falling. It was maintained at a high value when 0·02 *M* fumarate was added. When instead of fumarate NaCl was added in iso-osmotic concentration (0·03 *M*) the respiration was again

maintained at a higher value, even if not quite so well as by the fumarate. Part of the effect of the fumarate might, therefore, be ascribed to osmotic action. This consideration is not likely to affect the interpretation of Banga's experiments which were performed in solutions which were not very hypotonic

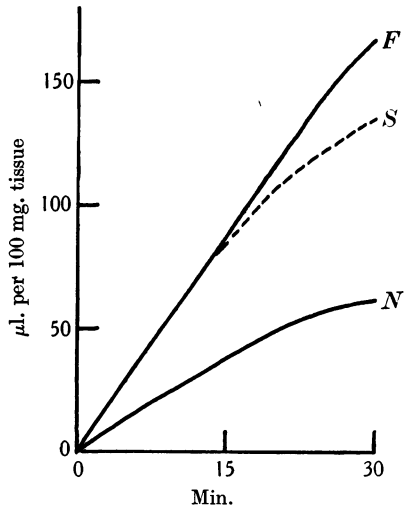

Fig. 1. Pigeon breast muscle (hypotonic solution).

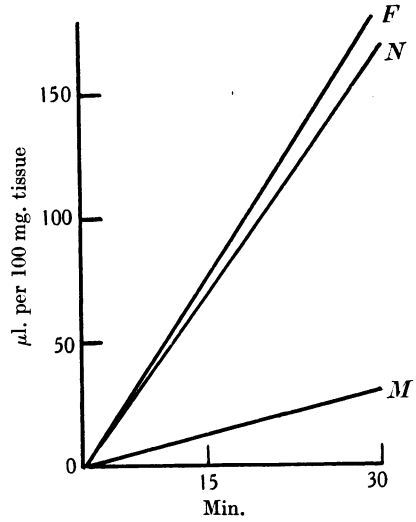

Fig. 2. Pigeon breast muscle (slightly hypertonic solution).

N, no addition; *F*, fumarate; *M*, malonate; *S*, NaCl.

(0·05 *M* phosphate). But in the experiment of Gözsy and Szent-Györgyi [1934], in which it was shown that fumarate raised the respiration without itself disappearing, the muscle was suspended in 0·022 *M* phosphate, and in view of this, the experiment loses much of its force.

The effect of calcium.

When the respiration of minced pigeon breast muscle is measured in isotonic phosphate-containing solutions, the addition of calcium in the concentration in which it occurs in blood or Ringer's solution causes a strong inhibition of the respiration. In the experiment shown in Fig. 3, the effect of the addition of 8·8 mg./100 ml. Ca and 20 mg./100 ml. K is shown. The solutions (1·9 ml. in each vessel) were isotonic, the phosphate concentration was *M*/36, and the curves correspond to the following additions:

	Fumarate (*M*)	Malonate (*M*)	Calcium (mg./100 ml.)	Potassium (mg./100 ml.)
F	0·02	—	—	—
M	—	0·01	—	—
CF	0·02	—	8·8	20
CFM	0·02	0·01	8·8	20
CN	—	—	8·8	20
CM	—	0·01	8·8	20

On the addition of calcium and potassium, fumarate and malonate have little effect on the respiration, which becomes very low and of the same order as the

malonate-inhibited respiration in the absence of calcium. A number of experiments showed that the effect was due to the calcium alone, the respiration being strongly inhibited by concentrations as low as 4 mg. per 100 ml.

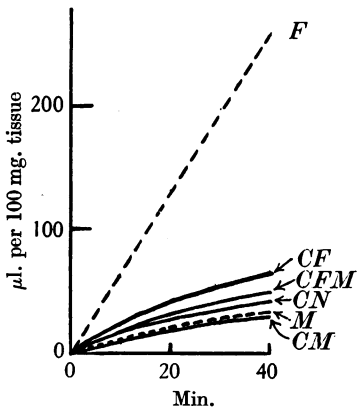

Fig. 3.

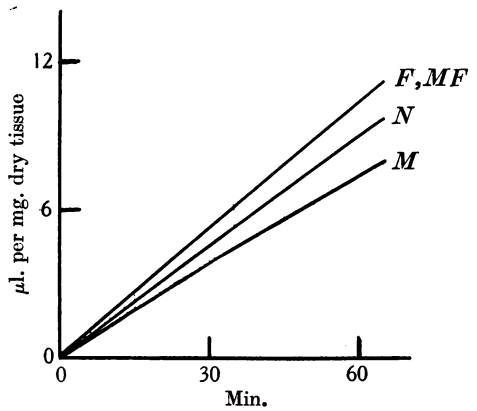

Fig. 4.

Fig. 3. Pigeon breast muscle.

Fig. 4. Rat diaphragm. 0·002 *M* $CaCl_2$, 0·005 *M* KCl. *N*, no addition; *F*, 0·02 *M* fumarate; *M*, 0·01 *M* malonate; *MF*, 0·01 *M* malonate + 0·02 *M* fumarate.

That calcium strongly inhibits the respiration of minced tissue was discovered by Thunberg [1909, 1, 2] and his observations have been confirmed and extended by others, including Meyerhof [1919] and Holck [1934]. It is now seen that this inhibition also occurs in the presence of fumarate.

It is interesting to compare the effects of cations on the minced pigeon muscle with their effects on the respiration of slices of rat brain cortex [Dickens and Greville, 1935]. With the latter material, calcium lowers the respiration, potassium accelerates it [compare Ashford and Dixon, 1935] and can overcome the effect of calcium. Magnesium also lowers the respiration. Experiments with minced pigeon breast muscle in isotonic solutions containing 0·02 *M* fumarate have shown that: (1) Potassium (concentrations up to 0·09 *M*) has little effect on the respiration. (2) Addition of potassium (0·003–0·15 *M*) does not relieve the inhibition due to calcium. (3) Magnesium (up to 0·005 *M*) has little effect on the respiration.

Dickens and Greville [1935] have suggested that calcium modifies the condition of the brain protoplasm so as to make the enzymes less available. If, as seems probable, the effect of calcium on the minced muscle is due to irreversible damage consequent on penetration of the calcium into the tissue, the differences in the effects of cations in the two materials are not surprising.

It is clear that the effects of the dicarboxylic acids have been studied using tissue in such an "unphysiological" condition that the respiration is heavily inhibited by the addition of "physiological" concentrations of calcium.[1]

[1] It may be thought that the sensitivity of the tissue towards calcium in these experiments is due to damage caused by the particular mincer used. The respiration in Szent-Györgyi's experiments in calcium- (and bicarbonate-) containing Ringer solution is only a little lower than that in the calcium-free phosphate. There is, however, no evidence of undue damage. The mincer conformed to Szent-Györgyi's specifications, and the oxygen uptakes observed in absence of added fumarate agree well with those given by Straub [Annau *et al.*, 1935].

Ahlgren [1925] remarks that "Ringer-Lösung für feinverteilte Gewebe wenigstens bei Atmungsversuchen eine sehr ungeeignete Suspensionsflüssigkeit ist". The more acceptable view would be that it is the state of the tissue rather than the fluid which is unsuitable. Hence it seemed desirable to test the effects of malonate and fumarate on tissue as little damaged as possible.

Effects of dicarboxylic acids on the respiration of diaphragm and heart muscle.

The most suitable material seemed to be the rat diaphragm. Warburg *et al.* [1924] showed that with young rats (100 g. or less) the tissue is thin enough to be completely saturated with oxygen; and Meyerhof and Himwich [1924] and Meyerhof *et al.* [1925] found that respiration is constant for 2 or 3 hours, being somewhat increased by the addition of pyruvate.

The diaphragms of rats of 80–100 g. were freed from the central tendon and divided into four. The respirations of the four pieces agreed well. Fig. 4 shows that in isotonic media containing phosphate, calcium and potassium, 0·01 *M* malonate has little effect on the respiration. In the absence of Ca and K similar curves were obtained: calcium did not inhibit the respiration. Malonate had no greater effect in the presence of pyruvate. But if, in the absence of added substrate, higher concentrations of malonate be added, and the respiration followed over longer periods, malonate is seen to exert a very definite effect (Fig. 5). Fig. 6 shows that 0·02 *M* fumarate overcomes the inhibiting action of

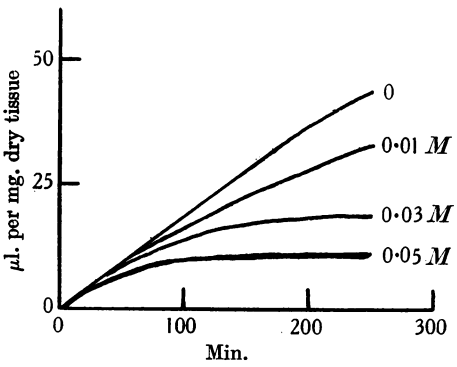

Fig. 5.

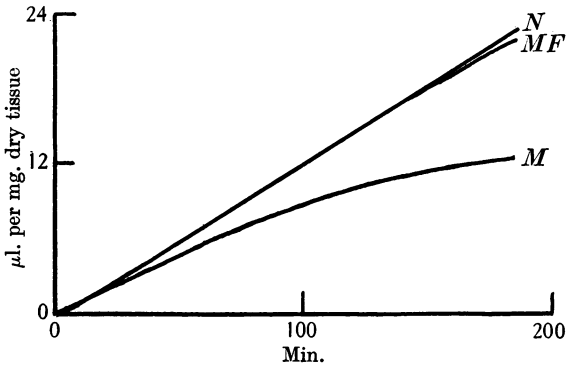

Fig. 6.

Fig. 5. Rat diaphragm. Inhibition by malonate.

Fig. 6. Rat diaphragm. *N*, no addition; *M*, 0·03 *M* malonate; *MF*, 0·03 *M* malonate + 0·02 *M* fumarate.

0·03 *M* malonate. Thus the events which are explained by Szent-Györgyi's theory can occur with tissue that has suffered minimum damage, the respiration of which is not reduced by the usual concentrations of calcium.

If now the diaphragm tissue be severely damaged, by being cut into small pieces, the lower concentration of malonate (0·01 *M*) has an immediate action, the respiration falls off in the absence of added fumarate, and calcium strongly inhibits the respiration in the presence of fumarate. Fig. 7 shows an experiment in which the pieces of diaphragm were weighed on a torsion balance, and then each piece rapidly cut with scissors to a fine brei. Control experiments showed that the respirations of four pieces of the same diaphragm cut in this way agreed

well. If the damage were not so severe, the cut particles being larger, the respiration did not fall off so rapidly in the absence of fumarate, calcium had not much effect, but malonate still inhibited strongly (Fig. 8).

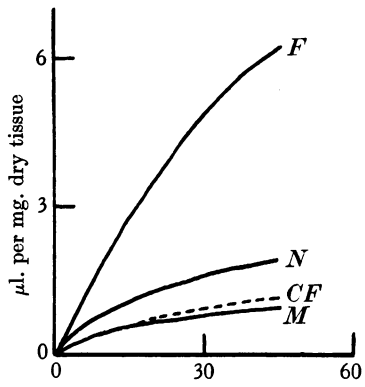

Fig. 7.

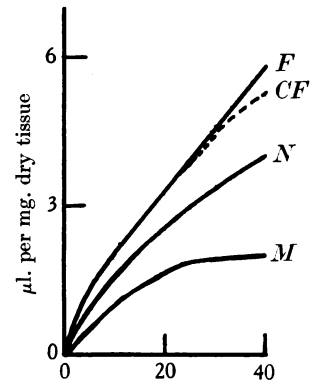

Fig. 8.

Fig. 7. Rat diaphragm. Severely damaged. Isotonic solutions. *F*, 0·01 *M* fumarate; *M*, 0·01 *M* malonate; *N*, no addition; *CF*, 0·01 *M* fumarate + 0·002 *M* $CaCl_2$ + 0·005 *M* KCl.

Fig. 8. Rat diaphragm, moderately damaged. (Legend as in Fig. 7.)

Finally, with muscle tissue cut in slices the effects of malonate and fumarate were very pronounced. This is shown by an experiment with sliced guinea-pig heart muscle:

	1	2	3	4
Phosphate (0·11 *M*)	0·6	0·6	0·6	0·6
Fumarate (0·1 *M*)	—	—	0·4	0·4
Malonate (0·1 *M*)	—	0·2	—	0·2
NaCl (0·9 %)	1·4	1·2	1·0	0·8
Q_{O_2} (first 40 min.)	**−5·5**	**−1·5**	**−13·7**	**−14·8**

Effect of dicarboxylic acids on the respiration of brain cortex and retina.

As a first step towards finding out whether carbohydrates can be oxidised in the tissues by means of fumarate catalysis, the effects of fumarate and malonate on tissues with a pure carbohydrate respiration have been tested. One such tissue is the brain cortex, the respiration of which is maintained only when it is supplied with[1] carbohydrates or carbohydrate derivatives, which are burnt completely [Loebel, 1925; Dickens and Šimer, 1930, 2]. Fig. 9 shows the effect of 0·05 *M* fumarate and 0·02 *M* malonate on the respiration of rat brain cortex slices in glucose and phosphate-containing medium. Fumarate accelerates the respiration; malonate reduces it to below half the control value. It may remain constant at this lowered value for at least 90 min. Fumarate only partially relieves the malonate inhibition. Similar results are obtained whether the tissue is prepared in solutions at room temperature, at 38°, or on ice.

Malonate has less effect on the respiration when the substrate is pyruvate than when it is glucose. Fig. 10 shows the effect of 0·01 *M* malonate on the oxygen consumption of rat brain cortex in the presence of 0·2 % glucose, of 0·2 % lithium

[1] Succinic, α-ketoglutaric and *l*(+)-glutamic acids are also vigorously oxidised [Quastel and Wheatley, 1932; Krebs, 1935; Weil-Malherbe, 1935].

pyruvate and of 0·2% glucose+0·2% lithium pyruvate. If Szent-Györgyi's explanation of inhibition by malonate is to apply to brain cortex, it would be necessary to say that since malonate has less effect on the respiration in the

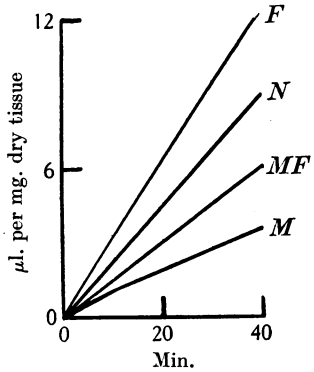

Fig. 9.

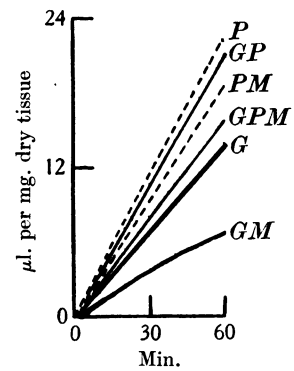

Fig. 10.

Fig. 9. Rat brain cortex (slices). *N*, no addition; *M*, malonate; *F*, fumarate; *MF*, malonate + fumarate.

Fig. 10. Rat brain cortex (slices). *G*, glucose; *GM*, glucose + malonate; *P*, pyruvate; *PM*, pyruvate + malonate; *GP*, glucose + pyruvate; *GPM*, glucose + pyruvate + malonate.

presence of pyruvate than in the presence of glucose, the reducing intensity (or the "Wasserstoffmobilisierung") is less when the former is substrate [Annau *et al.*, 1935, p. 12]. This would be possible. Again, since addition of pyruvate lessens the malonate inhibition in the presence of glucose (Fig. 10), addition of pyruvate to the glucose solution would have to lessen the "Wasserstoffmobilisierung" and the "Wasserstoffüberschuss" of the tissue; and a consideration of molecular collisions shows this to be possible also.

It will be seen from Fig. 9 that the addition of 0·02 *M* fumarate reduces the inhibition due to 0·01 *M* malonate, but that the relief is partial. It is essential, if Szent-Györgyi's explanation is to apply, that fumarate should relieve the malonate inhibition completely; but it is possible that a failure of the fumarate to diffuse adequately into the tissue prevents this from being observed. Evidence has been sought in several ways:

1. *Attempted alterations in the permeability of tissue.* The respiration of brain cortex is very dependent on the cations in the medium [Dickens and Greville, 1935]. The medium in the experiment of Fig. 9 contained Na, Ca, K, Mg. Removal of Ca, of Ca and Mg or of Ca, K and Mg did not appreciably modify the effects of fumarate and malonate (Table I; in these experiments the

Table I. $-Q_{O_2}$ *of rat brain cortex.*

Glucose-phosphate-medium. Cation concentrations as in the paper by Dickens and Greville [1935].

Cations	Duration min.	No addition	Malonate (0·01 *M*)	Fumarate (0·02 *M*)	Malonate (0·01 *M*) + Fumarate (0·02 *M*)
Na-K-Ca-Mg	40	14·0	6·3	15·5	10·4
Na-K-Mg	30	14·2	5·8	17·2	9·8
Na-K	35	19·4	7·7	21·6	12·2
Na	40	19·1	7·5	20·8	10·5

fumarate and malonate concentrations were the same as those in the pigeon breast muscle experiment).

2. *Cut tissue.* With diaphragm, cutting the tissue resulted in more marked actions by malonate and fumarate. When rat brain cortex slices were cut with scissors to a brei, the effect of malonate became rather less; and no evidence was obtained of increased relief by fumarate.

3. *Increased fumarate concentrations.* Relief was still only partial with 0·06 *M* fumarate (Table II).

4. *Malate.* Instead of fumarate, the effect of *l*-malate was tried. This should be brought into equilibrium with fumarate by the tissue fumarase [Quastel, 1931], and might possibly penetrate better. However, the relief was still incomplete (Table II).

Table II.

Substrate: glucose. Time: 60 min.

Malonate (*M*)	—	0·01	0·01	0·01	0·01
Fumarate (*M*)	—	—	0·06	—	—
l-Malate (*M*)	—	—	—	0·01	0·03
$-Q_{O_2}$	17·5	5·4	12·6	9·2	13·0
Malonate (*M*)	—	0·01	0·01	0·01	
l-Malate (*M*)	—	—	0·02	0·04	
$-Q_{O_2}$	17·7	7·3	13·4	14·4	

In order to see whether this imperfect relief was a peculiarity of brain tissue, the behaviour of another tissue with pure carbohydrate respiration was tested. With rat retina, fumarate was even less effective:

	Substrate: glucose.		
	No addition	Malonate (0·01 *M*)	Malonate (0·01 *M*) + fumarate (0·02 *M*)
$-Q_{O_2}$ (60 min.)	21·4	6·1	7·4

Tumour tissue.

It was found that 0·02 *M* malonate inhibited the respiration of slices of Jensen rat sarcoma in glucose medium by 20–40 %. Similar results have recently been published by Boyland and Boyland [1936].

Effect of malonate and fumarate on artificially-stimulated respiration.

Dinitrophenols accelerate the respiration of normal tissues [Dodds and Greville, 1933; Ehrenfest and Ronzoni, 1933; Euler, 1933] and tumour tissues [Dodds and Greville, 1934]. The increased respiration is strongly inhibited by malonate. In the following experiment with rat brain cortex, malonate and fumarate were added after 30 min. respiration (substrate: glucose).

Dinitro-*o*-cresol present initially ($M \times 10^{-5}$)	—	3	3	3	3
Added after 30 min.	—	—	Malonate (0·02 *M*)	Fumarate (0·02 *M*)	Malonate (0·02 *M*) + fumarate (0·02 *M*)
$-Q_{O_2}$ before addition (30 min.)	13·1	39·0	36·3	36·6	33·2
$-Q_{O_2}$ after addition (40 min.)	13·7	40·6	11·9	38·5	13·9

The next experiment, in which dinitro-*o*-cresol and malonate were added together, also shows that the latter abolishes the action of the former on the

respiration of rat brain cortex. The respiration during 30 min. after addition is expressed as a percentage of that during 30 min. before addition.

Fumarate present initially (M)	—	—	—	0·02	0·08
Added after 30 min.:					
Dinitro-*o*-cresol ($M \times 10^{-5}$)	—	3	3	3	3
Malonate (M)	—	—	0·01	0·01	0·01
% of initial respiration	**101**	**184**	**62**	**78**	**98**

Concentrations of fumarate as high as 0·08 M do not overcome the action of malonate in preventing the acceleration.

In tumour tissue (JRS) malonate prevents the acceleration by dinitro-*o*-cresol in the same way. In the experiment given below, malonate and dinitro-*o*-cresol were added after a preliminary period. The respiration after addition (35 min.) is expressed as a percentage of the respiration before addition (35 min.). With both brain and JRS, dinitro-*o*-cresol only accelerates the respiration in presence of carbohydrate or carbohydrate derivatives; so presumably here we have another case of carbohydrate respiration being inhibited by malonate.

Besides the nitrophenols, certain of the redox indicators accelerate the respiration of tumour tissue [Barron, 1930; Dickens, 1934]. The acceleration in respiration produced by one of the most efficient of these, brilliant cresyl blue [Dickens, unpublished], is not prevented by malonate.

JRS. Substrate: glucose.

Added after 35 min.:					
Malonate (M)	—	—	0·01	—	0·01
Dinitro-*o*-cresol ($M \times 10^{-5}$)	—	1	1	—	—
Brilliant cresyl blue ($M \times 10^{-4}$)	—	—	—	2	2
% of initial respiration	**89**	**204**	**85**	**218**	**207**

Although their effects are often similar, the modes of action of the redox indicators and nitrophenols on respiration are possibly quite different [Greville and Stern, 1935; see also Krahl and Clowes, 1935]. That malonate overcomes the action of the latter and not of the former may therefore be of interest.

Discussion.

According to Szent-Györgyi, fumarate acts as a hydrogen carrier for the tissue oxidations. In any particular tissue it might act as a hydrogen carrier for the whole of the respiration or for a part of the respiration. Before the former could be claimed for a given tissue it would be necessary to establish that:

(1) The tissue, killed at any time during full respiration in such a way that *post mortem* chemical changes could not occur, contains enough dicarboxylic acid (fumaric + malic + succinic + oxaloacetic) to be a catalyst for the whole respiration at that time.

(2) The tissue's own fumarate (or malate) can be oxidised to oxaloacetate in the tissue, and the tissue's own oxaloacetate can be reduced to fumarate (or malate) in the tissue.

(3) The tissue's own fumarate (or malate) is oxidised to oxaloacetate at a rate at least equivalent to the oxygen uptake during respiration.

(4) The tissue's own oxaloacetate is reduced to fumarate (or malate) at a rate at least equivalent to the oxygen uptake.

(5) Oxaloacetate reduction in the tissue is connected with substrate oxidation.

These conditions are essential, but it would be almost impossible to establish (3), (4) and (5). If the following further conditions, which are in themselves not essential, could be established in addition to (1) and (2), it would become highly probable that fumarate acts as a catalyst for the whole of the respiration.

(6) Added fumarate (or malate) is oxidised to oxaloacetate at a rate at least equivalent to the oxygen uptake during respiration.

(7) Added oxaloacetate is reduced to fumarate (or malate) at a rate at least equivalent to the oxygen uptake during respiration.

(8) The reduction of added oxaloacetate is accompanied by an equivalent oxidation of substrate.

(9) The dicarboxylic acid content (fumaric + malic + succinic + oxaloacetic) remains constant, either with or without addition of any of these acids, during respiration.

(6) and (7) are not essential because of the possibility of feeble penetration of the added acid.

If, on the other hand, fumarate is to act as a hydrogen carrier for only a part of the respiration, (2) and (5) would be essential, (8) and (9) would become a valuable addition to the evidence; but as to the rest, there would be no independent way of determining what proportion of the respiration was carried by fumarate.

For pigeon breast muscle, Annau *et al.* [1935] have produced evidence for (7) and (9). For kidney, liver and tumour none of these points has been established. The remark of Boyland and Boyland [1936] that "malignant tissue therefore seems to resemble muscle, kidney and liver in using C_4 dibasic acids as oxygen carriers" would therefore seem to be premature.

The results with brain cortex in this paper emphasise the necessity of considering the question of permeability in future work with the dicarboxylic acids.

It is to be hoped that many more facts about the effects of dicarboxylic acids on respiration will be established, so that it may be seen whether they receive their explanation from Szent-Györgyi's most stimulating theory.

Summary.

The effects of malonate and fumarate on the respiration of various kinds of surviving tissue have been studied. With minced pigeon breast muscle, the effects described by Szent-Györgyi and colleagues have been confirmed. The tissue was in so damaged a condition that its respiration, even in the presence of fumarate, was strongly inhibited by physiological concentrations of calcium. With tissue which had suffered minimum damage (rat diaphragm) the effects of malonate and fumarate could still be shown. Damage by cutting this tissue facilitated the demonstration of their actions. Whether any action would occur with perfectly undamaged tissue is not clear.

Pure carbohydrate respiration (rat brain cortex and retina) was also inhibited by malonate, but fumarate only partially relieved the inhibition. Respiration (presumably carbohydrate oxidation) artificially accelerated in brain cortex and tumour by dinitro-*o*-cresol was strongly inhibited by malonate. That produced in tumour by a redox indicator was not.

These experiments were a first step in studying the applicability of Szent-Györgyi's new theory that fumarate is a hydrogen carrier in respiration. Observations on malonate inhibition cannot confirm any particular theory; but the findings do not contradict Szent-Györgyi's views, if the failure of fumarate to overcome completely the malonate inhibition in brain and retina can be ascribed to insufficient penetration of the fumarate into the tissue.

The establishing of Szent-Györgyi's theory will be very difficult. Conditions necessary for establishing a claim that the theory applies to any particular tissue are discussed.

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