XVI. BIOLOGICAL OXIDATIONS IN THE SUCCINIC ACID SERIES.

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(*Received December 31st, 1930.*)

The oxidation of succinic acid can be accomplished by cells of very diverse types, ranging from the bacteria to those of mammalian muscle and brain. Early work on the course of this oxidation, by Batelli and Stern, Einbeck, and Dakin, showed that succinic acid was oxidised to fumaric acid and that this took up the elements of water to form *l*-malic acid. Hahn, Haarman and Fischbach [1929] demonstrated that in presence of muscle *l*-malic acid was further oxidised to oxaloacetic acid. In the case of bacteria it was clear [Quastel, 1924] that the main course of oxidation of succinic acid was through fumaric acid and pyruvic acid, oxaloacetic acid presumably being an intermediate step. The question, however, arose as to whether fumaric acid was oxidised directly or whether it was necessarily transformed to malic acid prior to oxidation. It was shown with bacteria, using the methylene blue technique [Quastel and Whetham, 1924], that malic acid was not a ready donator of hydrogen, a fact contrary to what would be expected if the oxidation of fumaric acid proceeded through malic acid. The evidence was sufficient to justify the opinion that fumaric acid might be oxidised directly. The discovery, however, that the donating powers of malic acid might be obscured by the accepting powers of the acid—presumably through the formation of fumaric acid which is a ready hydrogen acceptor—vitiated this conclusion, and later on the formulation of the theory of activation [Quastel, 1926] made it appear very unlikely that fumaric acid would be oxidised directly, an activated fumarate molecule, on the theory, tending to take up positive electricity (protons or hydrogen) rather than negative (oxygen). The question, therefore, was left quite open. Most tissues and organisms can bring about a change of fumaric acid to *l*-malic acid and can accomplish the oxidation of the latter acid, but neither of these facts constitutes evidence that the main line of oxidation of fumaric acid lies through *l*-malic acid. In the case of certain bacteria, for instance, fumaric acid gives rise (in presence of ammonium ions) to *l*-aspartic acid, an amino-acid capable of being oxidised by the bacteria in question. But it cannot be assumed from these facts that the main line of oxidation of fumaric acid, with these bacteria, lies through *l*-aspartic acid. The actual finding among the products of oxidation of succinate or fumarate

of any substance which is capable of further oxidation does not constitute evidence that this substance occurs on the main line of oxidation of the succinate or fumarate. Quantitative, in addition to qualitative, evidence is required in order properly to appraise the significance of any such substance.

An attempt is made in this communication to show, from a quantitative standpoint, that the biological oxidation of fumaric acid proceeds largely, if not entirely, through *l*-malic acid. This is done by comparing the rates of oxidation of fumaric and malic acids in presence of various organisms and tissues, by comparing the action of inhibitors on these oxidations and by measuring the malic acid produced from succinic acid. At the same time measurements have been made of the relative rates of oxidation of succinic, fumaric, malic and aspartic acids in presence of various tissues and organisms and of the influence these substances have on each other's oxidation. Results have been obtained showing very clearly how the organisms and tissues differ in significant ways from each other.

Of considerable importance to the problems of respiration is the inhibitory action of malonate on succinate oxidation, first found by the methylene blue method in the case of bacteria [Quastel and Whetham, 1925; Quastel and Wooldridge, 1928]. It will be shown that this inhibitory action applies not only to the oxidation of succinate by bacteria, but to that by muscle and brain tissue. The degree of inhibition varies widely, being greatest with muscle and brain. The reasons for this and the bearing of the results on the views held by Warburg are discussed.

Experimental.

Oxidation rates were measured in the Barcroft differential manometer, all experiments being carried out at 37°. Acids were used in the form of their sodium salts, the p_H of solutions being 7·4. The oxygen uptakes recorded are the net uptakes due to the substrates alone—the control oxygen uptake by the organism or tissue alone being subtracted from the uptake given by organism or tissue in presence of the substrate. On some occasions the same amount of organism or tissue was placed in each cup of the apparatus, the substrate under investigation being placed only in the right hand one. The oxygen uptake in such cases was that due to the substrate alone.

Phosphate buffer solution ($M/15$) was always present with the tissue, and substrates were present at an initial concentration of $M/15$. The total volume of solution in each cup of the apparatus was 3 cc.

The rates of oxidation of substrates were linear over the first 2 or 3 hours of the oxidation indicating that the initial concentration ($M/15$) used was sufficient to saturate the enzymes involved.

The various bacteria were prepared by 48 hours' growth either in tryptic broth or on nutrient agar plates, the organism in the first case being centrifuged and washed twice with 0·85 % saline, and in the second case being scraped off the plates, suspended in saline and then centrifuged and washed;

after washing, a homogeneous suspension of the organism in saline was prepared. 1 cc. of this suspension was used in each experiment. The organism was used as soon as possible after its preparation, its age being of great importance so far as the oxidations of fumarate, malate or aspartate are concerned. When not in use the organism was stored at 0°.

In the cases of muscle and brain, 0·5 g. of the tissue was always used. Muscle, after dissection from the animal, was cooled and minced. Brain was similarly treated, the whole brain in the case of the rabbit being used. With human brain only the grey matter of the cerebral cortex, after careful removal of the membranes and adhering blood vessels, was used.

In Table I in which most of the experimental results are set out, the

Table I. *Uptake of oxygen (at 37°) in mm.³ by organisms and tissues in presence of various substrates and mixtures of substrates, the initial concentration of each being* M/*15*.

Exp.	Organism or tissue	Succinate	Fumarate	*l*-Malate	*l*-Aspartate	Succinate + fumarate	Succinate + *l*-malate	Succinate + *l*-aspartate	Succinate + malonate
1	*B. coli*	1456	698	770	710	1202	1185	1312	1108
2	„	1372	662	651	—	1064	924	—	—
3	„ (few days old)	440	17	—	—	251	—	—	45
4	*B. acidi lactici*	1210	716	630	475	—	—	—	—
5	„	1072	—	—	—	842	889	806	—
6	„	210	5	—	—	123	—	—	37
7	*B. prodigiosus*	487	293	391	495	335	538	591	382
8	*B. proteus*	1818	984	1035	1270	1445	1578	1525	1550
9	„	552	237	—	—	406	—	—	345
10	*B. pyocyaneus*	470	550	447	532	—	—	—	—
11	„	429	—	—	—	370	403	533	—
12	„	497	—	—	—	—	—	—	279
13	*B. alkaligenes*	358	75	155	257	242	287	575	108
14	„	541	—	—	—	415	479	1362	—
15	„	720	248	—	—	496	—	—	386
16	*B. subtilis*	443	—	—	352	—	—	1136	—
17	„	889	715	971	1342	—	—	—	710
18	*M. lysodeikticus*	728	584	416	242	632	718	1160	645
19	Rabbit muscle	302	—	—	92	—	—	544	—
20	„	900	45	477	400	528	640	1050	137
21	„	691	40	10	—	405	300	769	0
22	Rabbit brain	445	50	102	0	365	439	385	3
23	„ (few days old)	410	41	21	—	—	—	—	37
24	Human brain (grey matter)	804	9	—	—	690	—	—	108
25	„	201	47	38	23	—	—	—	—

results of any one experiment are quantitatively comparable with each other, but not with those of a differently numbered experiment. The results given are representative of a large number of experiments.

Relative rates of oxidation of succinic, fumaric, malic and aspartic acids.

The rates of oxidation, as previously stated, are approximately linear over the first 2 or 3 hours. In most of the experiments a period of 2 hours was made the limiting time, and the actual oxygen uptakes of a number of substrates at the end of this period are a measure of the relative rates of oxidation of these substrates.

Now it follows that if fumaric acid can only be oxidised *via* *l*-malic acid, the velocity of oxidation of fumaric acid cannot be greater than that of *l*-malic acid for equivalent saturation concentrations of these substrates. A distinctly higher rate of oxidation for fumarate would indicate a separate line of oxidation.

The experimental results recorded in Table I show, on the whole, that the velocities of oxidation of fumarate and *l*-malate are of the same order, a much higher figure being recorded, however, in the case of a specimen of rabbit muscle for *l*-malate. It is an interesting fact that in certain cases the velocity of oxidation of fumarate exceeds that of *l*-malate. This is notably the case with *M. lysodeikticus* and it has been observed several times with brain tissue. The excess of oxidation, however, is usually small so that although this excess may point to some direct oxidation of fumarate, the effect may also be attributable to a secondary factor.

The fact that the oxidations of fumarate and *l*-malate are usually of the same order points to the rapid transformation of fumarate into *l*-malate and hence to the presence of fumarase in the organisms and tissues investigated. Experiment shows this to be the case.

The oxidation of aspartic acid is of interest. Aspartic acid may undergo (1) a direct oxidation, or (2) loss of ammonia, in presence of those organisms possessing the necessary enzymes, to form fumaric acid [Quastel and Woolf, 1926; Cook and Woolf, 1928] and oxidation *via* *l*-malic acid. If course (2) is the sole mode of oxidation followed, the velocity of oxidation of aspartic acid should not be greater than that of *l*-malic acid; if course (1) takes place the velocity may be greater. The results show that the strict aerobes *B. alkaligenes* and *B. subtilis* oxidise aspartate at a much greater rate than *l*-malate, whereas the facultative anaerobes, *B. coli*, *B. acidi lactici*, oxidise the amino-acid at about the same or a lesser rate.

The results suggest that the strict aerobes in question attack the amino-acid by course (1) and this is supported by the fact that neither of the organisms possesses the power of transforming *l*-aspartic acid into fumaric acid.

The relative rates of oxidation of succinate and fumarate vary very widely with different cells. The greatest variation is seen in the cases of muscle and brain tissue, with which the oxidation of fumarate is small compared with that of succinate. The reverse of this, when the oxidation of fumarate is seen to be even greater than that of succinate, occurs with *B. pyocyaneus*, an

observation entirely in harmony with the facts relating to the relative rates of fermentation of succinate and fumarate by this organism [Quastel, 1924].

The oxidations of fumarate and *l*-malate are very much more dependent on the age or condition of the organism than is the oxidation of succinate. Storing the organisms for a few days at 0° brings about a very marked decrease in the ability of an organism to oxidise fumarate or *l*-malate. It is this very high sensitivity of the oxidation to relatively slight changes in the cell which, doubtless, accounts for the fact that with muscle, even when used quite fresh, variable results are obtained for the oxidation of *l*-malate. To obtain reliable results with bacteria, these organisms must be used as soon as possible after their preparation. With *B. coli*, freshly prepared, the rate of oxidation of succinate is usually roughly twice that of fumarate; on storing the organism for a few days at 0° the rate of oxidation of succinate becomes as much as ten times that of fumarate—a ratio approximating to that found in muscle or brain tissue.

The effect on succinate oxidations of the addition of fumarate, malate or aspartate.

It is now well known both from studies of bacteria and of muscle that the enzyme accomplishing the activation of succinate as a hydrogen donator will bring about the activation of fumarate as a hydrogen acceptor. It follows that fumarate is adsorbed at the succinate-activating enzyme and hence that it should compete with succinate for this enzyme. The addition, therefore, of fumarate to succinate (both at "saturation" concentrations) should bring about a diminution in the velocity of oxidation of the latter, the amount of diminution being dependent upon the relative degrees of adsorption. Experiment shows this to be the case. Usually it is found (see Table I) that the oxygen uptake of a mixture of succinate and fumarate is the average of that due to succinate alone and that due to fumarate alone, this being the case whether the oxidation of fumarate is small or large compared with that due to succinate. An exception to this rule has been found with muscle and the interesting question arises as to whether the adsorption of fumarate at the succinate enzyme is the same whatever the source of the enzyme. There is not yet, however, sufficient experimental evidence available to decide this point.

Incidentally the fact that fumarate inhibits succinate oxidation is in harmony with the hypothesis that the main course of oxidation of succinate is through fumarate. Were there a different means of oxidising succinate, it would have been anticipated that the addition of fumarate would have increased the rate of oxidation found in presence of succinate alone.

Since fumarase is present in the organisms and tissues under investigation, the effect of adding *l*-malate is virtually the same as adding an equivalent concentration of fumarate, for both give rise to the same equilibrium mixture. Experiment shows that the inhibiting action of *l*-malate is rather less than that

of fumarate, an effect presumably to be accounted for by the time factor involved in the equilibrium concentration of fumarate being formed from the malate. For instance *M. lysodeikticus* is slow compared with *B. coli* in transforming fumarate into malate, and accordingly we find that the degree of inhibition of succinate oxidation by malate is less with the former organism than with the latter.

Aspartate, in the presence of those organisms which can convert aspartic acid into fumaric acid, inhibits the oxidation of succinate. With those organisms which do not possess this power, however, aspartate increases the rate of oxidation found with succinate. Thus with *B. coli*, *B. acidi lactici* and *B. proteus*, which are organisms capable of forming *l*-aspartic acid from fumaric acid, the effect of aspartate is to inhibit succinate oxidation. Now, were the aspartate directly oxidised, instead of passing through fumarate and malate, the action of aspartate would be to increase the rate of oxidation in presence of succinate. With the strict aerobes *B. alkaligenes*, *B. subtilis* and *M. lysodeikticus* this actually occurs, there being a marked increase in the rate of oxidation on the addition of aspartate to succinate. This shows clearly the difference in mode of attack on the amino-acid between the strict aerobes and the facultative anaerobes.

B. prodigiosus seems to behave, to a small extent, in this respect like a strict aerobe.

The interesting fact emerges, however, with the strict aerobes, that the rate of oxidation of a mixture of aspartate and succinate is often greater than the sum of the individual rates. This does not always occur and the phenomenon depends very largely on the condition of the organism.

The same phenomenon applies to muscle tissue. Here again the addition of aspartate increases the rate of oxidation due to succinate, the new rate sometimes being greater than the sum of the individual rates. The effect is reminiscent of Needham's observation [1930] that a mixture of glutamic and aspartic acids gives rise to more succinic and malic acids than the sum of the acids produced from glutamic and aspartic acids individually.

With brain tissue, *l*-aspartate appears to have but little effect on succinate oxidation.

Both with muscle and brain tissue fumarate and malate inhibit succinate oxidations, the effects being rather less than with bacteria.

It is quite clear, as far as aspartate oxidation is concerned, that, both with muscle tissue and the strict aerobes, the line of oxidation is not mainly through fumarate and malate. With the facultative anaerobes, on the other hand, this course would appear to be the predominant one. The alternative would be that for these organisms alone the aspartate molecule *per se* is highly adsorbed at the succinate enzyme—a hypothesis for which there is as yet no evidence.

The action of malonate.

It would be anticipated that if fumarate underwent a direct oxidation, this would take place at the enzyme which is known to activate the fumarate molecule, *i.e.* at the succinate dehydrogenase. Though the electrical theory of activation does not favour the possibility of the activated fumarate molecule taking up negative electricity (*i.e.* of becoming directly oxidised), there is no definite evidence so far that this does not in fact occur. Now it has been shown that malonate competes with succinate for the enzyme capable of activating the latter, and Cook [1930] has recently shown that this inhibitory action of malonate on succinate oxidation applies aerobically, as well as anaerobically, to *B. coli*. There is little doubt that the competing action of malonate with succinate for the latter dehydrogenase is a general phenomenon—applying to muscle and brain tissue (see Table I) as well as to various classes of bacteria.

It would be expected, therefore, that if fumarate is oxidised at the succinate enzyme, where it is known to be adsorbed, the presence of malonate would inhibit its oxidation by competition for the enzyme.

The action of malonate on fumarate oxidation provides, therefore, a good test of the possibility that fumarate undergoes an oxidation at the succinate enzyme.

Experiment shows that the action of malonate both on fumarate or malate oxidation is either nil or very small.

For instance with *B. coli*

Fumarate (*M*/37)	took up	423 mm.^{3} O_2	in 2 hrs.
Fumarate (*M*/37) + malonate (*M*/15)	,,	430	,,
l-Malate (*M*/37)	,,	427	,,
l-Malate (*M*/37) + malonate (*M*/15)	,,	400	,,

The fact that malonate has either little or no effect on fumarate and malate oxidations explains the wide variations observed in the degrees of inhibition by malonate on succinate oxidation with various organisms and tissues. In the case of those cells with which the rate of fumarate oxidation approaches that of succinate, malonate has a relatively small effect; for, in spite of the large effect of malonate on the succinate-fumarate reaction, fumarate is still produced in sufficient quantity to allow a relatively rapid uptake of oxygen. (It is worthy of note that the succinate-fumarate reaction requires only one atom of oxygen, whilst the complete oxidation of fumarate requires six atoms.) On the other hand, in the case of those organisms with which the fumarate oxidation is slow compared with that of succinate, malonate has a large effect, the action of the malonate on the succinate-fumarate reaction being predominant. This is the case with muscle and brain tissue with which the fumarate oxidation is normally small, or with organisms which have been stored so long or treated in such a way that the fumarate and malate oxidations have disappeared. Here the malonate inhibition ap-

proaches 90 % or an even larger value. With organisms, however, such as *M. lysodeikticus* or *B. pyocyaneus* where fumarate oxidation is relatively high the malonate inhibition may approach only 30 %.

The small or negligible action of malonate on fumarate oxidation is, definitely, evidence against the view that fumarate might undergo a direct oxidation at the succinate enzyme.

The action of oxalate.

If the oxidation of fumarate proceeds largely through *l*-malate it follows that an agent which inhibits malate oxidation will also inhibit fumarate oxidation. Such an agent is oxalate. The following results were obtained with *B. pyocyaneus*, all the substrates being initially present at a concentration of *M*/15 and the oxalate as the potassium salt.

l-Malate	took up	551	mm.^{3} O_2 in 90 mins.
l-Malate + oxalate	,,	330	,,
Fumarate	,,	600	,,
Fumarate + oxalate	,,	315	,,
Succinate	,,	547	,,
Succinate + oxalate	,,	409	,,

It will be seen that oxalate exercises an inhibitory action on both malate and fumarate oxidations—the inhibition being greater than on that of succinate. Oxalate has not a large inhibitory action in the succinate-fumarate reaction [Quastel and Wooldridge, 1928] but it is to be expected it will appreciably inhibit the total oxidation of succinate, since the latter passes through fumarate and malate.

The fact that the inhibitions of fumarate and *l*-malate oxidations by oxalate are of the same order of magnitude is clearly evidence in favour of the view that fumarate oxidation proceeds *via l*-malate.

It is worthy of note that toluene, which eliminates the oxidation of fumarate by *B. coli* [Cook, 1930], also eliminates the oxidation of *l*-malate by this organism.

Oxidation of succinate to *l*-malate.

Summing up the evidence so far it appears:

(1) the velocities of oxidation of fumarate and *l*-malate are usually of the same order of magnitude;

(2) malonate, which inhibits the succinate-fumarate reaction, has little action on the oxidation of either fumarate or *l*-malate;

(3) oxalate inhibits the oxidation of fumarate and *l*-malate to about the same extent, the inhibition being greater than that observed with succinate.

Though this evidence, on the whole, is in favour of the view that the oxidation of fumarate proceeds largely through *l*-malate there is as yet no direct evidence that succinate does not suffer another oxidation (*e.g.* direct to *dl*-malate) besides that of proceeding, in the first place, to fumarate.

This evidence may be secured from a quantitative study of the oxidation of succinate in presence of those tissues or organisms with which the oxidation of fumarate or malate is small or nil. In presence of such cells, one atom of oxygen should be taken up per molecule of succinate forming an equilibrium mixture of fumarate and *l*-malate in the ratio of 1:3. Thus the uptake of 1 g.-atom of oxygen should give rise to $\frac{3}{4}$ g.-mol. of *l*-malate. If succinate suffered an oxidation other than that through fumarate, the uptake of 1 g.-atom of oxygen would lead to less than $\frac{3}{4}$ g.-mol. *l*-malate. If, for instance, it proceeded first to *dl*-malate, and this to a mixture of *d*-malate, *l*-malate and fumarate the final rotation observed would not only be smaller than if fumarate were the first step but of the opposite sign.

For experiment, succinate at an initial concentration of $M/15$ was oxidised in presence of (1) muscle tissue, with which the fumarate oxidation was very small compared with that of succinate; (2) a suspension of *B. coli* which had been treated with toluene so as to eliminate the oxidation of fumarate.

The oxidation was carried out in the Barcroft apparatus and after a certain period the oxygen uptake was read, and the solution in the Barcroft cup made up to 5 cc. with water. 1 cc. glacial acetic acid was added and the whole mixed with 10 cc. 14·2 % ammonium molybdate solution. After filtering or centrifuging, the clear solution was examined polarimetrically. Using the mercury green line a rotation of 1° (2 dm. tube) was equivalent to 9·65 mg. *l*-malic acid. The experimental results were as follows.

(1) *With muscle tissue:*

Oxygen uptake at 37° and 760 mm.	357 mm.^{3}
Rotation observed	0·30°
Rotation calculated on the basis of succinate being oxidised entirely through fumarate...	0·30°

(2) *With* B. coli *treated with toluene:*

Oxygen uptake at 37° and 760 mm.	1222 mm.^{3}
Rotation observed	1·06°
Rotation calculated	1·02°

These results clearly indicate that if succinate normally undergoes some other oxidation, besides that of proceeding through fumarate, the amount of this oxidation must be exceedingly small.

The entire evidence is now in support of the view that the normal course of biological oxidation of succinate lies largely, if not entirely, through fumarate and *l*-malate.

Warburg's respiratory enzyme.

The inhibition of succinate oxidation by malonate in presence of the intact cell bears upon the views held by Warburg of the "respiration enzyme" of the cell. These views are best expressed in his own words [Warburg, 1930] "...if different oxidases occur in an extract of one kind of cell they are not

enzymes which were preformed in the living cell, but transformation and decomposition products of a substance uniform in life. Uniformity of respiration enzyme and multitude of oxidases in the extracts do not constitute a contradiction."

Presumably, then, on this view the system responsible for the oxidation of succinate in the intact cell is identical with that which is responsible for the oxidation of fumarate, or lactate, or (say) *p*-phenylenediamine. This view leaves no room for the existence of specific dehydrogenases in the intact cell and supposes that the effects of treatment, or extraction, of a cell, on the various oxidations, are not to eliminate certain dehydrogenases but to affect a common oxidase in such a way that the less "sensitive" substances are no longer oxidised whilst the more sensitive are still attacked.

That this view cannot be true follows not only from the fact that with the bacteria the dehydrogenases are selectively poisoned [Quastel and Wooldridge, 1927], but from quantitative evidence with the intact cell. It was shown for instance that the oxidase systems for succinate and glucose differed from each other. Were there a common oxidase for these substances in the intact cell, then the rate of oxidation of a mixture of the substances at their saturation concentrations would not be greater than the higher of the individual rates. Experiment showed that the oxidation rate was the sum of the individual rates.

In this communication it has been shown that with *B. alkaligenes*, *B. subtilis* and *M. lysodeikticus* the velocity of oxidation of a mixture of succinate and aspartate is greater than either of the individual rates—a fact showing the presence of two distinct activating systems in the intact cell.

Attention, however, might be drawn, in this connection, to the inhibiting action of malonate. It has been shown previously by the methylene blue method that this substance acts reversibly on *B. coli* [Quastel and Wooldridge, 1928]. Moreover malonate does not injure the cell, for the organism is capable of prolific growth in presence of it.

Now it would be expected, if succinate and *p*-phenylenediamine are oxidised by the same system in the intact cell, that malonate which inhibits succinate oxidation would also inhibit the *p*-phenylenediamine oxidation. Experiment shows that this is not the case.

Thus, with brain tissue (0·5 g.):

p-phenylenediamine (21 mg.) took up in 2 hrs. 1206 mm.^{3} O_2.
p-phenylenediamine (21 mg.) + malonate (*M*/15) took up in 2 hrs. 1201 mm.^{3} O_2.
p-phenylenediamine (21 mg.) + oxalate (*M*/15) took up in 2 hrs. 1132 mm.^{3} O_2.

Neither malonate nor oxalate appreciably inhibits *p*-phenylenediamine oxidation under conditions such that succinate oxidation is markedly inhibited by malonate.

The conclusion is clear that, for a consistent interpretation of the results of biological oxidations, it is necessary to picture the existence in the intact cell of distinct active centres, or dehydrogenase systems, each concerned with the activation of a certain type of hydrogen donator. Possibly, as already suggested [Quastel and Wooldridge, 1927], the process of treating or extracting a cell alters the activating range of each centre, but that more than one distinct activating centre exists in the intact cell there can be no question. The oxidase which is concerned with the activation of molecular oxygen is quite distinct from the systems already referred to. Whether there is one such oxidase or a number in the intact cell is still a problem for further investigation.

SUMMARY.

1. It has been shown from quantitative evidence that the normal course of biological oxidation of succinate proceeds largely, if not entirely, through fumarate and *l*-malate. The evidence results from

(*a*) a comparative study of the oxidations of fumarate and *l*-malate in presence of various organisms and tissues;

(*b*) a comparison of the action of malonate and of oxalate on the oxidation of fumarate and *l*-malate;

(*c*) measurements of *l*-malic acid formed from succinic acid; the calculated quantities are in close agreement with those observed.

2. Fumarate and *l*-malate inhibit the oxidation of succinate in presence of various bacteria and of muscle and brain tissues.

3. It is shown that the strict aerobes, *B. alkaligenes*, *B. subtilis* and *M. lysodeikticus*, and also muscle tissue, attack *l*-aspartate in a different manner from the facultative anaerobes, *B. coli*, *B. proteus* and *B. acidi lactici*. The latter oxidise the acid through fumaric and *l*-malic acids.

4. Malonate not only inhibits the oxidation of succinate by bacteria but also that by muscle and brain tissues, the degree of inhibition being greatest with these tissues. It is shown that the wide variation with different cells in the degrees of inhibition by malonate is associated with differences between velocities of oxidation of fumarate and those of succinate.

5. Neither malonate nor oxalate inhibits the velocity of oxidation of *p*-phenylenediamine by brain tissue.

6. These results are discussed in relation to Warburg's views on the "respiration enzyme" of the cell.

Our thanks are due to the Medical Research Council for a grant to this laboratory in aid of equipment and for a whole-time grant to one of us (A. H. M. W.). We also wish to express our appreciation of the very able assistance afforded us by Mr A. Dignam.

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