CCXL. STUDIES ON REVERSIBLE DEHYDROGENASE SYSTEMS.

III. CARRIER-LINKED REACTIONS BETWEEN ISOLATED DEHYDROGENASES.

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Whilst the study of the aerobic oxidation of organic substrates by cells has been prosecuted with increasing intensity during the last two decades, the mechanism of anaerobic oxidations has received scanty attention. In a series of papers by Quastel and Stephenson [1925], Quastel, Stephenson and Whetham [1925] and Quastel and Wooldridge [1929] the experimental groundwork was laid for a clear conception of the systems involved in anaerobic oxidations. The important facts and conclusions of these authors are the following. (1) Substances may be divided into two classes, those that act as hydrogen donators and those that act as hydrogen acceptors. (2) Reactions between certain hydrogen donators and acceptors are catalysed by bacteria. For example, in the presence of resting *Bact. coli* lactate and nitrate can react to form pyruvate and nitrite, and lactate and fumarate can react to form pyruvate and succinate. In some cases the same substance could function as either donator or acceptor of hydrogen, according to the conditions of the experiment. (3) Bacteria can grow anaerobically on a medium containing only a hydrogen donator and a hydrogen acceptor, provided that one of the substances can enter into the synthetic reactions of the cell. (4) The reaction between donator and acceptor is catalysed by specific dehydrogenase systems and does not proceed in the absence of the enzymes. (5) The suggestion was made that the reaction between dehydrogenase systems is not direct but proceeds through some intermediary in the cell, presumably a substance of the type of an oxidation-reduction indicator.

These pioneer investigations of Quastel and his collaborators described the oxidative reactions which take place anaerobically in bacteria but left unanswered the question of mechanism. We may conceive of two enzymes in such close proximity on the cell surface that the reaction between the two substrates is a direct one, or we may picture a random distribution of enzymes in the cell with diffusible carriers linking the reducing and oxidising systems. In this paper evidence will be presented that isolated dehydrogenase systems do not react directly but require the intermediation of some carrier which can be reduced by the negative system and oxidised by the positive system. The question of what substances in either animal cells or bacteria can function as carriers will also be considered.

Schott and Borsook [1933] in a brief note indicated that toluene-treated *Bact. coli* could not carry out the reaction between either formate and pyruvate

or lactate and fumarate, but that on addition of an oxidation-reduction indicator these reactions did proceed. They applied the term "coupled reaction" to these anaerobic oxidations requiring a carrier, but the choice is unfortunate as the term has been previously used with a different meaning (*e.g.* the oxidation of nitrite to nitrate by peroxidase during the oxidation of hypoxanthine by xanthine oxidase and oxygen). To avoid any confusion of terminology, we propose the expression "carrier-linked reaction" to describe reactions between dehydrogenases which require an intermediary hydrogen carrier.

Experimental methods.

The following dehydrogenase preparations were used in the various experiments:

(1) *Xanthine oxidase.* The dried ammonium sulphate precipitate from whey was used direct [Dixon and Kodama, 1926].

(2) *Succinoxidase.* Succinoxidase was prepared from heart muscle according to the directions of Borsook and Schott [1931].

(3) *Glucose dehydrogenase.* The enzyme and co-enzyme were prepared from liver by the method of Harrison [1933].

(4) *Hexosemonophosphate dehydrogenase system.* The enzyme was prepared from bottom yeast and the co-enzyme from horse red blood cells as described by Warburg and Christian [1932]. The co-enzyme solution was used in the crude state.

(5) *Formic dehydrogenase and nitratase preparation.* This was made from washed *Bact. coli* by the method of Stickland [1929]. The preparation contains both formic dehydrogenase and nitratase (the enzyme which activates nitrate as a hydrogen acceptor).

It is noteworthy that the nitratase has not been detected previously in this preparation. This is the first demonstration of a cell-free nitratase preparation.

(6) *Lactic dehydrogenase and nitratase preparation.* Lactic dehydrogenase was prepared by the method of Stephenson [1928]. This also contains nitratase in addition.

(7) In a few experiments crude acetone-dried liver was used as a source of various enzymes (xanthine oxidase, succinic dehydrogenase and lactic dehydrogenase).

Preparations (1), (3) and (7) were kept as dry powders in a desiccator, and (2), (4), (5) and (6) in solution at 0–2°.

Methods of estimation.

All solutions were deproteinised by mixing with an equal volume of 1·5 % uranium acetate solution, and indicators were removed by adsorption on kieselguhr. Uric acid was determined colorimetrically with Benedict's reagent, and nitrite colorimetrically with the Griess-Ilosvay reagent. Pyruvic acid was estimated roughly by the nitroprusside test.

Experimental results.

Reactions between isolated dehydrogenase systems.

In the case of each pair of dehydrogenases to be tested, two Thunberg tubes were set up with identical solutions, except that to one was added 1 ml. of some oxidation-reduction indicator (at a concentration of the order of $M/2000$) and

to the other 1 ml. of water. Each tube contained in addition 1 ml. of $M/5$ phosphate buffer, 1 ml. of each enzyme preparation and 1 ml. of each substrate at a concentration of $M/10$ (except in the case of hypoxanthine, which was used at $0{\cdot}03M$). The tubes were evacuated thoroughly and incubated at 40° for several hours. At the end of the incubation period the solutions were deproteinised and freed from indicator, and the concentration of one of the products of the reaction was estimated. It was observed in all cases that no pair of dehydrogenases will react together except in the presence of an oxidation-reduction indicator. The results are given in Table I. With impure preparations

Table I.

Negative system	Indicator	Positive system	p_H	Time hours	Product estimated	Conc. of product M
Formic dehydrogenase + formate	Ethyl Capri blue	Nitratase + nitrate	7·0	3	Nitrite	1×10^{-3}
Lactic dehydrogenase + lactate	Ethyl Capri blue	Nitratase + nitrate	7·0	3	Nitrite	5×10^{-5}
Xanthine oxidase + hypoxanthine	Methyl viologen	Succinoxidase + fumarate	7·0	3½	Uric acid	$7{\cdot}5 \times 10^{-3}$
Succinoxidase + succinate	Methylene blue	Nitratase + nitrate	7·5	5½	Nitrite	1×10^{-4}
Lactic dehydrogenase + lactate	Ethyl Capri blue	Succinoxidase + fumarate	7·5	5	Pyruvate	2×10^{-3}
Xanthine oxidase + hypoxanthine	Benzyl viologen	Lactic dehydrogenase + pyruvate	7·0	5	Uric acid	5×10^{-3}
Hexosemonophosphate system	Pyocyanine	Nitratase + nitrate	7·0	4	Nitrite	1×10^{-4}
Glucose dehydrogenase + glucose	Ethyl Capri blue	Nitratase + nitrate	7·0	5	Nitrite	5×10^{-5}

a very slight reaction did occur in the absence of an added indicator, but with purified dehydrogenase solutions not the slightest direct reaction could be detected. Presumably impure preparations contain a minute amount of a natural carrier, though quite insufficient to allow the reaction to proceed at maximum velocity. But even in the case of the largest blank reaction that we have observed, the acceleration in the presence of a suitable indicator was at least 2000 %.

The function of the indicator.

Proof that the indicator functions by being reduced by the negative system and oxidised by the positive system is given by the following experiments. Thunberg tubes with hollow stoppers were used. The reducing system (1 ml. of enzyme preparation and 1 ml. of substrate), 1 ml. of buffer and 1 ml. of the indicator solution were placed in the main tube and the oxidising system (0·5 ml. of enzyme and 0·5 ml. of substrate) in the stopper. After thorough evacuation the tubes were incubated at 40° until maximum reduction of the dye was attained. Then the contents of the stopper were tipped in. In every case the indicator was either partly or completely reoxidised, the extent of reoxidation being obviously determined by the relative activities of the oxidising and reducing systems. Controls with enzyme alone in the stopper showed no trace of reoxidation of the indicator. The results are summarised in Table II.

Table II.

Negative system			Positive system	
Enzyme	Substrate	Indicator	Enzyme	Substrate
Formic dehydrogenase (*Bact. coli*)	Formate	Benzyl viologen	Nitratase (*Bact. coli*)	Nitrate
Lactic dehydrogenase (*Bact. coli*)	Lactate	Ethyl Capri blue	Nitratase (*Bact. coli*)	Nitrate
Xanthine oxidase (milk)	Hypoxanthine	Methyl viologen	Succinoxidase (heart muscle)	Fumarate
Hexosemonophosphate dehydrogenase (yeast)	Hexosemono-phosphate	Ethyl Capri blue	Succinoxidase (heart muscle)	Fumarate
Glucose dehydrogenase (liver)	Glucose	Ethyl Capri blue	Succinoxidase (heart muscle)	Fumarate
Xanthine oxidase (liver)	Hypoxanthine	Ethyl Capri blue	Succinoxidase (liver)	Fumarate
Lactic dehydrogenase (liver)	Lactate	Ethyl Capri blue	Succinoxidase (liver)	Fumarate
Xanthine oxidase (milk)	Hypoxanthine	Methyl viologen	Lactic dehydrogenase (*Bact. coli*)	Pyruvate

The kinetics of carrier-linked reactions.

Several aspects of the kinetics of these reactions have been studied in the cases of three pairs of dehydrogenase systems, *viz.* formate-nitrate, hypoxanthine-fumarate and lactate-nitrate.

(*a*) *The potential of the carrier.* It is evident that an indicator which is either more positive than the oxidising system or more negative than the reducing

Table III.

Indicator	E_0' at 30° and p_H 7 (v.)	Indicator	E_0' at 30° and p_H 7 (v.)
Phenolindophenol	+0·227	Nile blue	−0·116
Lauth's violet	+0·062	Janus green	−0·256
Cresyl blue	+0·047	Benzyl viologen	−0·359
Methylene blue	+0·011	Methyl viologen	−0·446
Ethyl Capri blue	−0·072		

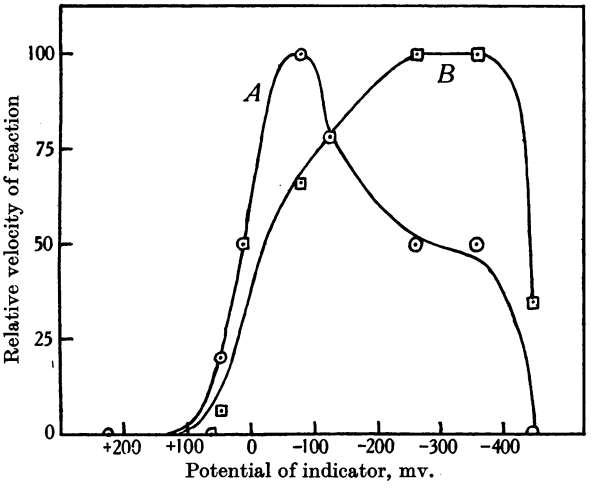

Fig. 1. Relation between potentials of indicators and their efficiency as carriers. System formate-nitrate, at p_H 7. Curves *A* and *B* represent different enzyme preparations.

system cannot function as a carrier. Theoretically any reversible indicator whose characteristic potential lies in the range between the two enzyme systems should be able to catalyse the interaction. Experiments were carried out to determine the relation between the potentials of indicators and their efficiency as carriers.

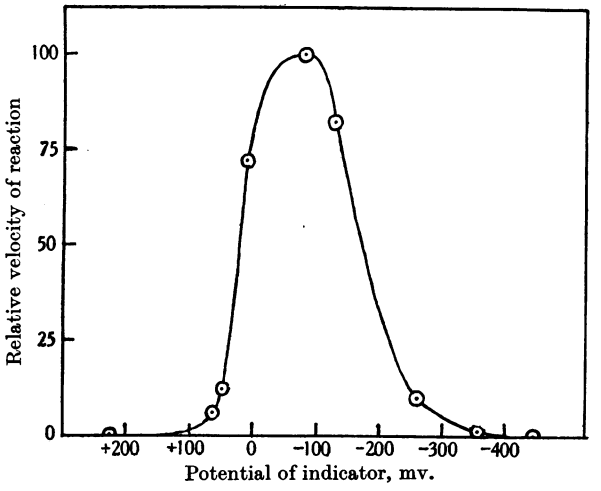

Fig. 2. Relation between potentials of indicators and their efficiency as carriers. System lactate-nitrate, at p_H 7.

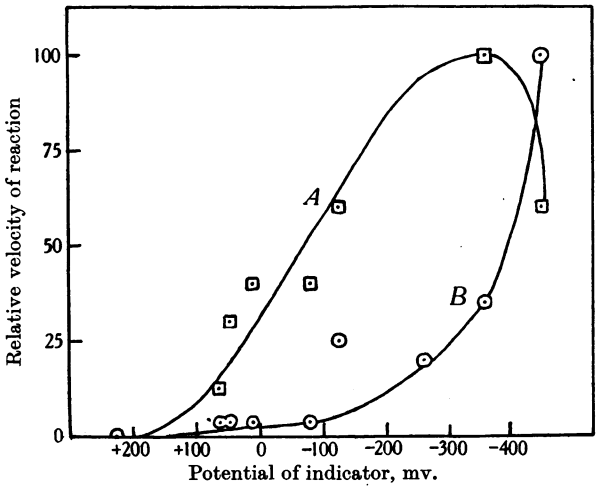

Fig. 3. Relation between potentials of indicators and their efficiency as carriers. System hypoxanthine-fumarate, at p_H 7. Curves *A* and *B* represent different preparations of succinoxidase.

The series of indicators used covered the range from r_H 0 to r_H 22 in convenient steps, as is shown in Table III. The experiments were carried out exactly as previously described, except that in a series of ten tubes one contained no indicator and the other nine the various indicators at a concentration of $M/10{,}000$. The concentration of one product of the reaction was estimated after from 3 to 5 hours' incubation at 40°. The results are shown in Figs. 1, 2 and 3.

It is clear that with any particular pair of dehydrogenase systems a rough relation exists between potential and the efficiency of a carrier. In every case a maximum velocity is observed at some point on the potential scale between the potentials of the two enzymes concerned, though sometimes the maximum

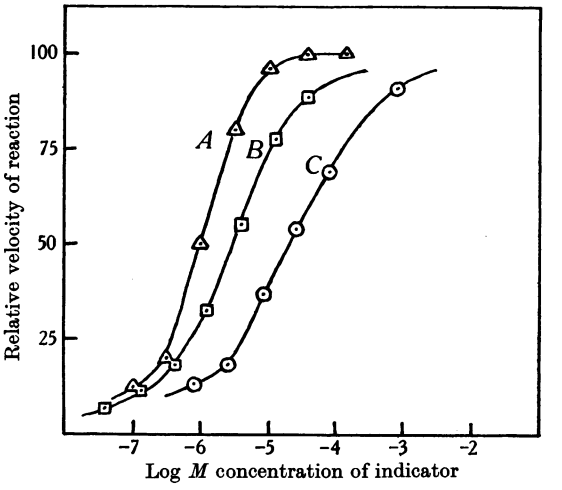

Fig. 4. Effect of dilution of indicators on the rate of reaction between formate and nitrate. Curve *A*, Nile blue; *B*, ethyl Capri blue; *C*, benzyl viologen.

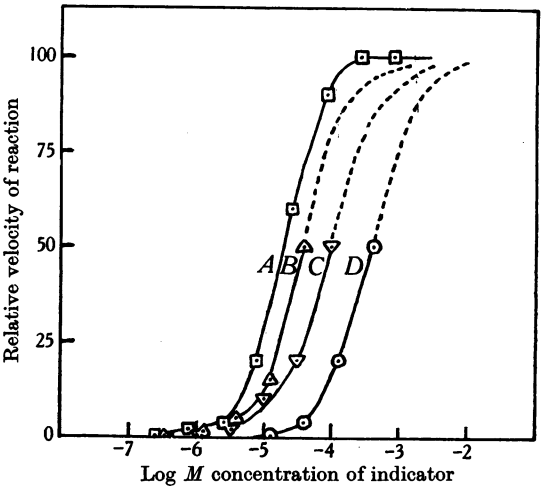

Fig. 5. Effect of dilution of indicators on the rate of reaction between hypoxanthine and fumarate. Curve *A*, benzyl viologen; *B*, ethyl Capri blue; *C*, methylene blue; *D*, methyl viologen.

covers a fairly wide range. There is always a decrease in velocity on each side of the maximum, until a zero value is reached with indicators which lie outside the range of potential of the two enzyme systems. The potential-velocity curves are naturally different for different pairs of enzymes and also differ with different preparations of the same enzymes. The significance and interpretation of these results will be discussed later.

(*b*) *The concentration of the carrier.* Experiments were carried out in the usual way, with a series of dilutions of the indicator. Figs. 4 and 5 show the effect of dilution of the carrier on the velocity of the reaction between dehydrogenases. It will be seen that as a rule the indicators can function as carriers at a very low concentration, especially in the case of the system formate-nitrate. With this system Nile blue and Capri blue show a definitely positive result at a concentration of $3 \times 10^{-7} M$, but the fumarate-hypoxanthine system requires in general higher concentrations. It is noteworthy that the dyes function even at dilutions at which their colours cannot be detected. Discussion of these results will be found later in the paper.

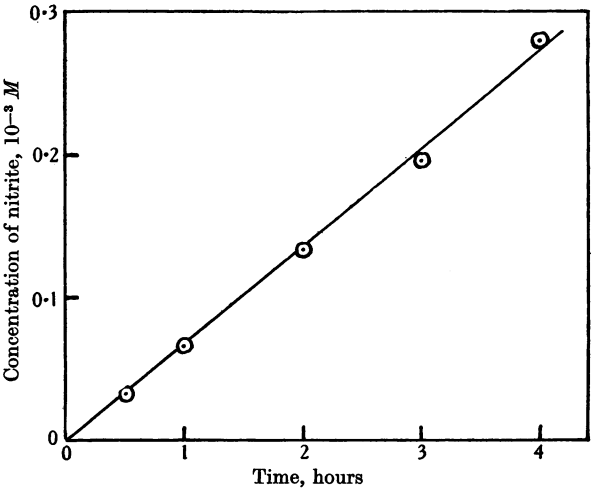

Fig. 6. Course of the reaction between formate and nitrate, with Nile blue as carrier.

(*c*) *The course of the reaction.* The reaction between formate and nitrate, linked by Nile blue, proceeds linearly. Five tubes were set up with the usual enzymes, substrates, buffer and indicator solutions, and the concentration of nitrite in the solutions was estimated after various time intervals. The result is shown in Fig. 6, and it justifies the use of single estimations as a measure of relative velocities.

(*d*) *Specificity.* It is noteworthy that all the members of the indicator series used to test the relation between the potential and the efficiency of a carrier were basic in character. If acid indicators were included, such as the indigo-sulphonates and rosindulin, they were found in every case not to fall on the same curve as the basic indicators (see Table IV). The general rule is that acidic indicators are reduced much more slowly than basic ones of the same potential. We may therefore conclude that a relation between potential and velocity will only hold for an indicator series all the members of which are basic, that is, chemical specificity as well as potential plays a part in determining the efficiency of a dye as a carrier. A striking example of specificity is that of pyocyanine, which in the cases of the formate-nitrate system (Table IV) and the hypoxanthine-fumarate system (Table V) is considerably more active as a carrier than its position on the potential scale should warrant.

Table IV. *Acidic indicators and pyocyanine tested as carriers, with the formate-nitrate system ($1\frac{1}{4}$ hours, 40°).*

Indicator	E_0' at 30° and p_H 7 (v.)	Nature of indicator	Relative speed of reaction
Methylene blue	+0·011	Basic	30
Pyocyanine	−0·034	Basic	130
Indigo tetrasulphonate	−0·046	Acidic	4
Capri blue	−0·076	Basic	90
Indigo trisulphonate	−0·081	Acidic	2
Nile blue	−0·125	Basic	100
Janus green	−0·258	Basic	80
Rosindulin	−0·282	Acidic	4
Neutral red	−0·325	Basic	4
Benzyl viologen	−0·359	Basic	80

The speed with Nile blue, the optimum of the normal series of indicators, is taken as the standard.

Table V.

Indicator	E_0' at 30° and p_H 7 (v.)	Relative speed of reaction
Methylene blue	+0·011	2
Pyocyanine	−0·034	140
Capri blue	−0·072	2
Nile blue	−0·125	25
Benzyl viologen	−0·359	40
Methyl viologen	−0·446	100

Reaction hypoxanthine-fumarate; $3\frac{1}{2}$ hours, 40°. The speed with methyl viologen, the optimum of the normal series of indicators, is taken as standard.

Natural hydrogen carriers.

Many substances derived from cells of animals, plants, moulds and bacteria have been described which are capable of reversible oxidation and reduction. The possibility is obvious that these substances might catalyse carrier-linked reactions in the same way as do artificial indicators. Tests were made with glutathione, ascorbic acid, cytochrome, pyocyanine, the yellow pigment of Warburg, lactoflavin, citrinin and ovoflavin, and the results are shown in Table VI. In the case of the Warburg hexosemonophosphate dehydrogenase system, experiments were carried out in Barcroft manometers to show that the

Table VI.

Enzyme systems: Positive	Enzyme systems: Negative	Carrier *Ca. M*/1000	Result
Nitratase-nitrate	Formic dehydrogenase-formate	Glutathione	−
		Ascorbic acid	−
		Cytochrome *c*	−
		Yellow pigment	−
		Lactoflavin	−
		Ovoflavin	−
		Citrinin	−
		Pyocyanine	+ + + +
Succinoxidase-succinate	Xanthine oxidase-hypoxanthine	Cytochrome *c*	−
		Lactoflavin	−
		Yellow pigment	−
		Pyocyanine	+ + + +
Nitratase-nitrate	Hexosemonophosphate-dehydrogenase system	Cytochrome *c*	−
		Lactoflavin	−
		Yellow pigment	(+)?
		Pyocyanine	+ + + +

various components of the system were active (see Table VII). The extraordinary conclusion follows from these results that none of the known natural oxidation-reduction indicators, pyocyanine excepted, can function as a carrier in linked reactions between the pairs of dehydrogenases studied.

Table VII.

	O_2 uptake in 3 hours (μl.)
Enzyme + yellow pigment + hexosemonophosphate	0
Enzyme + co-enzyme + hexosemonophosphate	0
Enzyme + co-enzyme + yellow pigment + hexosemonophosphate	120
Enzyme + co-enzyme + pyocyanine + hexosemonophosphate	35

Warburg and Christian [1933] have suggested that the yellow pigment plays no part in aerobic processes, but is directly concerned in anaerobic oxidations. The above results (Table VI) show clearly that there is no experimental basis for this suggestion.

Attempts to extract natural carriers from cells.

We have tried by mechanical, physical and chemical means to prepare from cells extracts which will function as carriers in linking dehydrogenases. Bacteria (*Bact. coli*, *Bact. dispar*, *M. lysodeikticus* and *S. lutea*), yeast and animal tissues (liver and heart muscle) have been employed, and the extracts prepared have been tested with the formate-nitrate and the hypoxanthine-fumarate systems. So far no active extracts have been obtained.

Velocity of carrier-linked reactions.

The velocity of these reactions will obviously depend on the activity of the less active of the pair of enzymes. The observed velocities are of the order of magnitude that would be expected from the activity of the weaker enzyme preparation, as the following examples show:

(1) 1 ml. succinoxidase + succinate reduced 1 ml. of $M/2000$ methylene blue in 6 minutes. $\therefore$ rate = 5 ml. of $M/1000$ per hour.

1 ml. of the same preparation, linked with excess of xanthine oxidase + hypoxanthine, produced 5 ml. of $M/130$ uric acid in 4 hours.

$\therefore$ rate = 10 ml. of $M/1000$ per hour.

(2) 1 ml. lactic dehydrogenase + lactate reduced 1 ml. of $M/5000$ ethyl Capri blue in 10 minutes. $\therefore$ rate = 1 ml. of $M/1000$ per hour.

1 ml. of the same preparation linked with excess of succinoxidase + fumarate produced 5 ml. of $M/500$ pyruvate in 5 hours.

$\therefore$ rate = 2 ml. of $M/1000$ per hour.

Discussion.

General.

The object of the work to which the present paper is an introduction is to decide whether, in intact cells, dehydrogenases react together directly or through the intervention of diffusible hydrogen carriers. The evidence produced so far shows conclusively that dehydrogenases, when isolated from cell structure, in every case fail to react together, but can be made to do so by the addition of an appropriate artificial hydrogen carrier.

The only alternative to the theory that in normal cells the dehydrogenases are linked by means of natural diffusible hydrogen carriers is that the enzymes are placed so close to one another that the activated substrate molecules react directly (the possibility of the enzymes themselves being freely diffusible in the inside of the cells is ruled out by the fact that they fail to react directly when extracted from the cell). This is a difficult conception, which, to explain the complete non-specificity of reaction between all hydrogen donators and all hydrogen acceptors, involves one of the two assumptions: (*a*) that molecules of all the oxidation-reduction enzymes are grouped together at the same spot, (*b*) that molecules of oxidising enzymes and dehydrogenases occur in pairs, every conceivable pair being represented. It is difficult to form a concrete picture of this state of affairs, but in our present state of ignorance of the nature of oxidising enzymes and their condition inside the cell we cannot exclude the possibility.

The analogy of aerobic oxidation systems is in favour of hydrogen carriers being involved. Two systems have been described in which carriers play a part in linking two oxidation-reduction enzymes: (1) oxygen—indophenoloxidase—cytochrome—dehydrogenases—substrates [Keilin, 1929] and (2) hydrogen peroxide—peroxidase—phenol—ascorbic acid—reducing systems [Szent-Györgyi, 1928]. Other systems (*e.g.* oxygen—yellow pigment—dehydrogenase co-enzyme—hexosemonophosphate [Warburg and Christian, 1932]) utilise a carrier, which is however autoxidisable, and only the reduction of which is catalytic.

Evidence that carriers are required for anaerobic oxidation-reduction reactions could be obtained by the isolation from cells of substances capable of linking pairs of cell-free dehydrogenases, but all the known natural substances which have been considered as respiratory carriers have proved inactive (except pyocyanine) and our own attempts at detecting such substances have so far been unsuccessful. The failure of these natural carriers to link dehydrogenases is due to various causes. Cytochrome, though reduced rapidly by most of the dehydrogenases, cannot be reoxidised by even the most positive dehydrogenase system. Warburg's yellow pigment is reduced only by the hexosemonophosphate system and is not reoxidisable except by oxygen. Except for the reduction of glutathione by glucose dehydrogenase, oxidised glutathione, dehydroascorbic acid, lactoflavin and ovoflavin are not reduced at an appreciable rate and the reduced forms are probably not reoxidised by any enzyme considered in this paper.

Considerations of potential show that, if carriers are involved in reactions of this type, then at least two such carriers must be present in certain cells. For instance, the two reactions succinate-nitrate (r_H range 15–>20) and hydrogen-pyruvate (r_H range 0–5), both of which are catalysed by intact *Bact. coli*, cannot be linked by the same carrier.

The kinetics of linked reactions.

The factors influencing the efficiency of a dye as a hydrogen carrier in linking two dehydrogenases may be classified under four headings:

(1) The potential of the dye.

(2) The relative activity of the two enzymes.

(3) The concentration of the dye.

(4) Certain factors of specificity in the relation between enzymes and indicators.

(1) There is a general empirical rule that the more negative an indicator is, the more slowly it is reduced and the more quickly its leuco-form is oxidised.

Moreover it is clear that the maximum rate of reaction will be obtained when the rate of reduction of the dye by the negative system and the rate of reoxidation of the leuco-dye by the positive system are roughly equal. Hence, if a series of indicators of different potentials is tested with a given pair of dehydrogenases, those lying near the more positive enzyme will be oxidised slowly, and those near the more negative reduced slowly, while somewhere between the two systems will be an indicator which is oxidised and reduced at comparable rates; this last will function most efficiently as a carrier. These points are illustrated in Figs. 1, 2 and 3.

(2) Exceptions to the above rule will be found when there exists a great disproportion between the activities of the reducing and oxidising enzymes. If the oxidising enzyme is present in only very small amount, then the rate of oxidation of the leuco-indicators all through the series will be correspondingly diminished, and the point at which there is a balance between the rate of reduction and the rate of oxidation will lie towards the more negative end of the scale. This is illustrated in the case of the reaction between hypoxanthine (very active xanthine oxidase) and fumarate (relatively inactive succinoxidase), where the optimum carrier lies at the level of the more negative system (see Fig. 3, curve *B*).

(3) In considering the concentration of indicator, it must be remembered that two concentrations, *viz.* those of the oxidised and reduced forms, have to be dealt with separately, as both are playing a part in the reaction. In the various experiments plotted in Figs. 4 and 5, the degree of reduction of the indicators concerned varied practically from 0 to 100 %. In the former case, the concentration of reduced dye, on which the oxidising enzyme acts, is practically *nil*, so one would expect the system to require a high total indicator concentration to make it work at maximum speed. This case is illustrated in Fig. 5, curve *D* (methyl viologen linking fumarate-hypoxanthine, where the dye was $<0{\cdot}5$ % reduced). In the other case the concentration of the oxidised form, on which the reducing enzyme acts, is again practically *nil*, so again the need for a high total concentration will be expected. This case is illustrated in Fig. 5, curve *C* (methylene blue linking fumarate-hypoxanthine, where the dye was $>99{\cdot}5$ % reduced). When, on the other hand, the extent of reduction lies between, say, 10 and 90 %, the concentration of both forms is of the same order as the total concentration, and both enzymes have a sufficient concentration to oxidise or reduce at a good speed, even when the total concentration of dye is very small.

The other factor which determines what concentration of indicator is required to give maximum velocity of reaction is obviously the affinities of the various enzymes for the dyes and leuco-dyes. Exact values for these affinities are not known, but it is evident from our results that on the whole the affinities of formic dehydrogenase for dyes and of nitratase for leuco-dyes are greater than those of xanthine oxidase and succinoxidase.

(4) The subject of specificity with regard to certain artificial indicators and biological oxidation-reduction carriers has already been discussed in the experimental section. We cannot at present give any explanation of the fact that while one indicator functions perfectly as a carrier, another of almost exactly the same potential does not function at all. In the case of the acidic (sulphonated) dyes, the reason cannot be toxicity, as Quastel [1931] has shown that basic dyes are in general much more toxic than acidic dyes to oxidising enzymes.

"Anaerobic potential" of cells.

If a series of indicators is micro-injected into cells or added to cell suspensions, it is found that there is a potential level below which indicators are not reduced [Needham and Needham, 1925]. Aerobically this level is fairly positive in potential, anaerobically fairly negative (in starfish eggs, for example, the values are −0·06 v. and −0·17 v. [Chambers *et al.*, 1933]); the two levels are called the aerobic and anaerobic potential respectively. Machlis and Green [1933] have demonstrated that the so-called "aerobic potential" is determined purely by kinetics and has no thermodynamic significance. The experiments described in this paper indicate that the "anaerobic potential" of cells must likewise be determined by kinetics. Suppose that methyl viologen is added to liver cells saturated with the substrates of the various dehydrogenase systems. Xanthine oxidase, formic dehydrogenase *etc.*, will reduce the dye to some extent, but more positive enzyme systems such as the lactic and succinic dehydrogenases will reoxidise the reduced methyl viologen. Whether the indicator will remain in the reduced or oxidised form will obviously be determined by the relative speeds of oxidation and reduction, and will not be directly connected with potential. Furthermore, it is possible to obtain two different values for the "anaerobic potential" of the same mixed enzyme system, merely by choosing two different indicators whose relative rates of oxidation and reduction differ. For instance, in one experiment with the system hypoxanthine-fumarate (Fig. 3, curve *B*), Nile blue was about 95 % reduced, indicating a potential of about −0·20 v., while benzyl viologen was 50 % reduced, indicating a potential of about −0·36 v. Obviously the ratio $\frac{\text{rate of oxidation by fumarate}}{\text{rate of reduction by hypoxanthine}}$ was not so much greater with benzyl viologen than with Nile blue as the difference in potential would lead us to expect.

SUMMARY.

1. Dehydrogenases isolated from cells will not react with one another, unless an oxidation-reduction indicator is added.

2. The indicator functions by being continually reduced by the negative enzyme system and oxidised by the positive enzyme system.

3. None of the known constituents of cells, except pyocyanine, which can be oxidised and reduced (glutathione, cytochrome, ascorbic acid, Warburg's yellow pigment, lactoflavin, ovoflavin, citrinin and pyocyanine), is able to link any pair of dehydrogenases,

4. Some aspects of the kinetics of such "carrier-linked" reactions are described.

5. The bearing of these facts on the mechanism of anaerobic oxidations in intact cells is discussed.

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