XVII. REDUCTION POTENTIAL, ENERGY EXCHANGE AND CELL GROWTH.

EXPERIMENTS WITH *B. COLI*.

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PART I.

INTRODUCTION.

So far biological interest in oxidation-reduction potentials has centred mainly on the determination and significance of intracellular r_H . The experimental method has been to inject into cells oxidation-reduction indicators (whose r_H ranges have been determined by Mansfield Clark and his colleagues) and to observe the equilibria obtained between their reduced and oxidised forms.

Needham and Needham [1926] came to the conclusion that "the r_H of the amoeba is probably widely independent of the concentration of oxygen in the external atmosphere" but that an anaerobic protozoon (*Nyctotherus cordiformis*) has "an internal r_H of 19 to 20 under aerobic conditions whilst under anaerobic conditions the latter value changes to r_H 9.5 to 10.5." They considered that "in the facultative anaerobe the oxidation-reduction potential can be adjusted to the environment whilst in the strict aerobe changes in the environment which would lead to such an alteration in the r_H bring about death." Their conclusion that the cell has "a definite, well poised and characteristic equilibrium" was based essentially upon the fact that certain oxidation-reduction indicators became oxidised when injected under anaerobic conditions into the cell in their reduced forms.

Rapkin and Wurmser [1927] have determined the r_H of a number of cells and found them all to be in the zone 19–20. This value was not altered by the injection into the cell of such substances as glucose, sodium pyruvate and succinate. They concluded that "the rate of dehydrogenation is, within wide limits, independent of the concentration of hydrogen donors."

Recently Cohen, Chambers and Reznikoff [1928] have injected twenty-five oxidation-reduction indicators in either oxidised or reduced form into *Amoeba dubia* and *Amoeba proteus* under controlled oxygen access. They found, contrary to the Needhams, that under anaerobic conditions the amoeba was able to reduce *all* the indicators used and that it could not oxidise six of the most easily oxidisable indicators. The phenomena were closely parallel to those observed by Cannan, Cohen and Clark [1926] in their reduction electrode studies on bacterial cultures.

At present, therefore, there is evidence by the Needhams pointing to the existence of a fairly well poised oxidation-reduction system in the cell and there is the opposing evidence of the American workers indicating the existence of an intracellular reduction intensity which increases as the neutralising effect of oxygen is eliminated.

It is difficult to estimate, however, how far r_H terminology can be applied to the living cell. The application of r_H terminology implicitly assumes the existence in the cell of equilibria arrived at almost instantaneously in a homogeneous reversible system. Yet the cell is a heterogeneous system; its enzymes and catalysts are in a heterogeneous condition. From the work of Brooks [1926], Rapkin and Wurmser [1926] and Cannan [1926] it is known, for example, that relatively high reducing potentials can be maintained in the

green cell apparently in the presence of free or molecular oxygen. It is doubtful therefore what significance can be given to the actual value of intracellular r_H ; its value (determined by the ratio of reduced to oxidised form of indicator) is a function of many factors, inevitable in a heterogeneous system of catalysts, and to perceive its significance it is necessary to disentangle and estimate the influence of each factor.

Michaelis and Flexner [1928] point out that when a tissue, naturally aerobic, is kept under anaerobic conditions it undergoes reactions which ultimately reach an equilibrium, the final potential obtained being in some way related to the chemical composition of the tissue in its living state. But, granting that such a final equilibrium potential can be determined, it is difficult to see how it can be interpreted to give some idea of the state of the cell under normal conditions; for it is with velocities of change that the normal events of the cell are primarily concerned and these velocities may in many cases be independent of the magnitude of a potential.

We have approached the problem of the relationship of r_H to events in the cell in a different manner from that which has hitherto been adopted.

Quastel and Stephenson [1926] suggested that the facts relating to the action of oxygen on the proliferation of a strict anaerobe such as *B. sporogenes* could be best interpreted by assuming that the cell must attain a certain limiting reduction potential before proliferation could occur. At a potential more oxidising than this the cell would fail to proliferate; below this potential proliferation was possible. This point of view has been confirmed by the recent work of Aubel, Aubertin and Genevois [1928].

The question arose as to whether a facultative anaerobe such as *B. coli* needed a definite r_H range within which to proliferate and this led to the enquiry: does the growth of the cell depend at all on the oxidising or reducing intensity, on the r_H , of the environment? The experiments on *B. sporogenes* seem to show that a particular value of reduction potential is necessary before proliferation of the anaerobe can occur. But is this phenomenon a general one? The following communication constitutes an attempt to answer this question.

EXPERIMENTAL METHOD.

Our experimental method, briefly, has been to determine how far the growth of *B. coli* on synthetic media is affected by changes in the reducing or oxidising power of the media in presence of the cell. The work has been made possible by a knowledge of the reactions brought about by the various constituents of the media in presence of the "resting" or non-proliferating organism.

Throughout the experiments described below, the inorganic medium employed was as follows:

0.4 g. $(\text{NH}_4)_2\text{HPO}_4$.	0.07 g. $\text{MgSO}_4, 7\text{H}_2\text{O}$.
0.1 g. NaCl .	Trace FeSO_4 .
0.1 g. KH_2PO_4 .	100 cc. distilled water.

This inorganic medium was usually made up to three times the strength given and kept as stock. When made up into a nutritional medium it was always so diluted that the final strength of salts was that given. The organic acids contained in the media were added in the form of their sodium salts and the final p_H of all media was 7.2. All experiments were done in duplicate, and series of experiments were repeated many times. 5 cc. of each medium, after autoclaving, were sown with 2 drops of a freshly grown tryptic broth culture of *B. coli*. Usually 20-hour growths were used but 6-hour growths are very satisfactory. It is advisable to use young cultures, results with old ones being inconsistent and variable. A heavy sowing, such as that given in 2 drops, was usually found to be necessary. A number of strains of *B. coli* have been used. It is preferable for consistent results to use a strain which gives a good growth, in tryptic broth at 37°, within 6 hours.

In certain experiments, especially when using slowly growing strains of *B. coli*, a trace of an alcoholic extract of marmite was added to the media, controls always being carried out to show that no perceptible growth occurred on the quantity of marmite alone.

Growths were usually examined after 20–40 hours' incubation (aerobic and anaerobic) at 37°. Details of the composition of the various media are given in the tables which summarise the results.

Figures are given in the tables which express relative growths.

The growths were usually estimated by comparison with standard barium sulphate suspensions or nephelometrically. In a number of cases bacterial counts were made but such absolute values were not so important for our purposes as the relative ones. The figures in one table representing relative growths cannot be compared with the figures given in another table, the tabulation being such that the results given in any one particular table can be strictly compared with each other but not with those in any other table.

SYNTHETIC MEDIA FOR ANAEROBIC GROWTH OF *B. COLI*.

The following three conditions, at least, appear to be necessary before proliferation can occur.

1. The organism must be able to secure energy either by anaerobic decomposition of the substrate (*e.g.* glucose into lactic acid) or by the oxidation of the substrate.

2. The organism must be able to activate the substrate so that the latter is capable of reaction, *e.g.* oxidation or reduction (which it does not necessarily undergo in absence of the cell).

3. The products of oxidation (or decomposition) of the substrate, or the substrate itself, must be capable of entering into the synthetic operations of the organism.

It follows that if molecular oxygen be replaced by some molecule which induces in or around the bacterial cell a state comparable with that which occurs under aerobic conditions and so long as the three conditions stated

above are fulfilled anaerobic growth should occur in presence of this molecule. Now *B. coli* activates nitrates and fumarates as hydrogen acceptors and the conditions enumerated above hold in the systems lactate-nitrate and lactate-fumarate. Anaerobic growth of *B. coli* was found to occur in both media, the lactate giving rise on oxidation to pyruvate which was utilised by the organism [Quastel, Stephenson and Whetham, 1925; Quastel, 1925]. Later it was shown that anaerobic growth occurred, as was anticipated, on glycerol-nitrate, glycerol-fumarate and glycerol-aspartate media and that it did not occur with a strict aerobe, such as *B. alkaligenes* which failed to show any perceptible activating action on nitrates or fumarates [Quastel and Stephenson, 1925; Quastel and Wooldridge, 1925].

In a lactate-fumarate medium, the lactate acts, in presence of the cell, as a hydrogen donator and must be regarded therefore as exerting a reduction potential relative to the cell. Similarly the fumarate must be considered as exerting, in presence of the cell, an oxidation potential. Therefore, although a lactate-fumarate medium, in the absence of the cell, has no observable reducing or oxidising power¹ and gives no potential discoverable at an electrode, it must be considered as exerting an oxidation-reduction potential, dependent on the relative concentrations of the two substances, in presence of the cell. This potential is not observable at an electrode because of the inaccessibility at the electrode of the cell enzymes in combination with their substrates—nevertheless it is clearly indicated by the fact that methylene blue, in presence of the system (lactate-fumarate), will be reduced to a particular value dependent on the relative velocities of reduction by lactate and oxidation by fumarate. Such a value would be as real an indication of the r_H of the medium relative to the cell, as is the intracellular r_H of a cell indicated by the method of microinjection. Now if the growth of *B. coli* is dependent on the r_H of the medium it should be dependent on the relative concentrations of lactate and fumarate in the medium.

ANAEROBIC GROWTH OF *B. COLI* ON A LACTATE-FUMARATE MEDIUM (TABLE I).

Table I.

Growth of *B. coli* anaerobically on media containing lactate and fumarate in varying concentrations. Each column represents a separate medium. Growths examined nephelometrically.

Conc. of lactate $\times M/10$	1	1	1	1	1	1	1	1.5	2	3	4	4
Conc. of fumarate $\times M/40$	0	0.5	1	1.5	2	3	4	1	1	1	1	2
Relative anaerobic growths after 20 hrs. incubation	0	0.5	1.5	2	2	2	1.5	2.5	1.5	0.5	0	0

The following conclusions may be drawn.

1. Growth increases with increase of lactate concentration until a concentration is reached above which growth diminishes with increase in concentration.

2. Growth increases with fumarate concentration until a maximum is reached. This concentration is about $M/40$. Above this concentration further

¹ On, say, the methylene blue system.

increase of fumarate appears to have but little inhibitory action. High concentrations of fumarate are however definitely inhibitory.

These results are in accordance with those found by Quastel and Stephenson [1925].

The fact that growth increases with increase in lactate and fumarate concentration until maxima in concentrations are reached is easily understood, for the rate of growth must be dependent on the rate of oxidation of the lactate to pyruvate (which is assimilated by the organism) and upon the rate of energy liberation (which is used for synthetic purposes). Such rates must be dependent on the amounts of lactate and fumarate present.

The inhibitory action of lactate at relatively high concentration is not due to any toxic effect of this substance on *B. coli*, for good aerobic growth will occur in lactate-fumarate media where the concentrations of lactate are such that anaerobic growth is markedly inhibited.

The inhibition of growth with increase of lactate concentration may be due, however, to an increased reduction potential being secured within the cell. If this be so it will be expected that increase in fumarate concentration, after attaining a critical value, will balance the inhibitory action of increased concentration of lactate and so result in an increased growth. This does not seem to occur. It may well be argued, however, that the failure of fumarate to balance the lactate effect is due to saturation of the fumarate enzyme with its substrate, so that increase in concentration of the latter does not exert any increased velocity of oxidation and hence any power of neutralising the lactate effect.

This difficulty may be surmounted in the following manner.

In the presence of *B. coli* an equilibrium is established between succinate, fumarate, methylene blue and leucomethylene blue, the equilibrium point being independent of the absolute quantities of succinate and fumarate present [Quastel and Whetham, 1924]. The oxidising action of fumarate is balanced by the reducing action of succinate in a reversible manner characteristic of the usual reduction-oxidation indicators.

If now to a lactate-fumarate medium succinate be added, the r_H of the medium (relative to the cell) must decrease, the fumarate action being opposed by that of the succinate. The evidence points to the same enzyme activating both succinate and fumarate [Quastel and Wooldridge, 1927, 1] so that the difficulty of enzyme saturation does not appear here. Clearly, whatever the concentrations of the two substances, their ratio will determine the reduction potential at their enzyme, *i.e.* in the presence of the cell. Hence by keeping the lactate concentration constant the r_H may be varied by varying the relative proportions of succinate to fumarate.

ANAEROBIC GROWTH OF *B. COLI* ON A LACTATE-
FUMARATE-SUCCINATE MEDIUM.

The results are shown in Table II.

1. Increase in succinate concentration results in a marked inhibition of growth.
2. This inhibition is relatively greater than that produced by increase in lactate concentration.

Table II.

Growth of *B. coli* anaerobically on lactate-fumarate-succinate media of varying compositions.

Conc. of lactate $\times M/10$	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Conc. of fumarate $\times M/40$	0.5	0.5	0.5	0.5	0.5	1	1	1	1	1	1	2	2	2	2	2	2	2
Conc. of succinate $\times M/40$	—	0.25	0.5	1	1.5	2	—	0.25	0.5	1	1.5	2	—	0.25	0.5	1	1.5	2
Relative anaerobic growths after 20 hours' incubation	2	2	1.7	1.3	1	0.5	3	3	2.2	1.9	1.4	0.5	3	2.9	1.7	1.1	0.8	0.75

THE SPECIFIC ACTION OF SUCCINATE.

The inhibiting action of succinate may be shown to be specifically connected with fumarate. Anaerobic growth of *B. coli* in tryptic broth, glucose solution, sodium pyruvate solution or a lactate-nitrate medium is not perceptibly inhibited by the presence of concentrations of succinate which markedly inhibit growth in a lactate-fumarate medium (see Table III). This specific action of succinates supports the view that the oxidising action of fumarate is being opposed by a reducing action of the succinate. It may be shown that the inhibition of growth is not due to toxicity of succinate, in this particular

Table III.

Growth of *B. coli* anaerobically in various media in the presence and absence of succinate.
Growths examined after 20 hours' incubation.

	Tryptic broth	Lactate- fumarate	Lactate- nitrate	Glucose $M/20$	Pyruvate $M/10$
Without succinate added	2	2	2	2	2
In the presence of succinate $M/10$	2	0.5	1.5	2	2

medium, for if nitrate be added to such a growth-inhibitive medium, an accelerated growth results. Again a lactate-fumarate-succinate medium exposed aerobically gives a good growth. The effect of the succinate is not due to osmotic action; the same molar concentration of NaCl has no effect on a lactate-fumarate medium. These results are noted in Tables IV and V.

Table IV.

To show anaerobic growths of *B. coli* in lactate-fumarate-succinate media accelerated by the presence of nitrates.

Conc. of lactate $\times M/20$	...	...	...	...	1	1	1	1	1	1
Conc. of fumarate $\times M/40$	...	...	...	...	1.5	1.5	1.5	3	3	3
Conc. of succinate $\times M/40$	...	...	...	...	2	2	2	2	2	2
Conc. of nitrate $\times M/10$	...	...	...	...	—	0.5	1	—	0.5	1
Relative anaerobic growth after 20 hrs. incubation	0.6	2	2	2	0.4	2	2	2	2	2

THE EFFECTS OF SUBSTANCES OTHER THAN SUCCINATE
ON THE ANAEROBIC GROWTH OF *B. COLI*.

So far the evidence would appear to favour the conclusion that the anaerobic growth of *B. coli* in a lactate-fumarate medium diminishes with increased concentration of succinate because the latter is responsible for an increase in reduction potential. The results do not show very clearly, however, that growth is dependent on any ratio of succinate to fumarate. There is remarkably little indication, on the whole, that increase of fumarate concentration neutralises the inhibitive action of increased succinate concentration.

Another explanation of the phenomenon is possible. The same effect of succinate on the anaerobic growth of *B. coli* in a lactate-fumarate medium would obtain if succinates were adsorbed at the fumarate enzyme so that the amount of active fumarate available for hydrogen acceptance would be reduced. Thus if the addition of succinate simply had the effect of competing with fumarate for its enzyme, there would result a diminution in the velocity of oxidation of the lactate. This would not only result in a diminution in the rate of liberation of energy but in a diminution in the rate of production of pyruvate necessary for assimilation. It is easy to see therefore how the presence of succinate would retard cell growth. If this view is true substances other than succinate, perhaps without any reducing action, would bring about the same effect—so long as they competed with either fumarate or lactate for their respective enzymes.

It is known from results on resting bacteria [Quastel and Wooldridge, 1928] that the following substances are among those which compete with lactate for its enzyme: glycollate, α -hydroxybutyrate, oxalate, hydroxymalonate. Malonate competes with succinate for its enzyme. None of these substances is comparable with succinate or lactate for reducing activity at relatively low concentrations; oxalate and malonate have no perceptible reducing action in presence of *B. coli*. Table V shows the effects of these substances on the anaerobic growth of *B. coli* in a lactate-fumarate medium. It will be seen that the substances which compete with lactate or fumarate for their enzymes inhibit growth. The same molar concentration of NaCl has no effect. The same molar concentration of acetate, which does not compete markedly with either lactate or fumarate, has relatively little effect¹.

The evidence points therefore to the action of succinate being just as much due to its competition with fumarate as to its exerting a reduction potential.

This is borne out by another experiment. As indicated earlier *B. coli* will grow anaerobically in a glycerol-fumarate medium. The growth in such a medium takes place with much lower concentrations of fumarate than will allow anaerobic growth in a lactate medium, *i.e.* a smaller number of activated fumarate molecules are necessary for growth on glycerol than for growth on

¹ Significance should not be attached to the results obtained on the addition of acetate to nitrate media since growth of *B. coli* occurs anaerobically in an acetate-nitrate medium.

lactate. (An explanation for this will be suggested later.) It would be possible, if the competition theory were true, that the addition of succinate to a glycerol-fumarate medium, whilst diminishing the total number of activated fumarate molecules, would still leave sufficient of the latter to induce relatively unaltered growth. Experiment (Table V) shows that a concentration of succinate which markedly inhibits growth in a lactate-fumarate medium has but little or no effect in a glycerol-fumarate medium, the amounts of fumarate being in each medium the same.

Table V.

To show the effect of the addition of various substrates to basal media, containing a hydrogen donator and acceptor, upon the anaerobic growth of *B. coli*. Each tube contains 5 cc. of medium consisting of the specified inorganic medium together with the concentrations of H-donator and H-acceptor given in the first column. The substrates are noted at the head of subsequent columns; each being present in *M*/20 concentration. Two drops of an 18-hour broth culture of *B. coli* were inoculated into each tube. The resulting growth after 30 hours' incubation at 37° anaerobically was estimated nephelometrically and is given by the figures in the table. The growths of each series are mutually comparable. ? = doubtful growth.

	Control	NaCl	Acetate	Oxalate	Hydroxy- butyrate	Gly- collate	Hydroxy- malonate	Malonate	Succinate
<i>M</i> /10 lactate*	3	3	2	1	0.25	0.25	1	1	0.5
<i>M</i> /20 fumarate									
<i>M</i> /100 lactate*	2	2	2	1.5	1.5	1.5	1.5	0.5	1.5
<i>M</i> /100 nitrate									
<i>M</i> /100 fumarate*	2	2	2	1.5	1.5	2	2	?	1
<i>M</i> /100 nitrate									
<i>M</i> /20 acetate*	1.5	2	1.5	2	1.5	2	3	0.75	2
<i>M</i> /20 nitrate									
<i>M</i> /50 glycerol	2	3	2	4	2	1	3	3	3
<i>M</i> /50 fumarate									
<i>M</i> /50 glycerol	3	3	3	4	2.5	2.5	4	1.5	3
<i>M</i> /50 nitrate									
<i>M</i> /20 glucose	2	2	1	2	2	2	1.5	2	2
<i>M</i> /10 pyruvate	2	2	1	2	2	2	3	3	3

* These series contain 0.5 cc. of alcoholic extract of marmite (1/250), control tubes showing no perceptible growth.

COMPETITIVE ACTION.

The specific succinate effect seems to be parallel to the effect of carbon monoxide in competing with oxygen for the oxidase system [Warburg, 1926; Keilin, 1927]. The reversibility exhibited by the CO-O₂-oxidase system, is not so easily demonstrable, however, in the succinate-fumarate system. At most we have only had indications that the inhibitive action of succinate is balanced by an increased concentration of fumarate. Further experiment is still needed to establish this finally. The experimental difficulty is that high concentrations of salts undoubtedly inhibit growth by their osmotic effect; so that only a restricted range of concentrations is possible within which to test for reversibility and the range may not be great enough for the purpose. Very low concentrations of fumarate cannot be used for experiment because at these concentrations a small increase of fumarate will, in the absence of succinate, bring about an appreciably increased rate of growth.

The competitions between succinate and malonate and between lactate and oxalate at their respective enzymes are reversible [Quastel and Wooldridge, 1928], the inhibition of reduction by a competing molecule such as malonate or oxalate not being directly proportional to the concentration of the latter but taking place at a rate which would be anticipated if adsorption occurred. Eadie [1928], who has confirmed the observation [Quastel and Whetham, 1925] that the velocity of reduction of methylene blue by a donator in presence of *B. coli* follows the logarithmic relationship

$$v = k + k' \log C,$$

(where k , k' are constants, C is concentration and v is velocity), concludes that this relationship is best interpreted on the basis of adsorption of the substrate at its enzyme.

It would seem a reasonable argument, in face of the evidence, that the succinate effect is due more to competitive adsorption at the fumarate enzyme than to the succinate exerting a reduction potential effect. The matter has been put to further test by investigating the action of succinates and other substances on a variety of media capable of supporting anaerobic growth. Table V summarises the results. It will be observed that inhibition of growth occurs where direct competition of the additional substance with a substrate of the medium is known to occur [Quastel and Wooldridge, 1928].

CONCLUSIONS ON THE ACTION OF SUCCINATE.

Part, at any rate, of the inhibiting action of succinate may be attributed to its competing with fumarate for its enzyme. There is left the possibility that some of the action is due to an increased reduction potential. It may be considered that the competition itself is synonymous with the production of an increased reduction potential owing to the diminution in number of activated fumarate molecules and hence in the velocity of oxidation. The intracellular r_H would indeed change to a more negative (reducing) value if the velocity of oxidation by fumarate within the cell were to decrease—assuming no marked poisoning action within the cell. But the same effect would occur by competition with the lactate molecules; here, in contrast to fumarate, the intracellular r_H would become more positive owing to a diminution in the velocity of reduction. In both cases a diminution of growth occurs. It may appear that there is just one value of intracellular r_H at which the rate of proliferation is greatest; that it diminishes as the r_H becomes more oxidising. But in presence of oxygen when presumably the r_H is still more positive the rate of growth is increased. Hence it becomes difficult, if not impossible, to relate the growth of the cell directly to its internal r_H .

PART II.

It is desirable now to consider the effects of the addition to the basal medium of a substance, normal to tissues, but independent of the cell or its enzymes for its reducing action. Such a substance is glutathione and the experiments to be described show the action of glutathione, cysteine and thioglycollate on the growth of *B. coli*. Details of the experimental technique will be found in Part I.

EFFECTS OF CYSTEINE, GLUTATHIONE AND THIOGLYCOLLATE ON THE GROWTH OF *B. COLI* ON A LACTATE-FUMARATE MEDIUM.

(A) *Anaerobic* (see Table VI).

It will be seen that these —SH compounds¹ affect the rate of anaerobic growth markedly. There is a considerable diminution in the growths, these results being clearly observed after 30–40 hours' anaerobic incubation.

(B) *Aerobic* (see Table VI).

The growths under aerobic conditions are, on the other hand, after 20–40 hours' incubation, at least as profuse as, and often greater than, the growths in the media containing no —SH.

Table VI.

To show effects of —SH compounds on aerobic and anaerobic growth of *B. coli* on lactate-fumarate media at p_H 7.2. Growths were examined nephelometrically after 40 hours' incubation. The —SH compounds were sterilised separately by steaming.

Conc. of lactate $\times M/20$...	1	1	1	1	1	1	1
Conc. of fumarate $\times M/40$...	1	1	1	1	1	1	1
Conc. of cysteine $\times M/156$...	—	0.5	1	—	—	—	—
Conc. of glutathione $\times M/250$...	—	—	—	0.5	1	—	—
Conc. of thioglycollate $\times M/92$...	—	—	—	—	—	0.1	0.2
Relative growths aerobic	2.5	5	3	4	4	2.5	3.5
Relative growths anaerobic	2	0.7	0.5	0.8	0.6	1.2	0.5

To account for this phenomenon it might be suggested that under aerobic conditions all the —SH is oxidised by molecular oxygen, so that there is no —SH left to give a high reduction potential. The maintenance in the cell of this potential might account for the diminution under anaerobic conditions in the rate of proliferation of the organism.

QUANTITY OF —SH PRESENT AFTER GROWTH UNDER AEROBIC AND ANAEROBIC CONDITIONS.

To test this view the amounts of —SH left in the medium after growth under aerobic and anaerobic conditions were estimated by titration with dilute iodine solution (Table VII).

In almost every case it was found that the amount of —SH left in the medium under aerobic conditions was equal to, and often greater than, the amount left under anaerobic conditions. Thus, in spite of the fact that there

¹ It is very convenient to speak of —SH as though it were a separate entity.

was present in the medium sufficient —SH to give a high negative reduction potential [Dixon and Quastel, 1923; Michaelis and Flexner, 1928], aerobic growth proceeded apparently unaffected whilst anaerobic growth was markedly inhibited.

Table VII.

To show quantities of —SH (estimated by titration with *N*/200 iodine solution) present in lactate-fumarate —SH media after 40 hours' incubation under aerobic and anaerobic conditions.

Lactate-fumarate media		Relative growths anaerobic	cc. <i>N</i> /200 iodine required for 5 cc. medium	Relative growths aerobic	cc. <i>N</i> /200 iodine required for 5 cc. medium
<i>Exp. 1.</i>					
Without —SH		2.6	0.1	3.7	0.1
With cysteine initial conc. <i>M</i> /312 ...		1.0	1.5	5.5	1.6
With glutathione initial conc. <i>M</i> /250		1.2	2.2	5.7	1.6
<i>Exp. 2.</i>					
Without —SH		2.0	0.1	2.5	0.1
With cysteine initial conc. <i>M</i> /156 ...		0.5	0.85	3.0	1.15
With glutathione initial conc. <i>M</i> /250		0.6	1.4	4.0	1.65

The fact that aerobic growth obtains under such conditions is reminiscent of the work of Brooks, Rapkine and Wurmser, and Cannan, which shows that high reduction potentials can occur within the green cell apparently in the presence of free or molecular oxygen. The fact, too, reminds us of the experiments of Hopkins [1925] on the oxidation of protein in presence of glutathione. Throughout the oxidation, even when the velocity of the latter was at its greatest, a small but definite concentration of —SH was maintained. As Hopkins said, the maintenance of this concentration was determined by the relative velocities of reduction of —S.S— by the protein and of oxidation of —SH by oxygen.

EFFECTS OF NITRATES AND OXYGEN.

If to a lactate-fumarate medium containing —SH, which therefore only feebly supports the anaerobic growth of *B. coli*, there be added nitrates, an accelerated growth of the organism is observed (Table VIII).

Table VIII.

To show the effects of nitrate on anaerobic growth of *B. coli* in lactate-fumarate —SH media

Conc. of lactate × <i>M</i> /20	...	...	...	...	1	1	1	1	1
Conc. of fumarate × <i>M</i> /40	...	...	...	...	1	1	1	1	1
Conc. of cysteine × <i>M</i> /156	...	...	...	...	—	0.5	1	0.5	1
Conc. of nitrate × <i>M</i> /1.7	...	...	...	...	—	—	—	0.5	0.5
Relative growths after 40 hours' anaerobic incubation					2.0	0.7	0.5	2.1	1.9

In the case of aerobic growth on the —SH media, there is usually a lag in the initial rate of growth which is dependent upon the amount of —SH initially introduced into the medium. But the lag disappears after 30 hours' or less incubation and then there results a very profuse growth. This lag we attribute to the medium being essentially anaerobic in the presence of excess

of —SH; as soon as the excess is removed by oxidation, molecular oxygen becomes freely available to the cells and aerobic growth proper commences.

The quantity of —SH measured after 30–40 hours' incubation does not diminish after further incubation either aerobically or anaerobically; rather does it tend to increase.

The aerobic growth of *B. coli* on the —SH media is not due simply to a growth on the —SH compounds introduced. Control experiments show that the growth on these substances in the concentrations used is small compared with the growths obtained in the lactate-fumarate —SH media. Thioglycollic acid¹, upon which the growth of *B. coli* is most feeble, has similar effects, aerobically and anaerobically, to glutathione.

INTERPRETATION OF THE EFFECTS OF —SH.

The fact that aerobic growth is unaffected and anaerobic growth is greatly diminished by the presence of —SH in a lactate-fumarate medium, and that in both cases the same amount of —SH is maintained, points to the conclusion that the ability of the cell to proliferate cannot be directly related to the reduction potential of the medium—if by reduction potential is meant the potential secured by the electromotively active substance present.

Now it has been shown by Kendall and Nord [1926] and by Michaelis and Flexner [1928] that the potential of cysteine at an electrode is markedly affected by traces of free oxygen. What, then, are we to consider the potential of the media which we have employed?

It is difficult to apply r_H terminology to such electromotively unbalanced systems as these. It can be argued that the —SH present in the medium maintains within the cell a reduction potential which becomes more positive in the presence of oxygen than in the presence of fumarate. Proliferation presumably would only occur normally at the more positive value. But if proliferation depends essentially on the maintenance of this more positive potential, why should anaerobic growth of *B. coli* occur on glucose? Here reducing conditions are so intense that, as is well known, hydrogen is evolved. Consideration of potential alone seems to give no satisfactory interpretation. This seems to be inevitable, for the rate of growth necessarily depends upon velocity relations, and only in so far as reduction potentials affect relative velocities can we expect to connect rates of growth with potential measurements.

THE QUESTION OF INTRACELLULAR HYDROGEN CARRIERS.

When *B. coli* grows anaerobically on a lactate-fumarate medium, by what means is the hydrogen transferred from the lactate to the fumarate? The two enzymes governing the activation of lactate and fumarate appear from the work on resting bacteria to be distinct from each other².

¹ To perceive the effects of these —SH compounds only relatively small quantities must be used.

² Some "overlapping" occurs in the case of the lactic and succinic enzymes when these enzymes are studied with normal *B. coli* [Quastel and Wooldridge, 1927, 2].

The enquiry resolves itself into the following problem: will a hydrogen donator react with a hydrogen acceptor in the presence of their specific enzymes but in the absence of any intermediate hydrogen carrier?

Now if such a reaction could occur in the absence of a carrier we would expect that a hydrogen donator, or acceptor, in the presence of its enzyme would be electromotively active, *i.e.* it would give a potential at an electrode in a manner similar to $-\text{SH}$ or methylene blue. The experiments bearing on this point do not, however, indicate that a hydrogen donator in the presence of its enzyme has a behaviour characteristic of an electromotively active system. Indeed the experiments of Kodama [1926] on hypoxanthine in presence of xanthine oxidase show that no such potential is produced. Keilin has been able to demonstrate that cytochrome plays the part of hydrogen carrier between oxygen activated by oxidase and hydrogen donators activated by dehydrases. It seems equally possible that in the cell of *B. coli* a carrier transfers hydrogen from the activated donators to the activated acceptors. It remains for experiment¹ finally to decide this, but in the meantime we feel justified in proceeding on the assumption that such intracellular hydrogen carriers are necessary. The scheme is represented in diagrams I and II.

The carrier² is presumably of the usual oxidation-reduction type. Let its oxidised form be represented by A and its reduced form by AH_2 . Then since it is diffusible in the cell (otherwise hydrogen carriage would be impossible) the level of oxidation-reduction potential in the cell will be determined by the ratio $[\text{A}]/[\text{AH}_2]$. This ratio will be determined by the relative velocities of the reducing and oxidising processes within the cell. Now the rate of oxidation of a hydrogen donator, say lactate, will be determined by the concentration of A and the rate of reduction of a hydrogen acceptor, say fumarate, by the concentration of AH_2 . The rates will, of course, be dependent also on the concentrations of donator and acceptor but we may assume that the cell enzymes are saturated with regard to their substrates, so that variation of substrate concentration above their saturation points will not affect velocities.

The growth of *B. coli* will be dependent on at least two factors:

- (a) the rate of production of pyruvate from lactate,
- (b) the rate of liberation of energy by oxidation.

¹ Such experiments are now being carried out.

² There may be a number of carriers but this does not affect the discussion.

These rates will be determined by the value [A] and hence by the oxidising and reducing processes present. If the value [A] be diminished by the introduction into a cell of another reducing factor, say —SH, the proliferation of the cell will be affected through changes in the rates of pyruvate and energy production. If the rate of reduction of A by —SH is greater than the rate of oxidation of AH_2 by the acceptor, the value [A] will decrease with a consequent decrease in the rate of proliferation.

This, we suggest, is a possible explanation of the effects of —SH on the aerobic and anaerobic growth of *B. coli* on a lactate-fumarate medium. It is known from work on the resting organism that the rate of oxidation of leuco-methylene blue by oxygen is much greater than by fumarate. Hence we may expect the rate of oxidation of AH_2 to be greater with oxygen than with fumarate. If therefore the amount of —SH in the cell remains constant, the value of [A] will be greater in the case of oxygen than in the case of fumarate. This value may be sufficiently large in the case of oxygen¹ to allow the proliferation of the cell to proceed unaffected, but in the case of fumarate too small for normal growth.

ENERGY REQUIREMENTS OF THE CELL.

The rate of growth, as has been stated, depends on at least two rates, those of production of pyruvate and of energy. Now it is possible that there may be two molecules, each capable of giving rise to pyruvate, but whose energy productions per molecule may differ from each other considerably. An example of a pair of such molecules is glycerol and lactate. The yield of energy per molecule in breaking down to pyruvic acid is shown in the following equations:

It is easily seen that if the rate of oxidation of glycerol is small compared with that of lactate the rate of energy production by glycerol may still be the same as that by lactate, and, so long as the pyruvate produced in both cases is sufficient for the organism's needs, the rates of proliferation on the two substances may be the same.

This means that if the concentration of A in the cell diminishes to such a small value that normal proliferation with lactate will not occur it may still be sufficiently large for a normal proliferation with glycerol. It has been stated earlier that, actually, anaerobic growth of *B. coli* in a glycerol-fumarate medium takes place at a lower concentration of fumarate than in a lactate-fumarate medium. We expect therefore that the presence of —SH in the cell

¹ We are informed by Keilin that the presence of —SH seems scarcely to affect the rate of oxidation of reduced cytochrome by oxygen.

will not affect the anaerobic growth on a glycerol-fumarate medium to as great an extent as on a lactate-fumarate medium.

Experiment confirms this (Table IX).

Table IX.

To show absence of effect of a small concentration of glutathione on growth of *B. coli* in a glycerol-fumarate medium.

Conc. of glycerol $\times M/20$	...	...	...	...	1	1
Conc. of fumarate $\times M/20$	...	...	...	...	1	1
Conc. of glutathione $\times M/250$	...	...	...	...	—	1
Relative growths after 16 hours' anaerobic incubation					2.0	2.5

PART III.

Let us now consider the effects of addition to the basal medium of a third substance—formic acid. This compound differs from succinic acid in being independent of the lactate and fumarate enzymes and from cysteine in requiring an enzyme to bring about its activation. It is specially useful for our purpose for not only is it the most powerful hydrogen donator in presence of *B. coli* (with the exception of the sugars) but it is inert as a nutritional source of carbon and its energy of oxidation is nearly that of hydrogen itself. Formates are, indeed, the most powerful source of active hydrogen so far as *B. coli* is concerned, and if the rate of proliferation of this organism is to be related to the presence in a medium of an active source of hydrogen, the inclusion of formates would be expected markedly to affect proliferation.

ANAEROBIC GROWTH OF *B. COLI* ON A LACTATE-FUMARATE-FORMATE MEDIUM (TABLE X).

The effects of relatively small quantities of formates on the anaerobic growth of *B. coli* on a lactate-fumarate medium are very striking. There is a greatly accelerated growth which seems to reach its maximum when the formate concentration is about $M/40$. Above this concentration, increase of formate tends to diminish the rate of growth. Now this effect of formates is not only to be observed in a lactate-fumarate medium but in a medium composed of fumarate alone, *i.e.* with no lactate present. The growth in a fumarate-formate medium is considerably less than in a lactate-fumarate-formate medium for the same concentration of formates (see also Table XI).

EFFECTS OF FORMATES ON ANAEROBIC GROWTH OF *B. COLI* ON LACTATE-MALATE AND LACTATE-ASPARTATE MEDIA.

It was shown by Quastel and Stephenson [1925] that anaerobic growth of *B. coli* on lactate-malate or lactate-aspartate media is doubtful or absent. Examination of the results given in Tables XII and XIII shows the powerful accelerating effects of formates on the anaerobic growth of *B. coli* in these media.

Now it seems very probable that the effect of formate is connected with its reducing activity, but to test this we have compared formate with a closely allied substance—formamide.

Table X.

To show effects of varying concentrations of formate on the anaerobic growth of *B. coli* in a lactate-fumarate medium. 17 hours' incubation. Details of all experimental technique in Part I.

Conc. of lactate $\times M/20$	1	—	—	1	1	1	1	1	1	1	1	1	1.5	2	1	—	1	1	—
Conc. of fumarate $\times M/20$	—	1	—	1	1	1	1	1	1	1	1	1	1	1	—	1	1	—	1
Conc. of formate $\times M/40$	—	—	1	—	0.1	0.3	0.5	0.7	1	2	3	5	—	—	0.5	0.5	1	1	1
Relative growths	0	0	0	1	3	5	5	6	6	3	2	0.5	1.5	1.5	0	2	6	0	2

Table XI.

Effects of formates on anaerobic growth of *B. coli* on lactate-fumarate media giving relative growths and relative bacterial counts*. 16 hours' incubation.

Conc. of lactate $\times M/20$	1	1	—	—	1	1
Conc. of fumarate $\times M/20$	—	—	1	1	1	1
Conc. of formate $\times M/40$	—	1	—	1	—	1
Relative growths	0	0	0	?	1	3
Relative bacterial counts	22	16	40	80	240	1080

* The bacterial counts (which were done in duplicate) were made by plating out two loopfuls of the medium diluted in sterile saline (1/200). These it must be remembered are viable counts.

Table XII.

To show effects of formates on anaerobic growth of *B. coli* on lactate-malate media. 40 hours incubation.

Conc. of lactate $\times M/20$	1	1	1	—	—	1	—
Conc. of malate $\times M/20$	—	1	1	1	1	1	1
Conc. of formate $\times M/40$	1	—	1	—	1	2	2
Relative growths	0	0	4	0	0	4	2

Table XIII.

To show effects of formates on anaerobic growth of *B. coli* on lactate-aspartate media. 40 hours' incubation.

Conc. of lactate $\times M/20$	1	1	1	—	—	—
Conc. of aspartate $\times M/20$	1	1	1	1	1	1
Conc. of formate $\times M/40$	—	0.5	1	—	0.5	1
Relative growths	0.5	3	3	0	?	?

ACTIVATION AND EFFECTS OF FORMAMIDE.

Formamide at low concentrations is not a powerful donator of hydrogen. In a previous communication [Quastel, 1926] it was considered that formamide had a strong reducing activity (on methylene blue in presence of *B. coli*) even at very low concentrations. Formamide purified according to the method of Willstätter and Wirth [1909] always exhibits such activity at low concentrations. If this preparation of formamide, however, be frozen at -8° , allowed partially to thaw and the liquid portion discarded, the solid portion shows a diminished reducing activity. By repeating this operation several times a specimen of formamide may be obtained which shows but little reducing activity at low concentrations ($M/500$). At appreciably greater concentrations the formamide again becomes highly reducing, but it is probable that much of this activity is due to traces of formates still remaining in

the formamide¹. Experiment shows that formamide does not inhibit reductions of methylene blue due to formate, *i.e.* it does not appear to be accessible to the formate enzyme.

The purest form of formamide which we were able to prepare was used in the experiments whose results are given in Table XIV. No acceleration in the anaerobic growth of *B. coli* was produced by formamide at concentrations in which formates produced powerful effects.

Table XIV.

To show relative effects of formate and formamide on anaerobic growth of *B. coli* in lactate-fumarate media. 18 hours' incubation.

Conc. of lactate $\times M/20$	1	1	1	1	1	1	1	1	1
Conc. of fumarate $\times M/40$	1	1	1	1	1	2	2	2	2
Conc. of formate $\times M/40$	—	1	2	—	—	—	1	2	—
Conc. of formamide $\times M/40$	—	—	—	1	2	—	—	—	1
Relative growths	1	3	3	1	1	1	5	5	1

ANAEROBIC GROWTH OF *B. COLI* ON LACTATE-FUMARATE MEDIA AT VARYING INITIAL HYDROGEN ION CONCENTRATIONS.

When growth occurs in a lactate-fumarate medium containing formates, the media usually become more alkaline owing to the oxidation of the formates. Grey [1914] has made use of the fact that formates are easily broken down by *B. coli* with liberation of alkali in his experiments on the fermentation of sugars. Now it seems conceivable that the accelerating action of formates noted above is due to their oxidation with resulting increased p_H and possibly therefore increased rate of proliferation.

This was tested by growing *B. coli* anaerobically in the presence and absence of formates on lactate-fumarate media, whose initial p_H ranged from 6.0 to 8.0 (Table XV).

Table XV.

To show anaerobic growth of *B. coli* on lactate-fumarate media of varying initial p_H , in the absence and presence of formates. 18 hours' incubation.

Conc. of lactate $\times M/20$	1	1	1	1	1	1	1	1	1	1	1
Conc. of fumarate $\times M/40$	2	2	2	2	2	2	2	2	2	2	2
Conc. of formate $\times M/40$	—	1	—	1	—	1	—	1	—	1	—
Initial p_H	6.0	6.0	6.4	6.4	6.8	6.8	7.2	7.2	7.6	7.6	8.0
Relative growths	0	0	0.9	2.4	1	1.7	0.7	2.1	0.8	1.7	0.7

It will be seen that the anaerobic growth of *B. coli* was not accelerated by increase in p_H between the range 6.4 and 8.0. The presence of formates always accelerated the growth in this range. It seems scarcely conceivable, therefore, that the formate effect is simply an effect due to change in hydrogen ion concentration.

¹ It is worth noting that the bacterial test for formate (by reduction of methylene blue in presence of *B. coli*) is more sensitive than any chemical test for formate with which we are acquainted.

Again it should be remembered in this connection that very good growth of *B. coli* occurs anaerobically on glucose and glycerol-fumarate media; and in these media growth is always accompanied by an increase in hydrogen ion concentration¹.

EFFECTS OF FORMATES ON THE ANAEROBIC GROWTH OF *B. COLI*
ON GLUCOSE AND SODIUM PYRUVATE MEDIA.

Formates at concentrations which markedly accelerate growth in lactate-fumarate media have no perceptible action on the anaerobic growth of *B. coli* in glucose and sodium pyruvate. But relatively high concentrations of formates have a definite inhibiting action (see Table XVI).

Table XVI.

To show effects of formates on anaerobic growth of *B. coli* in glucose and pyruvate media. 18 hours' incubation.

Conc. of glucose $\times M/200$	1	1	1	1	—	—	—
Conc. of pyruvate $\times M/40$	—	—	—	—	1	1	1
Conc. of formate $\times M/40$	—	1	2	4	—	1	4
Relative growths	4	4	4	1	4	4	1.5

Now it is well known that glucose on breakdown by *B. coli* produces formates, so that perhaps the lack of effect on the addition of small quantities of formate to a glucose medium is not surprising. On the other hand, the absence of formate action may be real, *i.e.* formates may definitely have no accelerating action. We have investigated the action of formate on glucose with the latter at a concentration of $M/1400$ and still obtained no accelerating action.

FERMENTATION OF PYRUVIC ACID BY *B. COLI*.

Investigation of the products formed from sodium pyruvate when a suspension of *B. coli* is placed in a solution of the latter shows that formates are produced in abundance. An examination of the products formed immediately after the pyruvic acid has been fermented indicates that the yield of the lower fatty acids and lactic acid does not account for all the pyruvic acid fermented. It has now been found that another substance is produced during the fermentation. This substance does not give the nitroprusside or guaiacol tests for pyruvic acid, but if a solution of it be heated in presence of a little alkali it breaks up to yield a solution once more giving the nitroprusside and guaiacol reactions. This phenomenon is being investigated further by one of us (W. R. W.).

The production of formate from pyruvate on fermentation by *B. coli* may be a reason why the former in low concentration does not accelerate the growth of *B. coli* on a pyruvate medium.

¹ Formates, as is shown later, do not perceptibly accelerate growth on a glycerol-fumarate medium. Such an acceleration would be expected if the formate effect was due to intracellular increase in p_H .

INTERPRETATION OF THE FORMATE EFFECT.

There is little doubt that the action of formates on growth in the lactate-fumarate medium is connected with their reducing action in presence of the cell; the growth is accelerated. Yet in the case of cysteine or glutathione, where again there was little doubt that the effects were connected with the reducing action of —SH , the growth was inhibited. It appears to be quite certain therefore, as has already been concluded, that the rate of proliferation of *B. coli* cannot be directly related to the reducing or oxidising action of the medium in presence of the cell. Nevertheless formates at relatively high concentrations do inhibit growth and this inhibition is not due to toxicity as can be shown by introducing nitrates into the medium when growth is found to be accelerated. Formate shares this inhibitory action at relative high concentration with similar high concentrations of lactate, succinate or —SH , and it seems quite possible that this action is due to a high reduction potential being set up which may be neutralised by the presence of nitrates or of oxygen.

Formates are therefore abnormal in exerting a marked accelerating action at relatively low concentrations on the growth of *B. coli*. It should be possible to account for this phenomenon on the views which have been put forward to explain the results observed with succinate and with —SH .

This can quite easily be done when it is remembered (1) that the energy of oxidation of formate is nearly that of hydrogen itself,

and (2) that formates are extremely active donators of hydrogen (in presence of *B. coli*) even at low concentrations.

It will be recalled that in interpreting the action of —SH on lactate-fumarate media and glycerol-fumarate media the suggestion was made that although the —SH brought about decreased rates of oxidation of lactate and glycerol, the energy production in the latter case was sufficiently large, but in the former case not sufficiently large, to allow the normal rate of proliferation to occur. It was assumed that sufficient pyruvate was produced for the organism's needs. The same suggestion may be made to account for the effects of formates. The formate may indeed have the effect of lowering the rate of oxidation of lactate, but, so long as this is not lowered so far that there is insufficient pyruvate produced for synthesis, the greatly increased energy production due to the oxidation of the formate itself would bring about the increased rate of growth. Thus formates should exhibit a maximum effect in their accelerating action, as is actually observed in experiment.

This explanation will account also for the accelerating action of formates on the anaerobic growth of *B. coli* on lactate-malate and lactate-aspartate media. Both malates and aspartates are hydrogen acceptors in presence of *B. coli*, though feeble compared with equal concentrations of fumarate. The fact that formates induce anaerobic growth on fumarate or malate alone is

explained when it is recalled that both fumarate and malate serve as nutritional sources of carbon in the presence of air or of nitrates. Presumably fumarate and malate can undergo self oxidation and reduction each giving rise to succinate and pyruvate (by decarboxylation of oxalacetate) and the presence of formates yield sufficient energy by their oxidation to make anaerobic growth possible. Formates will not induce anaerobic growth of *B. coli* on succinate, or lactate or glycerol or on mixtures of these substances.

This view can be put to further test. Clearly if the formate action is simply connected with the energy of oxidation of formate, the addition of a formate to a glycerol-fumarate medium should not have the same accelerating action as is observed in a lactate-fumarate medium. This is found to be the case (see Table XVII).

On these lines too we may account for the apparent absence of accelerating action of formates on growth in glucose and pyruvate media.

Table XVII.

To show the effects of formates on anaerobic growth of *B. coli* on glycerol-fumarate media. 18 hours' incubation.

Conc. of glycerol $\times M/20$	1	1	1	—	—	—	1	1	1
Conc. of fumarate $\times M/20$	—	—	—	1	1	1	1	1	1
Conc. of formate $\times M/40$	—	0.25	1	—	0.25	1	—	0.25	1
Relative growths	0	0	0	0	1	1	3	3	3

A COMPARISON OF *B. COLI* WITH *B. SPOROGENES*.

It is interesting at this stage to compare these two organisms.

Growth of *B. sporogenes* appears to depend on the acquirement by the cell of a reduction potential of such a value that certain oxidations can be eliminated¹. But it should be emphasised that it is not the magnitude of the potential which is of great significance; it is the fact that the substance responsible for this potential can reduce quickly enough to prevent certain oxidations (say by oxygen) bringing about a disturbance in the sequence of events which results in proliferation. If we could imagine the cysteine in a medium capable of supporting the growth of *B. sporogenes* to be entirely metal-free, we should expect no proliferation to occur in spite of the fact that metal-free cysteine has a high reduction potential [Harrison and Quastel, 1928]. The presence of —SH in a cell might prevent the oxidation by molecular oxygen of some grouping or constituent vital for the normal sequence of reactions in the cell. But this protection would depend not so much on the potential secured by the —SH as on the velocity of reduction due to the sulphydryl compound. The two, potential and velocity, are connected with each other (being both dependent on the concentration)—but the magnitude of the velocity will depend on the nature of the body catalysing the activity of the —SH.

Anaerobic growth of *B. coli* is inhibited in a lactate-fumarate medium by the presence of —SH. This we have suggested is due to the decrease in velocity

¹ Thus —SH (e.g. cysteine) will induce growth of *B. sporogenes* even under aerobic conditions.

of oxidation of lactate due to the presence of —SH . In effect the —SH competes with the lactate for the reduction of the fumarate. A definite velocity of oxidation of lactate—or of glycerol—is necessary for the proliferation of *B. coli*, and all the effects of succinate, —SH , and formate can be consistently interpreted on this view. The anaerobic growth of *B. coli*, as has been stated in earlier papers [Quastel, Stephenson and Whetham, 1925; Quastel and Stephenson, 1925] is not in any way fundamentally different from its aerobic growth¹.

SUMMARY AND CONCLUSIONS.

We have examined the effects of three substances, succinate, cysteine (with which are associated glutathione and thioglycollate) and formate on the anaerobic growth of *B. coli*, particularly in a lactate-fumarate medium. The first substance is known to be specifically associated for its activation with the fumarate enzyme; the second is independent of the cell for its reducing action and can act in a homogeneous phase; and the third is dependent on the cell for its reducing action but is independent of the lactate and fumarate enzymes.

It is now shown that:

(a) Succinate inhibits the anaerobic growth of *B. coli* in the lactate-fumarate medium. The effect may be accounted for by its competition with fumarate for its enzyme but it is possible that part of the effect may be attributed to its securing a high reduction potential within the cell.

(b) Cysteine (or —SH) inhibits the anaerobic growth of *B. coli* in the same medium and the effect is accounted for by its inhibiting action on the velocity of oxidation of the lactate. This may be considered a true reduction potential phenomenon but it is emphasised that the effect is due rather to the speed of reduction by the —SH than to the magnitude of the reduction potential it secures.

(c) Formate at relatively low concentrations (when it is, however, highly reducing) increases the rate of proliferation of *B. coli* in a lactate-fumarate medium and this is accounted for by the rapid oxidation of formate with high energy yield. All the other evidence given supports these conclusions.

We may now attempt to answer the question we have set ourselves: does the growth of the cell depend on the reduction or oxidation intensity, on the r_H , of the environment? We feel justified in concluding from these experiments on succinate, —SH , and formate that the rate of growth of *B. coli* is not dependent on the oxidation or reduction intensity of its environment. Relatively high concentrations of these substances do produce, however, a similar inhibitory action on the anaerobic growth of *B. coli* and

¹ It is worth emphasising that oxygen is simply one of a number of hydrogen acceptors, each possessing its characteristic properties and each important from the point of view of cellular metabolism. It is a study of all hydrogen acceptors, which induce growth, not only that of oxygen, which is essential if a true picture of the normal life of the cell is to be obtained.

it is possible that with these concentrations certain limiting conditions are secured above which growth cannot take place. These conditions are associated probably more with kinetic considerations than with potential for it is not so much the magnitude of a reduction potential which is of significance as the activity or reducing power of the substance responsible for the potential. Cysteine, for instance, gives a high reduction potential but its reducing power (velocity of reduction) is greatly dependent on the presence of traces of metals which have little or no effect on the magnitude of its potential.

It is with the greatest pleasure that we express our gratitude to Sir F. G. Hopkins for the interest he has taken in this work.

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