# XCVIII. DEHYDROGENATIONS PRODUCED BY RESTING BACTERIA. III.

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The two previous communications on the activating powers of resting or non-proliferating bacteria were confined exclusively to *B. coli comm.* This paper deals with the activating powers of *B. prodigiosus*, *B. proteus* and *B. faecalis alkaligenes.*

The experimental technique was that adopted in the work on *B. coli* [see Quastel and Whetham, 1924, 1925, 1].

## *B. prodigiosus.*

Resting *B. prodigiosus* was prepared by growing the organism in tryptic broth in Roux bottles for two days at 37°. The organism was centrifuged and washed well with normal saline. The washed *B. prodigiosus* after aeration was made up with normal saline to a suspension of which a suitable quantity (say 0·5 cc.) did not reduce a standard quantity of methylene blue in two hours. To determine the activating powers of this organism (as well as other organisms) a standard series of substances was used. All substances were brought to a $p_H$ of 7·4 prior to investigation. Table I gives the results with *B. prodigiosus*, *B. proteus* and *B. faecalis alkaligenes.*

It will be seen that in practically all respects *B. prodigiosus* is similar to *B. coli comm.* in its dehydrogenating powers. One marked difference appears, however, and this is the effect with glycine. *B. coli* so far has never been found to possess any marked activating power with glycine. As the organism (*B. prodigiosus*) becomes older its activating power on glycine appears to fall off rapidly.

## *B. proteus.*

The organism was prepared in the same way as *B. prodigiosus.*

*B. proteus* like *B. coli* appears to have no appreciable effect on glycine. It seems to resemble *B. coli* in its dehydrogenating powers. In the presence of high concentrations of the fatty acids *B. proteus*, like *B. coli*, causes rapid reduction of methylene blue. With low concentrations of the fatty acids no decoloration of methylene blue occurs.

## Table I.

Each vacuum tube contained 2 cc. of phosphate buffer $p_H$ 7·4, 1 cc. of 1/5000 methylene blue solution, a quantity of the suspension of the organism and 1 cc. of a solution of the substance under investigation. The volume of solution in each tube was made up to 6·5 cc. with distilled water. The reduction was carried out *in vacuo* at 45°.

| | | Reduction time in minutes | | |
|---|---|---|---|---|
| Donator | Concentration of donator | 0·5 cc. *B. prodigiosus* | 0·5 cc. *B. proteus* | 0·2 cc. *B. alkaligenes* |
| None | Control | Not reduced in 2 hrs. | Not reduced in 2 hrs. | 31·0 |
| Formic acid | *M*/30 | 4·0 | 2·0 | 7·25 |
| Acetic acid | *M*/13 | 32·4 | | 12·9 |
| ,, | *M*/6·5 | | 2·5 | |
| Propionic acid | *M*/13 | 64·0 | | 16·1 |
| ,, | *M*/6·5 | | 16·2 | |
| Butyric acid | *M*/13 | 74·0 | | 14·9 |
| ,, | *M*/6·5 | | 90·0 | |
| Isobutyric acid | *M*/13 | 19·4 | | 22·2 |
| ,, | *M*/6·5 | | 13·4 | |
| Oxalic acid | *M*/13 | Not reduced in 2 hrs. | Not reduced in 2 hrs. | 40·0 |
| Malonic acid | *M*/13 | ,, | ,, | 40·0 |
| Succinic acid | *M*/130 | 6·0 | 5·3 | 21·0 |
| Glutaric acid | *M*/13 | Not reduced in 2 hrs. | | |
| Lactic acid | *M*/13 | 6·2 | 5·7 | |
| ,, | *M*/130 | 18·8 | 13·7 | 9·0 |
| α-Hydroxybutyric acid | *M*/13 | 12·0 | 3·7 | 4·4 |
| ,, | *M*/130 | 36·0 | 25·0 | |
| β-Hydroxybutyric acid | *M*/13 | 4·3 | 2·4 | 3·8 |
| ,, | *M*/130 | 3·25 | 80 % reduced in 90 minutes | |
| Mandelic acid | *M*/13 | 15·7 | Not reduced in 2 hrs. | 14·8 |
| ,, | *M*/130 | Not reduced in 2 hrs. | | 26·0 |
| Tartaric acid | *M*/13 | 27·0 | Not reduced in 2 hrs. | 32·0 |
| ,, | *M*/4·3 | | 63·0 | |
| Glycollic acid | *M*/13 | Not reduced in 2 hrs. | Not reduced in 2 hrs. | 31·0 |
| ,, | *M*/4·3 | | 90·0 | |
| Glycine | *M*/10 | 6·0 | Not reduced in 2 hrs. | 26·0 |
| Alanine | *M*/12 | 35·8 | ,, | 22·3 |
| Glutaminic acid | *M*/19 | 15·6 | | |
| ,, | *M*/13 | | 7·7 | 12·4 |
| Glycol | *M*/13 | 51·2 | Not reduced in 2 hrs. | 31·0 |
| Glycerol | *M*/6 | 5·6 | 7·0 | 29·0 |
| Erythritol | *M*/6 | Not reduced in 2 hrs. | | |
| ,, | *M*/13 | | Not reduced in 2 hrs. | 31·0 |

### *B. faecalis alkaligenes.*

The organism was prepared in the same way as the previous two. A marked feature of well-washed *B. alkaligenes* suspensions is the high reducing activity of the organism itself. In this it differs markedly from *B. coli* and *B. proteus*.

It is clear from the results with *B. alkaligenes* that this organism has only a very slight activating power, this power being apparently greatest with the hydroxy-monobasic acids and formic acid (as seems also to be the case with *B. coli*, *B. prodigiosus* and *B. proteus*).

*The activations of fumaric, malic and aspartic acids.*

There is a marked difference in activating power between *B. alkaligenes* and *B. prodigiosus* or *B. proteus*; the differences, however, between the latter two organisms are not so apparent. Apart from the effect with glycine the differences appear to be simply of a quantitative nature. This quantitative aspect of the differences in activating power between the two organisms is shown most clearly in the case of fumaric acid.

It was shown by Quastel and Whetham [1924] that fumaric acid is activated by *B. coli*, this activation being demonstrated by the power of fumaric acid to oxidise leucomethylene blue only in the presence of the resting organism. *B. prodigiosus* possesses this power of activating fumaric acid to a marked degree; *B. proteus* possesses the same power but apparently to a much smaller extent. It was shown in the case of *B. coli* [Quastel, Stephenson and Whetham, 1925] that activated fumaric acid can play a dual rôle—it can act as a hydrogen acceptor (*e.g.* lactic acid will transfer its hydrogen to fumaric acid in presence of *B. coli*) and it can act as a hydrogen donator (or oxygen acceptor, *e.g.* fumaric acid is oxidised by nitrates in presence of *B. coli*). In the methylene blue experiments with *B. coli*, no reduction of methylene blue by fumaric acid was ever observed. It is clear, however, that if fumaric acid can both accept and donate hydrogen then the reduction velocity of methylene blue in the vacuum tube will be dependent on the velocities of the two reactions which fumaric acid can bring about. (In speaking of fumaric acid as a hydrogen donator the usual nomenclature of reduction phenomena is being adopted. The reduction does not necessarily mean that hydrogen is being transferred from the fumaric acid molecule to methylene blue; it is far more likely in this case that the elements of water play a part in the reaction.)

The rate of reduction of methylene blue by *B. prodigiosus*, like that of *B. coli*, is strongly retarded by fumaric acid. For instance, 2 cc. of a thick suspension of *B. prodigiosus* reduced 1 cc. of 1/5000 methylene blue (without the addition of any donator) in 15 minutes. In presence of fumaric acid (0·8 %) complete reduction was never obtained; the methylene blue was reduced to an equilibrium point which remained stationary. Again, 0·5 cc. of a suspension of *B. prodigiosus*, which did not reduce 1 cc. of 1/5000 methylene blue in two hours, but which in the presence of succinate (0·15 %) reduced in six minutes, did not in the presence of a mixture of succinate (0·15 %) and fumarate (0·8 %) bring about a complete reduction of the methylene blue after several hours; an equilibrium point was reached. Just as with *B. coli*, therefore, *B. prodigiosus* strongly activates fumaric acid, so that the velocity with which the latter oxidises leucomethylene blue is greater than that with which reduction of the dyestuff occurs. It is possible by using a sufficiently large amount of organism in the presence of an active donator to produce an extremely rapid rate of reduction of methylene blue—this reduction being so rapid that even if fumaric acid is present the oxidising power of the latter

does not exhibit itself until after a few minutes, when possibly all the methylene blue has been reduced. For instance, 2 cc. of a thick suspension of *B. prodigiosus* reduced 1 cc. of 1/5000 methylene blue solution in 15 minutes; in presence of succinate (0·15 %) it reduced in five minutes; in presence of a mixture of succinate (0·15 %) and fumarate (0·8 %) it reduced the methylene blue almost to completion in six minutes and then oxidation of the leucomethylene blue commenced, for the colour of the solution instead of disappearing completely quickly became bluer until it reached an intensity which remained stationary for several hours. This phenomenon will be referred to as "reversal." (Reversal was first noticed by Quastel and Whetham in the case of *B. coli* and β-hydroxybutyric acid and will be discussed in detail elsewhere.) If a sufficiently large quantity of *B. coli* be used, reversal can be demonstrated in a mixture of succinate and fumarate, just as with *B. prodigiosus*. In the absence of an active donator such as succinate neither *B. coli* nor *B. prodigiosus* has been found to exhibit reversal with fumaric acid. This demonstrates clearly the high oxidising power of the latter acid in presence of these organisms.

With *B. proteus* an activation of fumaric acid is produced; but in this case reversal can be observed with fumaric acid alone. For instance, 1 cc. of a *B. proteus* suspension did not alone reduce 1 cc. of 1/5000 methylene blue in two hours; in presence of fumarate (0·8 %) it reduced practically to completion in six minutes and then reversal occurred, the colour of the solution becoming deep blue by the end of two hours. In the presence of succinate (0·15 %) it reduced completely in four minutes and in the presence of both succinate (0·15 %) and fumarate (0·8 %) a complete reduction occurred in eight minutes, a slight reversal only being observed after a considerable time. It is clear, therefore, that in the case of *B. proteus* the velocity with which fumaric acid oxidises leucomethylene blue is small compared with that in the case of *B. prodigiosus*—or of *B. coli*. This difference is well illustrated by the results given in Table II.

## Table II.

Each vacuum tube contained 2 cc. phosphate buffer $p_H$ 7·4, 1 cc. 1/5000 methylene blue solution, 0·5 cc. of the resting organism, 1 cc. *M*/20 succinate and *X* cc. of 5 % fumarate. The solution was made up to 6·5 cc. with distilled water and the reduction carried out *in vacuo* at 45°. Controls (in the absence of succinate or fumarate) showed no reduction in two hours.

| Organism | *X* cc. | Observation |
|---|---|---|
| *B. prodigiosus* | 0 | Reduced completely in 6 mins. |
| ,, | 0·1 | Stationary equilibrium point reached after 19 mins. Complete reduction not attained. No reversal observed |
| ,, | 0·2 | Equilibrium attained. No complete reduction. No reversal |
| ,, | 0·4 | Do. |
| ,, | 0·8 | Do. |
| *B. proteus* | 0 | Reduced completely in 6 mins. 25 secs. |
| ,, | 0·1 | Reduced completely in 8 mins. No reversal observed after 1 hour |
| ,, | 0·2 | Reduced to completion in 10 mins., and then slight reversal |
| ,, | 0·4 | Reduced almost to completion and then reversal |
| ,, | 0·8 | Do. |

With malic and aspartic acids similar effects appear. It was shown in the case of *B. coli* that both malic and aspartic acids have the effects of weak hydrogen acceptors [see Quastel and Whetham, 1924; 1925, 2]. *B. prodigiosus* behaves similarly to *B. coli*. For instance, 1 cc. of a thick suspension of *B. prodigiosus* alone reduced 1 cc. 1/5000 methylene blue solution in 14 minutes, whilst in presence of malate (0·8 %) complete reduction was not observed but an equilibrium point was attained; in the presence of aspartate (0·8 %) there was reduction almost to completion followed by reversal. With succinate (0·15 %), the presence of malate (0·8 %) retarded the velocity of reduction, reversal not being observed after one hour. Aspartic acid (0·8 %) had no appreciable effect on the velocity of reduction due to succinate. In the case of *B. proteus* there appeared to be no marked effects on malic or aspartic acid.

*B. alkaligenes* showed no appreciable activating power on fumaric, malic or aspartic acid.

### *The effect of malonic acid.*

It was observed by Quastel and Whetham [1925, 1] that malonic acid had a retarding effect on the velocity of reduction of methylene blue by *B. coli* in the presence of succinate. Neither oxalic nor glutaric acid has such an effect. *B. prodigiosus* and *B. proteus* have now been examined and malonic acid again shows the same anomalous behaviour (see Table III).

### Table III.

Conditions as in Table II, but with the addition of 1 cc. of *M*/2 oxalic or malonic acid instead of *X* cc. of fumaric acid.

| Organism | Acid | Reduction time |
|---|---|---|
| *B. prodigiosus* | None (control) | 6 mins. 5 secs. |
| ,, | Oxalic | 6 ,, 5 ,, |
| ,, | Malonic | 18 ,, 43 ,, |
| *B. proteus* | None (control) | 6 ,, 20 ,, |
| ,, | Oxalic | 8 ,, |
| ,, | Malonic | Not completely reduced in 1 hour |

### Table IV.

Conditions as in Table II, but with 1 cc. of 5 % $KNO_3$ or 1 cc. *M*/10 $KClO_3$ instead of *X* cc. of fumarate.

| Organism | Oxidant | Reduction time |
|---|---|---|
| 0·5 cc. *B. prodigiosus* | None (control) | 6 mins. |
| ,, | Nitrate | Not reduced after 2 hrs. |
| ,, | Chlorate | ,, |
| 0·5 cc. *B. proteus* | None (control) | 3 mins. |
| ,, | Nitrate | Not reduced after 2 hrs. |
| ,, | Chlorate | ,, |
| 0·2 cc. *B. alkaligenes* | None (control) | 20 mins. |
| ,, | Nitrate | 15 ,, |
| ,, | Chlorate | 16 ,, |

*Activations of nitrates and chlorates.*

Like *B. coli* [see Quastel, Stephenson and Whetham, 1925] both *B. prodigiosus* and *B. proteus* strongly activate nitrates and chlorates, so that these are able to oxidise leucomethylene blue in presence of the organism. *B. alkaligenes* appears to have no appreciable activating power on nitrates and chlorates.

Table IV illustrates the experimental results.

## DEMONSTRATION OF THE THERMOLABILITY OF THE MECHANISMS DEALING WITH FUMARATE, NITRATE AND CHLORATE.

The demonstration can be carried out by means of vacuum U-tubes [see Quastel and Whetham, 1924], but the following experiments, which are dependent on the fact that *B. alkaligenes* does not activate (to any appreciable extent) nitrate, chlorate or fumarate, are equally convincing. Three solutions are made up; the first containing *B. alkaligenes* (which itself reduces methylene blue very quickly) and, say, the nitrate; the second containing *B. alkaligenes*, *B. prodigiosus* heated for five minutes at 100°, and the nitrate; and the third *B. alkaligenes*, *B. prodigiosus* unheated, and the nitrate. The effects of these solutions on the reduction of methylene blue are observed. It is found that the solution containing the *B. alkaligenes* alone and that containing the mixture of *B. alkaligenes* and heated *B. prodigiosus* reduce the methylene blue in approximately the same time, whilst that containing the unheated *B. prodigiosus* remains blue permanently. Table V gives a complete set of results.

Table V.

Each vacuum tube contained 2 cc. phosphate buffer $p_H$ 7·4, 1 cc. 1/5000 methylene blue, 0·5 cc. *B. alkaligenes* suspension, 1 cc. of *B. prodigiosus* or *B. proteus* (heated or unheated), 1 cc. of the oxidant. The volume was made up to 6·5 cc. with distilled water and reduction carried out *in vacuo* at 45°.

| *B. prodigiosus* | | *B. proteus* | | | |
|---|---|---|---|---|---|
| Unheated | Heated | Unheated | Heated | Oxidant | Reduction time |
| — | — | — | — | None (control) | 4 mins. 30 secs. |
| — | — | — | — | $M/10$ $KClO_3$ | 4 mins. 30 secs. |
| 1 cc. | — | — | — | ,, | Not reduced after 2 hrs. |
| — | 1 cc. | — | — | ,, | 4 mins. |
| — | — | 1 cc. | — | ,, | Not reduced after 2 hrs. |
| — | — | — | 1 cc. | ,, | 3 mins. 13 secs. |
| 1 cc. | — | — | — | 5 % $KNO_3$ | Not reduced after 2 hrs. |
| — | 1 cc. | — | — | ,, | 4 mins. 13 secs. |
| — | — | 1 cc. | — | ,, | Not reduced after 2 hrs. |
| — | — | — | 1 cc. | ,, | 6 mins. |
| 1 cc | — | — | — | 5 % fumarate | Reduction in 6 mins. 20 secs. followed by strong reversal |
| — | 1 cc. | — | — | ,, | Reduction in 6 mins. 30 secs. *not* followed by any reversal |
| — | — | 1 cc. | — | ,, | Reduction in 2 mins. followed by reversal |
| — | — | — | 1 cc. | ,, | Reduction in 9 mins. *not* followed by reversal |

*The activation of the sugars.*

The reducing power of a number of the sugars and the corresponding alcohols in the presence of the three organisms discussed in this paper have been examined. Table VI illustrates the results.

Table VI.

Conditions as in Table I, but with different organisms.

| Sugar (etc.) concentration = $M/130$ | Reduction time with 0·5 cc. *B. prodigiosus* | Reduction time with 0·5 cc. *B. proteus* | Reduction time with 0·2 cc. *B. alkaligenes* |
|---|---|---|---|
| None (control) | Not reduced in 2 hrs. | Not reduced in 2 hrs. | 24 mins. |
| Glucose | 5 mins. 4 secs. | 14 mins. 49 secs. | 22 ,, |
| Laevulose | 5 ,, 53 ,, | 16 ,, 6 ,, | 18 ,, |
| Mannose | 7 ,, 40 ,, | Not reduced in 2 hrs. | 23 ,, |
| Galactose | 4 ,, 42 ,, | ,, | 20 ,, |
| Sucrose | 33 ,, 42 ,, | ,, | 18 ,, |
| Lactose | 11 ,, 15 ,, | ,, | 19 ,, |
| Maltose | 9 ,, 12 ,, | ,, | 12 ,, |
| Xylose | 10 ,, 58 ,, | ,, | 16 ,, |
| Arabinose | Not reduced in 1 hr. | ,, | 35 ,, |
| Sorbitol | 10 mins. 2 secs. | ,, | 24 ,, |
| Mannitol | 8 ,, 9 ,, | ,, | 26 ,, |
| Dulcitol | 44 ,, | ,, | 19 ,, |

The effects on the sugars may be summarised briefly:

1. *B. prodigiosus* activates powerfully all the compounds investigated, with the exception of dulcitol, sucrose and arabinose which it activates only slightly. It resembles therefore *B. coli* [see Quastel and Whetham, 1925, 2] which, however, does not activate dulcitol or sucrose to any appreciable extent.

2. With the exception of glucose and laevulose *B. proteus* appears not to activate the sugars to any considerable extent.

3. *B. alkaligenes* has a slight effect with maltose but none appreciably on the other sugars.

## Summary.

An account is given of the activating powers of *B. prodigiosus*, *B. proteus* and *B. faecalis alkaligenes*.

1. *B. alkaligenes* shows only feeble activating powers, reducing effects (with methylene blue as hydrogen acceptor) being greatest with formic, lactic, $\alpha$- and $\beta$-hydroxybutyric acids. No oxidising action of fumarates, malates, aspartates, nitrates or chlorates can be demonstrated with this organism. Its power of activating the sugars is also very slight.

2. *B. prodigiosus* possesses powerful activating properties, which appear in the cases of fumarates, malates and aspartates to be greater than those of *B. coli*. It activates all the sugars tested, its effects with sucrose, arabinose and dulcitol being relatively weak.

3. *B. proteus* is less powerful than *B. prodigiosus* or *B. coli* in activating fumarates, malates or aspartates as hydrogen acceptors. Its action on nitrates

and chlorates is similar to that of these organisms. Only glucose and laevulose, among the sugars, appear to be activated by *B. proteus* to any considerable extent.

4. The thermolability of the mechanisms in *B. prodigiosus* and *B. proteus* which activate fumarates, nitrates and chlorates is demonstrated.

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REFERENCES.

Quastel and Whetham (1924). *Biochem. J.* **18**, 519.
—— —— (1925, 1). *Biochem. J.* **19**, 520.
—— —— (1925, 2). *Biochem. J.* **19**, 645.
Quastel, Stephenson and Whetham (1925). *Biochem. J.* **19**, 304.