XCVII. DEHYDROGENATIONS PRODUCED BY RESTING BACTERIA. II.

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In Part I of this series [Quastel and Whetham, 1925] an account was given of the behaviour of the following groups of substances as hydrogen donators in the presence of resting *B. coli*.

1. Fatty acids.
2. Saturated dibasic acids.
3. Hydroxy-acids.
4. Polyhydric alcohols.
5. Monohydric alcohols.

A table was given of the relative reducing powers or reducing coefficients of the hydrogen donators.

This communication deals with the behaviour of a number of the amino-acids and the sugars in the presence of resting *B. coli*. The technique adopted throughout the work has been described fully in earlier papers. It consists, briefly, in measuring the rate of reduction of methylene blue under standard conditions of temperature and hydrogen ion concentration, the measurements being made under anaerobic conditions in vacuum tubes.

The experimental results given below are typical examples of a large number of observations. A number of different strains and suspensions of *B. coli* were used and these are referred to as *coli* 1, *coli* 2, etc. The results obtained with *coli* 1 can be compared strictly quantitatively with one another, but only qualitatively with the results obtained with *coli* 2, etc.

The substances under investigation were always brought to a p_H of 7·2.

The Amino-acids.

Glycine appears to be almost inert as a hydrogen donator to methylene blue. It gives indications of a slight activity at very high concentrations but in no case has it been found to produce a complete reduction of the standard quantity of methylene blue used in these experiments. Alanine, on the other hand, is a fairly active donator, its activity being comparable with that of acetic acid. Table I illustrates results with these amino-acids. A succinate reduction is included for comparison.

Table I.

Each vacuum tube contained 2 cc. phosphate buffer p_H 7·2, 1 cc. 1/5000 methylene blue solution, 0·5 cc. *coli* 1, and *X* cc. of the amino-acid solution. 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°. A control tube showed no reduction in 6 hours.

Amino-acid	*X* cc.	Conc. of amino-acid	Reduction time
5 % alanine	1	*M*/11·6	65 minutes
5 % glycine	1	*M*/9·7	Not reduced in 6 hours
Succinic acid (control)	—	*M*/130	10′ 10″

Fig. 1 illustrates the variation in times of reduction of 1 cc. of 1/5000 methylene blue with varying concentrations of alanine at p_H 7·2 and 45° in presence of the same amount of organism.

Leucine gives no sign of reducing activity even at high concentrations.

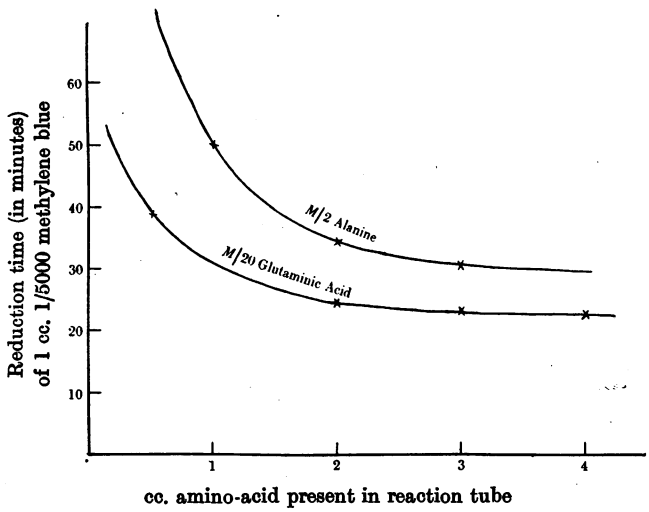

Fig. 1.

Histidine is slightly active at relatively low concentrations, whilst tryptophan is inactive at low concentrations but fairly active at high concentrations (see Table II).

Table II.

Conditions as in Table I.

Amino-acid	Concentration of amino-acid	Organism	Reduction time
Tryptophan	*M*/130	*Coli* 1	Not reduced in 3 hours
,,	*M*/26	,, 1	24′ 5″
Leucine	*M*/17	,, 2	Not reduced in 3 hours
Histidine	*M*/130	,, 3	169′
,,	*M*/65·5	,, 3	125′
,,	*M*/43·3	,, 3	80′
Succinate *M*/20 (control)	*M*/65·5	,, 3	5′ 15″

Glutaminic acid appears to be the most active amino-acid yet investigated. Its activity is comparable with that of succinic acid (Fig. 1 gives an example of its reduction time-concentration curve.) Aspartic acid, on the other hand, gives no indication of being a donator of hydrogen but has the properties of

a weak hydrogen acceptor. In this respect it resembles malic acid [see Quastel and Whetham, 1924]. Mixtures of succinic acid and aspartic acid, instead of reducing methylene blue completely, reach an equilibrium point which is dependent on the relative concentrations of succinic and aspartic acids. Experimental results with aspartic acid are demonstrated in Table III.

Table III.

Each vacuum tube contained 1 cc. of phosphate buffer p_H 7·2, 1 cc. of 1/5000 methylene blue solution, 2 cc. *coli* 4, 2 cc. of 4 % aspartic acid solution (neutral), and X cc. of $M/20$ sodium succinate. The volume was made up to 7 cc. with distilled water and the reduction carried out *in vacuo* at 45°.

X cc.	Equilibrium point attained in terms of cc. of methylene blue (1/5000)
0·5	0·24
1·0	0·17
1·5	0·10

Aspartic acid is far less active than fumaric acid as a hydrogen acceptor. It is difficult to understand how aspartic acid accomplishes the oxidation of leucomethylene blue unless it undergoes a preliminary deamination to fumaric acid. If this is true the system becomes analogous to the deamination of histidine to urocanic acid [Raistrick, 1917].

It is interesting that the amino-acids which show greatest activity in presence of *B. coli*, viz. glutaminic acid and alanine are also those which show considerable activity in presence of muscle [Thunberg, 1920].

The sugars and related substances.

Glucose appears to be an extremely vigorous donator of hydrogen to methylene blue in presence of *B. coli*. Its activity is greater than that of formic acid (see Table IV).

Table IV.

Each vacuum tube contained 1 cc. phosphate buffer p_H 7·2, 1 cc. of 1/5000 methylene blue solution, 1 cc. *coli* 5, and a quantity of the hydrogen donator. The volume was made up to 7 cc. with distilled water. A control tube did not reduce in 3 hours.

Donator	Concentration of donator	Reduction time
Formic acid	$M/3500$	17′ 30″
,,	$M/1750$	4′
Glucose	$M/3500$	6′ 36″
,,	$M/1750$	5′ 30″

An investigation of the influence of the concentration of the sugars on the reduction time of a quantity of methylene blue shows that at extremely low concentrations the velocity of reduction reaches a value which only very gradually increases with increase in concentration (see Fig. 2). This is typical of the reduction time-concentration curves of most of the sugars.

If glucose donates two atoms of hydrogen per molecule of glucose to methylene blue, then the least quantity of $M/100$ glucose solution which can completely reduce 1 cc. of 1/5000 methylene blue is 0·063 cc. Experiment shows,

however, that the least quantity of glucose which will completely reduce 1 cc. of 1/5000 solution of methylene blue (at p_H 7·2 and in presence of *B. coli*) is 0·02 cc. This means that a single molecule of glucose donates at least four (and possibly six) atoms of hydrogen to methylene blue in presence of *B. coli*. The least quantity of formic acid which accomplishes complete reduction of 1 cc. 1/5000 methylene blue solution is 0·1 cc. of a *M*/100 solution, an amount which would be expected if formic acid donated two atoms of hydrogen per molecule.

Laevulose is a good hydrogen donator with *B. coli*, though not so powerful as glucose (see Fig. 2). The lowest concentration of it which reduces 1 cc. of 1/5000 methylene blue solution completely is 0·02 cc. of a *M*/100 solution. Like glucose, therefore, laevulose donates at least four (and possibly six) atoms of hydrogen per molecule. The velocity of reduction due to laevulose at low concentration is less than that due to glucose. A point of practical importance in connection with glucose and laevulose may be mentioned. 0·02 cc. of *M*/100 solution of the sugar corresponds to 0·036 mg. of the sugar which in a volume of 7 cc. readily reduces 1 cc. 1/5000 methylene blue solution; *i.e.* a concentration of glucose corresponding to 1 part in 200,000 may be readily detected by the resting bacteria-methylene blue method.

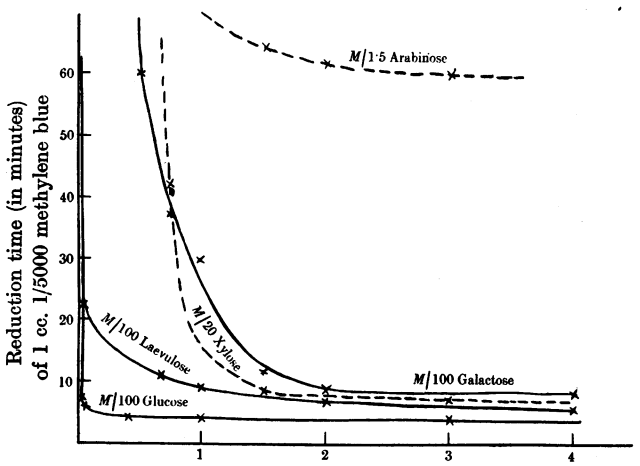

Fig. 2.

Galactose is less active than glucose or laevulose (see Fig. 2) and it is impossible to state by the methylene blue method whether galactose donates more than two atoms of hydrogen per molecule. Mannose is a good donator of hydrogen but this sugar has not yet been examined in detail. It is at least as active as galactose.

Xylose and arabinose, among the pentoses, have been examined. Their effects are indicated in Fig. 2. Arabinose is an extremely poor donator of hydrogen compared with xylose, whilst the latter is not as active as galactose (see Table V).

Table V.

Conditions as in Table IV. *Coli* 6.
A control showed no reduction in 3 hours.

Donator	Concentration of donator	Reduction time
Galactose	*M*/1400	60′
,,	*M*/700	30′
,,	*M*/350	8′ 25″
Maltose	*M*/1400	Not reduced in 2 hours
,,	*M*/280	30′
,,	*M*/140	8′
Arabinose	*M*/21	Not reduced in 2 hours
,,	*M*/10·5	110′
,,	*M*/7	87′
Xylose	*M*/280	Not reduced in 2 hours
,,	*M*/186	41′
,,	*M*/93	8′
Mannitol	*M*/28,000	17′
,,	*M*/35,000	Not reduced in 2 hours
Glucose	*M*/14,000	8′
Laevulose	*M*/14,000	22′
Succinic acid	*M*/700	26′
,,	*M*/70	10′

Among the disaccharides, sucrose appears to be inert. Both lactose and maltose, however, are good hydrogen donators (see Tables V and VI).

Table VI.

Conditions as in Table V. *Coli* 7.
Control showed no reduction in 1 hour.

Concentration of lactose	Reduction time
M/7000	27′ 25″
M/1750	11′ 35″
M/583	9′ 30″

It is difficult, with the disaccharides, to decide how far the reduction may be due to traces of glucose produced by hydrolysis. Harden and Zilva [1915] found no reducing effect with lactose, although a positive effect was obtained with maltose. Under the conditions of our experiments, however, no specimen of lactose has been found to be devoid of reducing activity.

Of the hexahydric alcohols, sorbitol, mannitol and dulcitol have been examined. Both mannitol (see Table V) and sorbitol show considerable reducing activity, whilst dulcitol seems to be practically inert (see Tables VII and VIII).

Mannitol appears to be almost as active as glucose and donates at least four atoms of hydrogen per molecule to methylene blue in presence of *B. coli*.

Only adonitol and arabitol have been examined among the pentahydric alcohols. The former is devoid of any appreciable reducing activity, whilst the latter which was prepared by reduction of arabinose with sodium amalgam seems to be a better donator of hydrogen than arabinose.

Raffinose and rhamnose have been examined and these do not seem to possess any reducing activity.

Table VII.

Each vacuum tube contained 2 cc. phosphate buffer p_H 7·2, 1 cc. 1/5000 methylene blue, and the hydrogen donator. Volume made up to 6 cc. with distilled water.

Donator	Concentration of donator	Reduction time
	1 cc. *coli* 8.	
None (control)	—	46′
Glucose	*M*/120	5′ 20″
Mannitol	*M*/120	8′ 15″
Sorbitol	*M*/120	7′ 40″
Saccharic acid	*M*/120	15′ 45″
Succinic acid	*M*/120	5′ 15″
	1 cc. *coli* 9.	
None (control)	—	22′ 30″*
Mannitol	*M*/120	9′ 10″
Sorbitol	*M*/120	9′ 35″
Dulcitol	*M*/120	20′
Adonitol	*M*/120	20′

* *Coli* 9 was a highly reducing organism.

Among the acids corresponding to the sugars, saccharic, gluconic and mucic acids have been investigated only in a qualitative manner. Saccharic and gluconic acids are poor but definite hydrogen donators whilst mucic acid, up to a concentration of *M*/24, is inert.

An examination has been made of several glucosides. Crude α-methylglucoside is a good donator of hydrogen but after being recrystallised a number of times, the pure material is practically devoid of reducing power. So far a specimen of β-methylglucoside has not been obtained which is inert. Amygdalin, if purified by several recrystallisations, is almost inert, although the crude material is nearly as active as glucose. Salicin and phloridzin have not been found to possess any reducing activity.

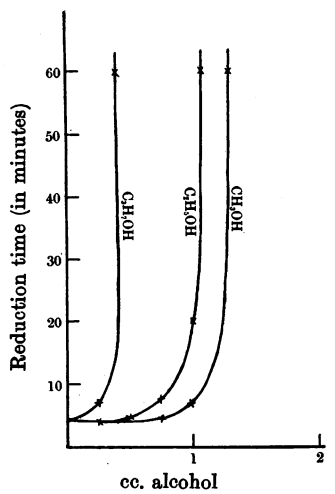

Fig. 3. Effects of methyl, ethyl and propyl alcohols on reduction times of 1 cc. 1/5000 methylene blue by 1 cc. *M*/20 glucose in presence of *B. coli*.

The alcohols—methyl, ethyl and propyl—have powerful inhibitory effects on the reduction of methylene blue due to the sugars. The inhibitory effect of methyl alcohol is less than that of ethyl alcohol and this less than that of propyl alcohol (see Fig. 3). The inhibitions are of a similar order to those noted in the case of other hydrogen donators.

The reducing coefficients of some of the amino-acids and sugars with respect to *B. coli* are given below (see Quastel and Whetham [1925] for details with regard to reducing coefficients). They are only to be regarded as approximate and as expressing the correct order of magnitude.

Reducing Coefficients (with respect to B. coli).

Succinic acid	100
Formic acid	700
Alanine	1·0
Glutaminic acid	25
Glycine	less than 0·8
Glucose	5000
Laevulose	5000
Galactose	200
Mannitol	5000
Xylose	20
Arabinose	less than 0·8
Maltose	50

Summary.

An account is given of the behaviour of a number of amino-acids, sugars and related substances in the presence of resting *B. coli* as the activating source and of methylene blue as the hydrogen acceptor. A table is given of the relative reducing powers or reducing coefficients of some of the donators.

We wish to express our appreciation of the interest taken by Sir F. G. Hopkins in this work and the thanks of one of us (M. D. W.) are due to the Medical Research Council for a grant held during this work.

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