LXXXVIII. HYDROGENLYASES.
BACTERIAL ENZYMES LIBERATING MOLECULAR HYDROGEN.

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THE earliest work dealing with the bacterial decomposition of formic acid was that of Hoppe-Seyler [1876], who dealt with its decomposition into carbon dioxide and hydrogen by mixed cultures obtained from mud, and showed that calcium formate was decomposed according to the equation

$$(H.COO)_2.Ca + H_2O \rightarrow CaCO_3 + CO_2 + 2H_2.$$

Later Pakes and Jollyman [1901, 1] showed that without exception those organisms which, in the presence of peptone water, fermented sugars with the production of hydrogen, also decomposed formic acid into carbon dioxide and hydrogen, and they drew the conclusion that the hydrogen produced in the fermentation of sugars arose from the decomposition of intermediately formed formic acid. The evidence of Harden [1901], Grey [1914] and many others confirmed this view, and it has been considered established that the hydrogen from sugar fermentations arises solely from decomposition of formic acid.

Thus the earlier work dealing with the bacterial attack on formic acid concerned itself entirely with the production of molecular hydrogen. Along with the study of bacterial dehydrogenases a second mode of attack on formic acid came into view [Quastel and Whetham, 1925]. Here it was shown by the use of washed suspensions of bacteria and the methylene blue technique that there existed an enzyme catalysing the reaction

$$H.COOH + X = CO_2 + XH_2$$

where X represents, in the experiments, methylene blue, and, in the normal metabolism of the cell, probably an intracellular hydrogen carrier. The behaviour of formic acid in this reaction is the same as that of a large number of other substances which donate hydrogen to acceptors but do not give off molecular hydrogen.

Stickland [1929] attempted to find the relation between these two modes

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of decomposition of formic acid by *Bact. coli*. He found that washed suspensions rapidly dehydrogenated formic acid in the presence of methylene blue, but did not carry out the reaction resulting in the liberation of hydrogen gas until they had been in contact with the formate solution for some 24 hours. Viable cell counts showed that the appearance of hydrogen did not begin until the surviving cells had begun to grow on the autolysed remains of the dead ones. This was shown more conclusively in an experiment in which the initial viable count was reduced to 0·0001 % of its former value by the action of ultra-violet light; the evolution of hydrogen coincided with the growth that took place after the dead cells had autolysed. A provisional theory was put forward suggesting that the production of molecular hydrogen was a function associated with the actual growth of the cells, but the true explanation will be made clear in the course of this paper.

This re-investigation of the subject is due to a stimulus received by the present authors from the recent work of Karström [1930]. The latter, confirming the early work of Dienert [1900] on yeast, has shown conclusively that, among the bacterial enzymes (or enzyme systems) attacking the sugars, some form an invariable part of the make-up of the cell (these he terms *constitutive* enzymes), while others are definitely absent unless the cells have actually been grown in the presence of the substrate (*adaptive* enzymes). For instance, *Bact. aerogenes*, grown in whey and washed and suspended in saline, failed to ferment xylose; if a source of nitrogen, permitting the growth of the washed cells, were added, fermentation set in after a varying latent period. If on the other hand the organism were grown on a medium containing xylose, fermentation of this sugar by the washed suspension set in immediately. Fermentation of glucose by washed suspensions was independent of the medium on which the cells had been grown, so that the glucose enzyme was constitutive, the xylose one adaptive.

Karström's paper recalled to us the fact that the cell suspensions used in Stickland's work on formate decomposition had been grown on tryptic broth in the absence of formate; it seemed possible that the introduction of formate into the medium might so modify the cells that a washed suspension would be able to decompose formate into carbon dioxide and hydrogen, and allow of a study of the enzyme concerned apart from growth.

The production of the enzyme formic hydrogenlyase.

The organism used was *Bact. coli* (Escherich) grown for not more than 20 hours in Roux bottles containing tryptic caseinogen broth at p_H 7 with or without 0·5 % sodium formate. The cells were centrifuged out and washed twice with saline or Ringer's solution and finally uniformly suspended in the same solution. The evolution of hydrogen was measured in the Barcroft differential manometer in the usual way, the cups being twice evacuated and filled with nitrogen purified by passage over heated copper, and the apparatus being shaken in a bath at 40°.

Fig. 1 gives the result of a typical experiment, and shows clearly that the cell suspension grown in broth *plus* formate subsequently decomposes formate with evolution of hydrogen, while that grown on plain broth does not. The presence of hydrogen in the cup at the end of the experiment was verified on several occasions by analysis of a sample of the gas in the Haldane apparatus.

Before definitely attributing this difference to the presence of formate in the broth, two other possibilities which might account for its occurrence must be eliminated, *viz.* the facts that (1) the decomposition of the formate renders the medium practically anaerobic, (2) the base liberated from the sodium formate makes the medium progressively more alkaline.

(1) If the hydrogenlyase were labile to oxygen, aerobic conditions during growth would prevent its appearance, and the formate might act merely by

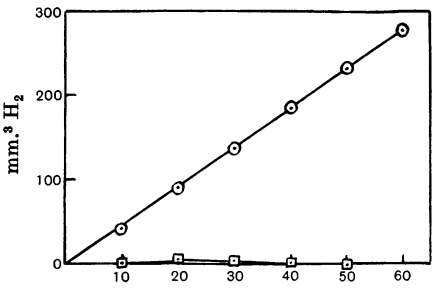

Fig. 1.

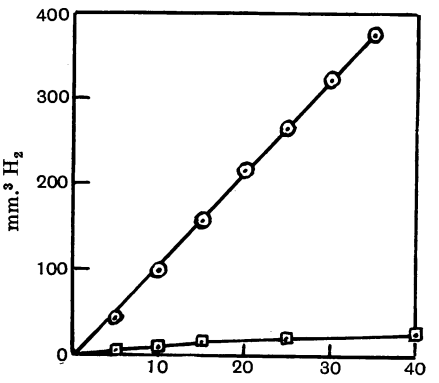

Fig. 2.

Fig. 1. Action on $M/30$ formate at p_H 7·0 of suspensions of *Bact. coli* grown aerobically. ⊙———⊙ in presence of formate. ⊡———⊡ in absence of formate.

Fig. 2. Action on $M/30$ formate at p_H 7·0 of suspensions of *Bact. coli* grown anaerobically. ⊙———⊙ in presence of formate. ⊡———⊡ in absence of formate.

keeping the medium partly anaerobic. Actually aeration of a suspension possessing the enzyme has little harmful effect:

	Velocity ($mm.^3$ H_2/hour)
Original suspension	580
Suspension after 45 mins. vigorous aeration at 20°	495

A further test was carried out by growing cultures on plain broth and formate broth under strictly anaerobic conditions, and testing the evolution of hydrogen by the washed suspensions as before. The results (Fig. 2) show that the organisms grown anaerobically on plain broth give a slow evolution of hydrogen, very small in comparison with that produced by the control suspension grown anaerobically on formate broth. This disposes of the possibility that anaerobiosis can account for the previous results. The slow decomposition by the suspension from plain anaerobic broth might be due to traces of formic acid known to be produced from the amino-acids of the broth.

(2) The final p_H of a broth medium containing 0·5 % sodium formate on which *Bact. coli* has been growing for 20 hours is about 8·0 or higher. That this change of p_H accounts for the production of the hydrogenlyase is contradicted by the fact, to be shown later, that cells grown in the presence of glucose also possess the enzyme, although the change in reaction is here in the opposite direction, the medium reaching a final p_H of 5·5–6·0.

We therefore conclude that the enzyme in question is an adaptive one in the sense used by Karström, and occurs as the result of growing the cells in the presence of formate. We propose the name *hydrogenlyase* for enzymes which liberate molecular hydrogen, to distinguish them from the dehydrogenase type; the evidence for the existence of different hydrogenlyases will be presented later.

The production of formic hydrogenlyase by growth on substances other than formate.

The introduction of glucose or glycerol (0·5 %) into the broth also results in the production of a suspension containing formic hydrogenlyase. As is well known, however, both these substrates are decomposed by *Bact. coli* with production of formic acid [Harden, 1901; Braak, 1928], so this phenomenon is sufficiently explained.

Some properties of formic hydrogenlyase.

(1) Fig. 3 gives the rate of action of the enzyme acting on $M/30$ formate, plotted against p_H, and needs no further comment. All other experiments were done at the optimum, p_H 7·0.

(2) Fig. 4 shows the affinity curves of formic dehydrogenase and formic hydrogenlyase (velocity plotted against logarithm of substrate concentration). The much lower affinity of the latter enzyme will be noted.

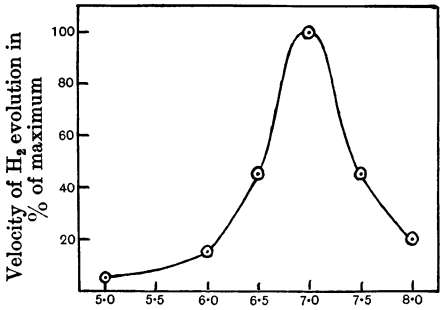

Fig. 3.

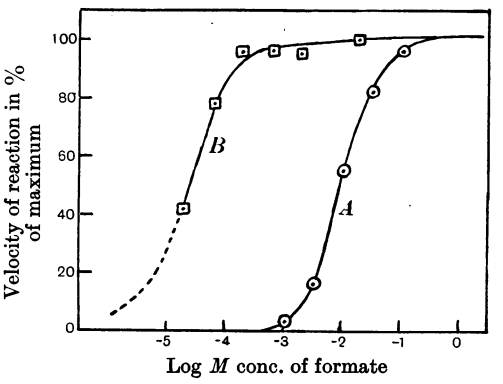

Fig. 4.

Fig. 3. Effect of p_H on action of formic hydrogenlyase.

Fig. 4. Affinities of *A*, ⊙———⊙ formic hydrogenlyase; *B*, ⊡———⊡ formic dehydrogenase.

(3) The enzyme is very labile to poisons of all kinds. Toluene, 1 % sodium fluoride, 2 % urethane and carbon monoxide at one atmosphere pressure all cause 100 % inhibition. Hydrogen from a Kipp's apparatus gave 100 % inhibition even at 0·2 atmosphere pressure, but this was found to be due to a trace of some poison which was not removed by passing the gas through silver nitrate solution, since hydrogen from a cylinder gave a much smaller inhibition (about 40 % at 1 atmosphere); this latter, and the inhibition by carbon monoxide mentioned above, may also have been due to some impurity not removed by passing the gas through silver nitrate solution or over heated copper, but the point has not been further investigated.

The enzyme is also very sensitive to the action of cyanide, 50 % inhibition being produced by a concentration of 10^{-5} *M*, as is shown in Fig. 5.

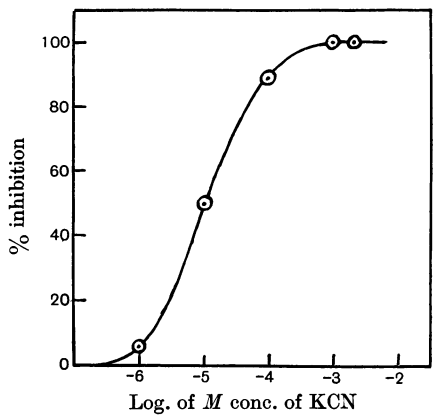

Fig. 5. Effect of cyanide on formic hydrogenlyase.

(4) Pakes and Jollyman [1901, 2] noted that, in the presence of nitrate, bacteria which normally decompose formate into hydrogen and carbon dioxide no longer do so, but instead oxidise the formate with reduction of nitrate to nitrite. We shall show later that there is no connection between formic dehydrogenase and formic hydrogenlyase, so it is difficult to see why the presence of a hydrogen acceptor should completely suppress the formation of hydrogen; we should expect the two enzymes to work side by side.

When oxygen is used as the hydrogen acceptor, *i.e.* when a suspension of bacteria containing formic hydrogenlyase is placed with formate in a Barcroft manometer filled with air there are three reactions taking place simultaneously, *viz.*

$$H.COOH \rightarrow H_2 + CO_2,$$

$$HCOOH + \tfrac{1}{2}O_2 \rightarrow CO_2 + H_2O,$$

and

$$H_2 + \tfrac{1}{2}O_2 \rightarrow H_2O.$$

The manometer readings express the balance of these three reactions, and it is impossible to deduce what is happening. In actual experiments the change

of pressure was practically nil, showing that the hydrogen given off and the oxygen taken up roughly balanced. Without enormous complications of experimental procedure nothing more can be learnt on this point.

With nitrate as the hydrogen acceptor the measurements might have been easier, as initially only one gas reaction is taking place, *viz.*

$$H.COOH \rightarrow H_2 + CO_2.$$

Eventually however the reaction

$$KNO_3 + H_2 \rightarrow KNO_2 + H_2O$$

will produce an apparent inhibition, and more important still the reaction

$$H.COOH + KNO_3 \rightarrow CO_2 + KNO_2 + H_2O$$

is rapidly making nitrite which gradually poisons the formic hydrogenlyase. Hence the measurement of velocity of hydrogen production in the presence of nitrate is not very accurate, but an estimate may be made before the nitrite poisoning becomes very appreciable.

The results of determinations of velocities of hydrogen production at different concentrations of formate and nitrate are given in Table I. It will be seen that the amount of inhibition varies very little with either the formate concentration or the nitrate concentration; the complete inhibition reported by Pakes and Jollyman is not observed.

Table I.

Formate concentration	Nitrate concentration: 0	*M*/60	*M*/180	*M*/600	*M*/6000	Inhibition %
	Velocity (mm.^{3} H_2/hour)					
M/6	456	156	144	—	—	67
M/12	480	216	—	228	—	54
M/15	354	90	—	108	84	73
M/30	1020	432	—	384	360	62
M/60	756	264	—	304	—	62

It has been stated already that this effect of nitrate cannot be due to its action as a hydrogen acceptor, as formic dehydrogenase and formic hydrogenlyase are completely separate enzymes (direct experimental confirmation of this might have been obtained by inhibiting nitrate activation by potassium cyanide but for the fact that cyanide inhibits formic hydrogenlyase even more strongly than it does nitrate activation). It seems therefore that the inhibiting effect of nitrate on the reaction

$$H.COOH \rightarrow H_2 + CO_2$$

is most probably due to a direct combination with the formic hydrogenlyase, the formate thus being prevented from reaching the enzyme. This reminded us of the work of Mann and Woolf [1930] on the action of salts on fumarase, in which various salts acted by changing the p_H-activity curve of the enzyme. Similar experiments to those of Mann and Woolf were done on formic hydrogenlyase, the salts used being nitrate, sulphate, fumarate, citrate and oxalate;

nitrate and sulphate produced the effects shown in Fig. 6, the others having no effect at all. It is significant that sulphate, which is not activated as a hydrogen acceptor, inhibits, while fumarate, which is activated as a hydrogen acceptor, does not. We consider that the action of nitrate and sulphate is similar to the salt effects described by Mann and Woolf with fumarase, and that the results of Pakes and Jollyman were probably due to destruction of the formic hydrogenlyase by the nitrite formed during their experiments.

(5) The theoretical amount of hydrogen is obtained when *Bact. coli* acts on known quantities of formate. For these experiments the substrate was placed in a small tube hooked on the small cup of the Barcroft apparatus, and tipped into the suspension of bacteria after the apparatus had reached equilibrium in the bath.

Amount of formate cc. $M/10$	H_2 (measured) mm.^{3}	H_2 (theoretical) mm.^{3}	% yield
0·5	1110	1120	99
0·2	472	448	106
0·2	448	448	100
0·1	218	224	97

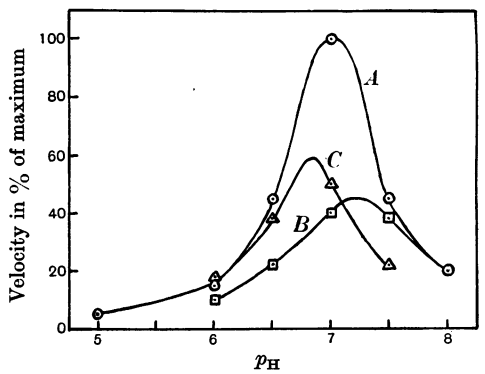

Fig. 6. Effect of salts on p_H-velocity curve of formic hydrogenlyase.
A. ⊙———⊙ normal. *B*. ⊡———⊡ $+KNO_3$ $M/30$. *C*. ◬———◬ $+Na_2SO_4$ $M/30$.

The production of hydrogen from glucose.

As *Bact. coli* is able to ferment glucose with formic acid as one of the decomposition products, it is to be expected that a suspension will liberate hydrogen from glucose also, in the same circumstances as it does from formate. This expectation is realised (Fig. 7).

It has generally been held that organisms fermenting sugars with production of hydrogen do so by virtue of the fact that formic acid is an intermediate product. We are now in a position to investigate this hypothesis more closely. The questions to be answered are (1) does the hydrogen arising by the action of a washed suspension of *Bact. coli* on glucose come from formic acid produced by preliminary fermentation of the sugar, or does it come from glucose direct or perhaps from some other intermediate product? and (2) in the latter case are we dealing with the same enzyme acting on two different substrates

(formic acid and glucose or some other intermediate compound) or with two separate enzymes?

With regard to the first question the evidence is conclusively against the view that hydrogen comes from glucose by decomposition of preliminarily formed formic acid. In the first place the production of hydrogen from glucose proceeds linearly and is not subject to any lag, as would be expected if a preliminary decomposition to formic acid were taking place, especially considering the low affinity of formic hydrogenlyase. Secondly, the affinity of the enzyme liberating hydrogen from glucose is very much higher than that of formic hydrogenlyase; the affinities of the two enzymes are compared in Fig. 8, that of the glucose enzyme being too large to be measured. At low

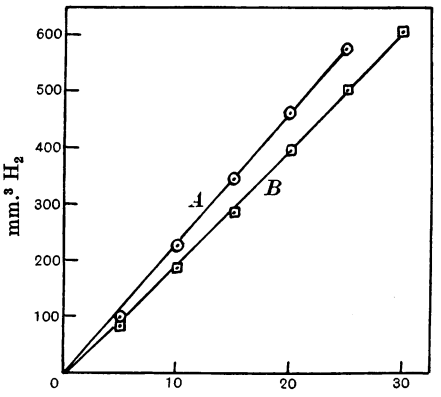

Fig. 7.

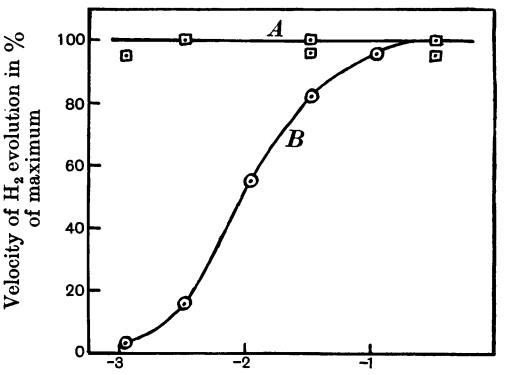

Fig. 8.

Fig. 7. Action of suspension of *Bact. coli* grown in presence of formate on *A*, ⊙———⊙ *M*/30 formate at p_H 7·0; *B*, ⊡———⊡ *M*/30 glucose at p_H 6·2.

Fig. 8. Comparison of affinity of glucose hydrogenlyase at p_H 6·2 (*A*, ⊡———⊡) with that of formic hydrogenlyase at p_H 7·0 (*B*, ⊙———⊙).

substrate concentrations the rate of evolution of hydrogen from glucose is actually greater than that from the same molar concentration of formate, *e.g.*

	Velocity (mm.³/hour)
From *M*/300 formate at p_H 7·0	252
„ glucose „	360

These facts taken in conjunction make it quite certain that the hydrogen liberated from glucose does not arise by the intermediate formation of formic acid.

The second question now arises, *viz.* whether the same enzyme acts on glucose and on formate, or whether there is a separate specific hydrogenlyase for each. This question may be investigated in two ways; first, by so treating the suspension that it contains one enzyme but not the other; second, by determining whether, in a saturating concentration of both substrates, the rate of evolution of hydrogen is equal to the greater of the rates on the two substrates separately, or to their sum.

The former of these experiments gave results indicating two specific enzymes for the two substrates. A suspension grown on formate broth liberates hydrogen from formate at p_H 7·0 at about the same rate as it does from glucose at p_H 6·2 (Fig. 7). A suspension grown aerobically on plain broth, as has already been shown, does not decompose formate, but this suspension is able to bring about a slow liberation of hydrogen from glucose (Fig. 9). The ratio

$$\frac{\text{rate of liberation of } H_2 \text{ from glucose}}{\text{rate of liberation of } H_2 \text{ from formate}}$$

is infinitely greater in the latter suspension than it is in the former, and this fact is not consistent with the theory that the same enzyme catalyses both reactions.

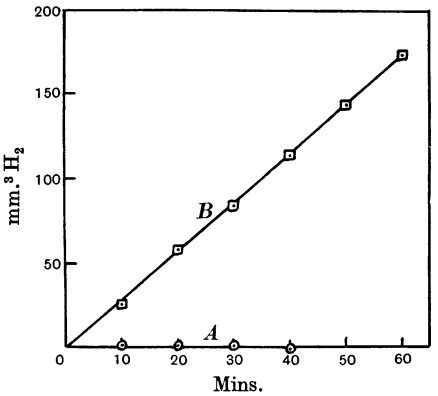

Fig. 9.

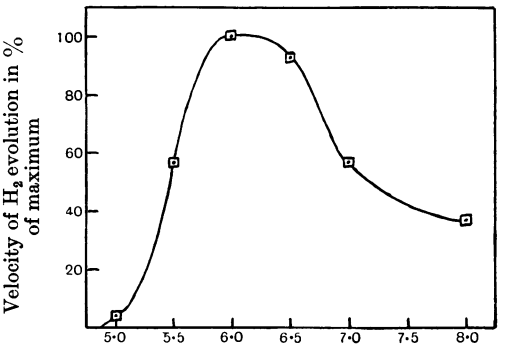

Fig. 10.

Fig. 9. Action of suspension of *Bact. coli* grown aerobically in absence of formate on *A*, ⊙———⊙ *M*/30 formate, p_H 7·0; *B*, ⊡———⊡ *M*/30 glucose, p_H 6·2.

Fig. 10. Effect of p_H on glucose hydrogenlyase.

The second line of investigation has given results pointing to the same conclusion. It is difficult to choose the right conditions for measuring the effect of the two substrates together, as the two reactions have different p_H optima, that for formate being at p_H 7·0 and that for glucose at p_H 6·2 (the relation between p_H and velocity of liberation of hydrogen from glucose is given in Fig. 10; that for formate is given in Fig. 3). Experiments done at p_H 7·0 and p_H 6·2 both show that the velocity of hydrogen evolution from a mixture of the two substrates is equal to the sum of the velocities from the two separately. The present state of our knowledge on this point is therefore that

	Velocity (mm.³ H_2/hour)	
(1) At p_H 7·0. Formate *M*/30	300	363
Glucose *M*/30	63	
Formate *M*/30 + glucose *M*/30		348
(2) At p_H 6·2. Formate *M*/30	234	411
Glucose *M*/30	177	
Formate *M*/30 + glucose *M*/30		413

the hydrogen from glucose does not come through formic acid and that it is liberated by an enzyme which is not formic hydrogenlyase; whether it is liberated direct from the glucose molecule or from some other intermediate compound we do not know, though the absolute linearity of the reaction indicates that the former may be true.

One further piece of evidence has been obtained about the origin of this hydrogen. If *Bact. coli* is allowed to act on glucose in two Barcroft manometers, one with potash in the small cup and the other without, the rate of gas production is greater in the latter, and this must indicate simultaneous evolution of carbon dioxide. If acid is spilt into the solutions after a given period of reaction, a further quantity of carbon dioxide is liberated, the total excess over the experiment where the carbon dioxide is absorbed representing roughly one molecule of carbon dioxide per molecule of hydrogen. This does not at present throw any light on the chemistry of the reaction.

In all recent schemes setting out the course of hexose fermentation by bacteria of the *coli-typhosus* group it has been assumed that the hydrogen appearing among the fermentation products arises from intermediately formed formic acid. It is now clear that this is not necessarily the case since hydrogen may be liberated from glucose by bacterial preparations which are unable to produce hydrogen from formate. The origin of this hydrogen is still obscure. In our experiments it was always attended by a simultaneous evolution of carbon dioxide in roughly equimolecular proportions; it seems therefore to be of the nature of a decarboxylating process but whether acting on a six carbon compound or on some unstable product of smaller carbon chain is not clear.

The production of hydrogen from other substances.

Glucose, fructose and mannose all yield hydrogen at about the same rate; lactose, galactose, arabinose, glycerol and mannitol all react much more slowly; sucrose, lactate and succinate do not react at all.

Glucose, fructose and mannose appear to act at the same enzyme, for mixtures of two substrates do not produce hydrogen faster than either substrate alone:

	Velocity (mm.³/hour)	
	(1)	(2)
Glucose *M*/60	168	135
Mannose *M*/60	126	—
Glucose *M*/60 + mannose *M*/60	162	—
Fructose *M*/60	—	140
Glucose *M*/60 + fructose *M*/60	—	144

It is possible that the other substances, and even other sugars not so closely related structurally as these three, may require other specific enzymes, especially as different bacterial species have very different powers of fermenting various sugars and alcohols, but we have not yet tested this point experimentally.

Comparison of rates of action of hydrogenlyases with those of dehydrogenases.

The dry weight of bacteria present in many of the suspensions has been estimated by determination of total nitrogen. By this means the dry weight of bacteria in any suspension may be calculated from a small sample, and the waste of material involved in the direct estimation is avoided. The nitrogen content of the dried bacteria is the same whether the suspensions are grown with or without formate:

	N content %	Average %
Suspension grown on plain broth	12·0, 13·5	12·7
,, ,, formate broth	12·1, 11·7	11·9
		12·3

Our values are all higher than that quoted for *Bact. coli* (10·3 %) by Nicolle and Alilaire [1909]. To express the results the symbol Q_{H_2} has been used with a meaning analogous to that of the widely used Q_{O_2}, *viz.* mm.^{3}/hour/mg. dry weight of cells. The values of Q_{H_2} obtained varied over a rather wide range, *viz.* for formate 340–960, with an average of 615 (6 determinations) and for glucose 170–400, with an average of 280 (5 determinations), the reaction being carried out at a substrate concentration of $M/30$, at 40° and at the optimum p_H. This variation is perhaps to be expected with adaptive enzymes, and could not be correlated with any controllable factor operating during the growth of the organisms.

For comparison with these values of Q_{H_2} for formate some estimations of Q_{O_2} for the same substrate were made on suspensions grown in the absence of formate (these values could not be obtained for suspensions containing formic hydrogenlyase). The average for two experiments gave Q_{O_2} (formate) = 225, and, allowing for the fact that formic acid gives off two atoms of hydrogen while its oxidation requires only one atom of oxygen, this means that the decomposition of formate into carbon dioxide and hydrogen takes place rather faster than its oxidation, when the appropriate suspensions of *Bact. coli* are used. Our value of Q_{O_2} (formate) = 225 is considerably higher than that of 40 found by Cook and Haldane [1931].

For suspensions grown aerobically on plain broth the Q_{H_2} for formate was always zero, while the slow evolution of hydrogen from glucose by these suspensions gave values of 31 and 42 (about 10–15 % of the normal average value of 280 obtained when the organism is grown in the presence of formate).

The relation between hydrogenase, formic dehydrogenase and formic hydrogenlyase.

Three bacterial enzymes are now known which act on formic acid or hydrogen:

(1) hydrogenase, which activates molecular hydrogen

$$H_2 + X \rightleftharpoons XH_2;$$

(2) formic dehydrogenase, which catalyses the reaction

$$H.COOH + X \rightleftharpoons XH_2 + CO_2;$$

(3) formic hydrogenlyase, which catalyses the reaction

$$H.COOH \rightleftharpoons H_2 + CO_2.$$

where X represents an intracellular hydrogen carrier, or experimentally a dye such as methylene blue. In a former communication [Stephenson and Stickland, 1931] we suggested the possibility that reaction (3) might be a combination of reactions (1) and (2), pointing out that such a view would be contradicted by the existence of an organism possessing the enzyme (3) but lacking (1) or (2), or even possessing both (1) and (2) but lacking (3). We have now further investigated the distribution of these enzymes, and the relevant results are summarised in Table II.

Table II.

Species	Medium grown on	Hydrogenase	Formic dehydrogenase	Formic hydrogenlyase
Bact. coli	Broth	+	+	0
Bact. coli	,, +formate	+	+	+
Bact. lactis aerogenes (4 strains)	,,	0	+	0
Bact. lactis aerogenes (4 strains)	,, +formate	0	+	+
Bact. dispar	,, +formate	+	+	0

Hydrogenase was tested for by putting the suspension in a Thunberg tube with 1 cc. 1/5000 methylene blue at p_H 6·5, filling the tube with hydrogen and noting the reduction time; formic dehydrogenase by adding 1 cc. $M/10$ sodium formate to a similar tube and simply evacuating and taking the reduction time; and formic hydrogenlyase by the method already described in this paper. The negative result for hydrogenase with *Bact. lactis aerogenes* might have been due to inability of the dye to reach the enzyme, though this was improbable since the formic dehydrogenase was always very vigorous in the same suspensions. This result was however of the greatest importance, so it was confirmed by testing the reaction

$$H_2 + KNO_3 = H_2O + KNO_2.$$

There can be no question whether hydrogen was able to permeate the cell, for hydrogen was given off copiously in the formic hydrogenlyase test; moreover, nitrate was rapidly reduced by other donators, so that if hydrogenase were present there should have been rapid uptake of hydrogen in the presence of nitrate. Experimentally, with *Bact. lactis aerogenes* there was none; so we feel justified in saying that hydrogenase is absent.

From the data given above it can be seen that all strains of *Bact. lactis aerogenes* when grown in presence of formate possess formic hydrogenlyase but no hydrogenase; conversely, *Bact. coli* grown on plain broth and *Bact. dispar* even when grown with formate present have hydrogenase and formic

dehydrogenase but no formic hydrogenlyase. Thus their distribution, in conjunction with the other evidence, makes it certain that these three enzymes are distinct entities.

SUMMARY.

1. The enzyme liberating molecular hydrogen from formic acid is distinct from formic dehydrogenase and from hydrogenase. The name formic hydrogenlyase is proposed for this enzyme.

2. In the case of *Bact. coli* formic hydrogenlyase is an adaptive enzyme, *i.e.* it is formed only when the organism is grown in the presence of formate.

3. Some properties of formic hydrogenlyase are recorded.

4. Formic hydrogenlyase has been shown to be distinct from the hydrogenlyases liberating hydrogen from the sugars.

5. In the conditions of the experiments here reported the evolution of hydrogen from glucose does not occur through formic acid as an intermediate product.

REFERENCES.

Braak (1928). Onderzoekingen over vergisting van glycerine. Diss. Delft.
Cook and Haldane (1931). *Biochem. J.* **25**, 880.
Dienert (1900). *Ann. Inst. Past.* **14**, 139.
Grey (1914). *Proc. Roy. Soc. Lond.* B **87**, 472.
Harden (1901). *J. Chem. Soc.* **79**, 610.
Hoppe-Seyler (1876). *Pflüger's Arch.* **12**, 1.
Karström (1930). Thesis. Helsingfors.
Mann and Woolf (1930). *Biochem. J.* **24**, 427.
Nicolle and Alilaire (1909). *Ann. Inst. Past.* **23**, 547.
Pakes and Jollyman (1901, 1). *J. Chem. Soc.* **79**, 386.
—— —— (1901, 2). *J. Chem. Soc.* **79**, 459.
Quastel and Whetham (1925). *Biochem. J.* **19**, 520.
Stephenson and Stickland (1931). *Biochem. J.* **25**, 205.
Stickland (1929). *Biochem. J.* **23**, 1187.