CCXXI. HYDROGENLYASES.

II. SOME FACTORS CONCERNED IN THE PRODUCTION OF THE ENZYMES.

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It has long been known that yeasts can be trained to ferment sugars upon which they are unable to act previous to training. The work of Dubourg [1899] on the training of yeasts to act upon cane sugar was criticised by Klöcker [1900]. But Dienert [1900] gave an account of some work on the acclimatisation of yeasts to galactose which is noteworthy. After growing yeasts on galactose, which, at the outset, they were not able to ferment, it was found that in circumstances where growth was impossible galactose was now fermented. The training was very rapid, 24 hours being sufficient to produce this property. The acclimatisation was lost only after growing on some other fermentable sugar for a long time. Yeasts which previously were unable to grow at all with galactose as the only carbon source, were acclimatised to galactose by growing in the presence of glucose and galactose. They were then able to grow with galactose as the sole carbon source.

Harden and Norris [1910] and Willstätter and Sobotka [1922] also trained yeasts to ferment galactose. The former showed that the press juice from acclimatised yeasts was able to ferment galactose. Abderhalden [1925] found that this property is not lost even if the yeast is grown for a long time on other carbon sources.

Similar work has been done on many other reactions induced by yeasts, moulds and bacteria. Thus Klotz [1906] trained a bacterium of the colon group to ferment lactose, and Twort [1907] was able to train *Bact. typhosus* to ferment lactose, and *Bact. dysenteriae* (Flexner) to ferment sucrose.

Karström [1930] described the adaptation of several species of bacteria to certain substrates. He concluded that bacterial enzymes may be classified as either constitutive or adaptive. The former are invariably present in the bacteria; the presence of the latter depends on the presence, in the medium on which the culture is grown, of the substrate upon which they act. Sometimes, the presence during growth of a certain substance inhibits the formation of an enzyme which is present in cultures grown on other media. For example, one of Karström's strains of *Bact. coli* contained saccharase when grown

on any sugar other than glucose, or when grown in the absence of sugar. Growth on glucose, however, gave a culture which had no saccharase.

It is necessary here to distinguish between "training" and "adaptation." First, it is clear that a given strain of bacterium can perform certain chemical reactions and cannot perform certain others, and that these properties normally remain constant. For example, a strain of *Bact. coli* growing on peptone and glucose ferments the glucose, but growing on peptone and sucrose does not ferment the sucrose. The phenomenon which has been called "training" consists in obtaining a strain which carries out a reaction which it was previously incapable of doing, by growing it for a large number of sub-cultures in a medium containing the new substrate. The new reaction then becomes a normal property of the strain. The phenomenon called "adaptation" is totally different, and is concerned with reactions which are already normal to the bacteria concerned. Of these reactions, some are catalysed by enzymes which are invariably present in the cells, on whatever medium they are grown; some by enzymes which are formed only when the cells are growing in the presence of the particular substrate. These two types are called respectively "constitutive" and "adaptive" enzymes. For example, one of Karström's strains of *Bact. coli* was able to ferment maltose immediately it was inoculated into a medium containing this sugar. It did not require to be trained to perform this reaction. However, a washed suspension of the bacteria grown in the absence of maltose was not able to ferment it, whilst a suspension of bacteria which had been grown in its presence did induce a fermentation, that is to say the enzyme is "adaptive." The training of organisms may proceed very rapidly as when Dienert [1900] acclimatised yeast to galactose in 24 hours. But once the organism has been trained, the newly acquired property is lost very slowly, if at all [Abderhalden, 1925]. On the other hand, an adaptive enzyme, which seems to be formed as a response to a chemical stimulus, is no longer produced when the stimulus is removed. This point is more fully discussed later.

Following the work of Karström, Stephenson and Stickland [1932] investigated the hydrogenlyases of bacteria, *i.e.* enzymes which liberate molecular hydrogen from formate, glucose, *etc.* It was found that washed suspensions of *Bact. coli* and *Bact. lactis aerogenes* possess formic hydrogenlyase when sodium formate is present in the medium on which they are grown, but not in its absence. The following work was undertaken with a view to determining further the factors concerned in the formation of these adaptive enzymes.

Experimental.

In all the experiments to be described, the bacteria in question were inoculated from an 8-hour culture in caseinogen digest broth into the medium concerned. The amount of sodium formate, glycerol or glucose added was always 0·5 %. Growth was allowed to continue for 15–18 hours (unless

otherwise stated) at 37°. The culture was washed twice with Ringer's solution by centrifuging and then made up in uniform suspension in Ringer's solution. The resulting preparation of "non-proliferating bacteria" was investigated as follows in the Barcroft manometer at 40° [Stephenson and Stickland, 1932]. 1 cc. of bacterial suspension and 1 cc. of $M/20$ phosphate buffer (p_H 7·0) were placed in each cup. In one cup was placed 1 cc. of the substrate, usually in $M/10$ solution, and in the other 1 cc. of water. The inner small cups were half filled with 40 % NaOH for absorption of carbon dioxide. The apparatus was twice evacuated and each time filled with nitrogen which had been passed over heated copper to remove small amounts of oxygen.

After equilibration readings were taken at intervals of 5 or 10 minutes.

In the case where gas is being evolved from glucose or glycerol, the rate is constant for a long time—certainly more than an hour. However, when formic hydrogenlyase is under investigation, the rate is rarely constant for longer than 20 minutes. The reason for this falling off is partly due to the alkalinity which soon manifests itself, and partly due to the fact that the formic hydrogenlyase has a low affinity [Stephenson and Stickland, 1932]. Thus the concentration of formate soon falls below the point where the enzyme is fully saturated.

The activity of the hydrogenlyases is expressed as Q_{H_2} (analogous to Warburg's Q_{O_2} for oxygen uptake), that is, it is the number of mm.^{3} of hydrogen evolved by 1 mg. dry weight of bacteria in 1 hour. The samples of the suspensions used were subjected to a nitrogen estimation by the micro-Kjeldahl method. Nitrogen determinations on dried bacteria (washed twice with distilled water) were also made and thus the dry weight of bacteria in any suspension could be arrived at by a nitrogen estimation. The dried bacteria were prepared by drying in a steam-oven to constant weight.

Nitrogen content of dried bacteria.

In order to determine whether the nitrogen content of bacteria varies according to the medium on which they are grown, a series of estimations was made which is summarised in Table I. The medium upon which the cells were grown is indicated in column 2. The broth used was a tryptic digest of caseinogen, containing about 3 % hydrolysed protein. The inorganic medium is that given by Stephenson [1930, p. 275]. Column 3 gives the conditions in which the bacteria were grown. "Roux" indicates that the culture was grown in Roux bottles lying flat, each containing 150 cc. of medium; "flask, aerated" means that the culture was grown in 500 cc. of medium in a litre flask with air bubbling through; "flask, not aerated" means that the preparations were grown similarly, but without air bubbling through.

It will be seen that the effect of the medium or of the growth conditions on the nitrogen content is at most very small. So also is the difference between the various organisms studied. Nicolle and Alilaire [1909], who estimated the nitrogen content of several species of bacteria, found that there

were "différences relativement faibles d'une bactérie à l'autre." Their value for *Bact. coli*, however, was 10·32 % N. They do not state how they dried their bacteria.

Table I. *Nitrogen content of dried bacteria.*

Bacterium	Medium	Conditions	N as % of dry weight
Bact. freundii	Broth formate glycerol	Roux	**12·4**
Bact. lactis aerogenes (strain 124)	Broth	Roux	13·3
	Broth formate	Roux	12·9
	Broth glucose	Roux	13·6
	Broth formate	Flask, aerated	13·0
	Broth glucose	Flask, not aerated	12·2
	Broth glucose	Flask, aerated	12·0
		Mean	**12·8**
Bact. dispar	Broth formate	Roux	12·2
	Broth glucose	Roux	13·6
		Mean	**12·9**
Bact. cloacae (strain 259)	Broth glucose	Roux	12·0
	Inorganic glucose	Roux	11·1
		Mean	**11·5**
Bact. cloacae (strain 402)	Broth glucose	Roux	13·2
	Broth glucose	Flask, aerated	12·1
	Broth glucose	Flask, aerated	11·9
	Broth glucose	Flask, not aerated	10·3
		Mean	**11·9**
Bact. coli	Broth	Flask, aerated	12·3
	Broth	Flask, aerated	12·5
	Broth	Flask, not aerated	11·9
	Broth formate	Flask, aerated	12·4
	Broth formate	Flask, aerated	12·0
	Broth formate	Flask, not aerated	12·2
	Broth glucose	Flask, aerated	12·3
	Broth glucose	Flask, not aerated	11·3
		Mean	**12·1**

Influence of growth conditions on the formation of the hydrogenlyases.

(i) *Bact. coli.* A summary of the results obtained with *Bact. coli* is found in Table II. It is there seen that the production of both hydrogenlyases studied is powerfully influenced by aeration. The condition when the culture is grown on broth formate or broth glucose in flasks is practically anaerobic owing to the rapid evolution of gas and to the small surface exposed. In these conditions the production of hydrogenlyases is at its maximum; with broth formate Q_{H_2} values of 190 and 76 are obtained for formic and glucose hydrogenlyase respectively, while with broth glucose these give Q_{H_2} values of 240 and 185. In Roux bottles, where a large surface is exposed, less enzyme is formed—with broth formate the Q_{H_2} values fall to 52 and 48, and with broth glucose to 215 and 120. With air passing through the medium, no hydrogenlyases are formed, except some glucose hydrogenlyase with broth glucose.

It is seen from Table II that aerobiosis in the formate medium results in an increase in alkalinity. This is explained by the increased oxidation of the formate in aerobic conditions. It might be thought that the decreased amount

Table II. *Hydrogenlyases of* Bact. coli.

Medium	Conditions	Final p_H	Formic hydrogenlyase: mm.³ of H_2 per mg. N	Formic hydrogenlyase: Q_{H_2}	Glucose hydrogenlyase: mm.³ of H_2 per mg. N	Glucose hydrogenlyase: Q_{H_2}
Broth	Anaerobic	7·5	290	35	400	48
,,	F.N.A.	7·4	0	0	Small	Small
,,	R.	8·0	0	0	100	12
,,	F.A.	8·3	0	0	0	0
Broth formate	F.N.A.	7·6	1580	190	630	76
,,	R.	8·0	425	52	400	48
,,	F.A.	8·6	0	0	0	0
Broth glucose	F.N.A.	5·4	1970	240	1530	185
,,	R.	5·0	1780	215	980	120
,,	F.A.	5·1	0	0	370	45
Broth glycerol	R.	—	510	62	310	38
Inorg. glucose	F.N.A.	7·0	0	0	0	0
,,	R.	—	0	0	0	0
Inorg. glucose chalk	R.	—	0	0	0	0
Inorg. glycerol	R.	—	0	0	0	0
Inorg. glycerol chalk	R.	—	0	0	0	0

R. = Roux. F.A. = Flask, aerated. F.N.A. = Flask, not aerated.

of the enzymes in these last experiments was due to the concomitant alkalinity. However, aeration in the case of the glucose medium also results in diminished hydrogenlyase activity. Here there is a production of acid, but the different degrees of aerobiosis cause little difference in the final p_H. Increased aerobiosis in this medium again however results in decreased hydrogenlyase activity. It seems, therefore, that the amount of enzyme produced varies inversely with the amount of air present during growth.

Apart from aeration, the formation of formic hydrogenlyase is seen to be most powerfully influenced by the presence of formate. A small amount is formed in strictly anaerobic conditions in broth alone ($Q_{H_2} = 35$) but as formic acid always occurs during the bacterial decomposition of amino-acids, this requires no special explanation. Glucose and glycerol also favour the production of the formic hydrogenlyase, the former to a greater and the latter to a less extent than formate ($Q_{H_2} = 240$ and 62 respectively). Both these substances are, however, known to be decomposed by *Bact. coli* with the formation of formic acid.

Formate also favours the production of glucose hydrogenlyase ($Q_{H_2} = 76$) but not so markedly as glucose itself ($Q_{H_2} = 185$).

Thus formate and glucose in the medium both result in the formation of both hydrogenlyases. The fact that the presence of glucose involves also the presence of formate, accounts for the crossways action in one direction. The production of the glucose enzyme by formate may be due to some characteristic common to the formic and glucose molecules.

The estimation of the Q_{H_2} of the organism grown on an inorganic medium shows that the washed suspensions of such cultures are lacking in both the enzymes. This result was unexpected in view of the fact that hydrogen is formed by *Bact. coli* while growing on an inorganic medium in the presence

of glucose. The reason for this result is not clear. It may be that the absence of the enzyme in the washed suspension is due to the absence of some stabilising substance which is present in the broth.

(ii) *Bact. lactis aerogenes.* The results for two strains of this bacterium are shown in Table III. In calculating the Q_{H_2} values for strain 418, the rate

Table III. *Hydrogenlyases of* Bact. lactis aerogenes.

Medium	Conditions	Final p_H	Formic hydrogenlyase: mm.³ of H_2 per mg. N	Formic hydrogenlyase: Q_{H_2}	Glucose hydrogenlyase: mm.³ of H_2 per mg. N	Glucose hydrogenlyase: Q_{H_2}
Strain 124						
Broth	R.	7·5	0	0	0	0
Broth formate	F.N.A.	7·3	2100	270	440	57
,,	R.	—	0	0	0	0
,,	F.A.	7·8	0	0	0	0
Broth glucose	F.N.A.	5·6	Very small	Very small	Very small	Very small
,,	R.	7·1	1800	230	100–440	13–57
,,	R.	—	1600	205	200–610	26–77
,,	F.A.	7·1	17	2	9	1
Inorg. glucose	F.N.A.	6·0	400	50	400	50
,,	R.	6·3	350	45	500	65
Strain 418						
Broth	R.	7·5	0	0	0	0
Broth formate	F.N.A.	7·4	3650	465	500	72
,,	R.	7·6	0	0	0	0
,,	F.A.	7·8	0	0	0	0
Broth glucose	F.N.A.	5·3	0	0	0	0
,,	R.	7·0	4300	550	1400	180
,,	R.	7·1	5500	700	640	82
,,	F.A.	7·1	0	0	400	50
Inorg. glucose	R.	6·0	0	0	1750	225
,,	R.	—	0	0	950	120

R. = Roux. F.A. = Flask, aerated. F.N.A. = Flask, not aerated.

per mg. N was multiplied by the nitrogen content of strain 124. It was found impossible to determine the nitrogen content of strain 418 owing to difficulty in centrifuging. The bacteria came down moderately well when Ringer's solution was used for washing, but when distilled water was used in order to get a preparation of dried bacteria, the cells did not come down even after an hour's centrifuging. Since bacteria of different species have such small differences in their nitrogen content, it is very improbable that there is any appreciable difference between two strains of the same species.

The two strains give exactly similar results. When grown on broth alone, the enzymes are not produced. On broth formate, the bacteria form the enzymes only when little air is present, showing in this respect a greater sensitivity than *Bact. coli.* In Roux bottles, or aerated in a flask, the cultures do not produce the enzymes. The maximum amount of enzyme formed in the case of broth glucose occurs when the culture is grown in moderately aerobic conditions. The very small amounts of the enzymes formed by strain 124, and the absence of the enzymes from strain 418, when the cells are grown in a flask and not aerated, are probably due to the production of acid in these

conditions. The presence of air ensures more complete oxidation of the glucose, and consequently less acid production.

The glucose hydrogenlyase of a suspension of strain 124 which is grown on a broth glucose medium in Roux bottles, shows an initial low output of hydrogen. This increases after about 30 minutes to a new constant rate.

In striking contrast to *Bact. coli*, when grown on an inorganic medium *Bact. lactis aerogenes* produces quite appreciable amounts of the enzymes. Strain 124 forms both hydrogenlyases whilst strain 418 gives only the glucose enzyme. (Both strains produce gas while growing on an inorganic medium with glucose.)

(iii) *Bact. dispar.* This organism produces neither a formic nor a glucose hydrogenlyase no matter on what medium the culture is grown. It does not form gas from formate or glucose in Durham tubes. It is therefore entirely unable to decompose formate or glucose with the liberation of hydrogen, either while growing or when in washed suspension.

(iv) *Bact. cloacae.* Two strains of this species were used. The results are summarised in Table IV.

Table IV. *Hydrogenlyases of* Bact. cloacae.

			Formic hydrogenlyase		Glucose hydrogenlyase	
Medium	Conditions	Final p_H	mm.^{3} of H_2 per mg. N	Q_{H_2}	mm.^{3} of H_2 per mg. N	Q_{H_2}
Strain 259						
Broth	R.	7·1	0	0	0	0
Broth formate	F.N.A.	—	0	0	0	0
,,	R.	7·4	0	0	0	0
Broth glucose	F.N.A.	5·5	3450	410	Very small initial	Very small initial
,,	R.	7·1	2900	335	170	20
,,	F.A.	—	Very small	Very small	0	0
Inorg. glucose	R.	6·0	0	0	0	0
,,	F.N.A.	—	0	0	0	0
Strain 402						
Broth	R.	7·1	0	0	0	0
Broth formate	F.N.A.	7·0	0	0	0	0
,,	R.	7·4	0	0	0	0
Broth glucose	F.N.A.	5·5	1560	200	1610	190
,,	R.	7·4	3100	370	200	24
,,	F.A.	7·3	170	20	395	47
Inorg. glucose	R.	—	0	0	0	0
,,	F.N.A.	—	0	0	0	0

R. = Roux. F.A. = Flask, aerated. F.N.A. = Flask, not aerated.

Broth alone or broth formate give no hydrogenlyases in whatever conditions growth takes place. The only medium which results in the formation of the enzymes is broth glucose. The formic hydrogenlyase of strain 259 is best formed in the presence of little air. Strong aerobiosis almost completely inhibits its formation. The glucose enzyme on the other hand is most developed in moderately aerobic conditions. In the case of strain 402, the converse occurs. Here it is the formic enzyme which is at its maximum in

moderately aerobic conditions, and the glucose enzyme which is best formed when little air is present.

No enzymes are formed on an inorganic medium, although, as with *Bact. coli* and *Bact. lactis aerogenes*, gas is formed during growth on such a medium containing glucose.

(v) *Bact. freundii.* This is an organism of the coli-typhosus group. It was first described by Braak [1928]. Its chief biochemical interest lies in the fact that it is able to grow anaerobically in an inorganic medium with glycerol as sole carbon source. This is attributed to its power of oxidising one molecule of glycerol at the expense of a second, which is simultaneously reduced to trimethyleneglycol.

Table V gives the results of a number of experiments undertaken to discover what media are necessary for the production of the hydrogenlyases by this organism. In all cases except those specifically mentioned, growth was allowed to take place for 15–18 hours in Roux bottles.

Table V. *Hydrogenlyases of* Bact. freundii.

Exp. No.	Medium	Final p_H	Formic hydrogenlyase: mm.³ of H_2 per mg. N	Formic hydrogenlyase: Q_{H_2}	Glycerol hydrogenlyase: mm.³ of H_2 per mg. N	Glycerol hydrogenlyase: Q_{H_2}
1	Broth	7·3	1710	210	330	40
2	,,	7·3	2100	260	600	75
3	Broth formate	7·8	3500	430	—	—
4	,,	8·0	7400	920	690	85
5	,,	—	4300	535	290	36
6	Broth formate glycerol	7·3	3020	375	900	110
7	,,	7·4	2700	335	750	93
8	,,	7·3	3600	445	1350	170
9	,,	7·4	2900	360	1020	125
10	,,	7·3	—	—	1600	200
11	,,	—	2450	305	1000	125
12	Broth glucose	—	410	50	(240	30)
13	Inorg. glycerol (aer.)	—	0	0	0	0
14	,, (anaer.)	—	0	0	0	0
15	,, chalk (aer.)	—	0	0	0	0
16	,, ,, (anaer.)	—	0	0	0	0
17	Inorg. glucose (aer.)	—	0	0	0	0
18	,, chalk (aer.)	—	340	42	(340	42)
19	Inorg. acetate	—	0	0	0	0
*20	Inorg. broth glycerol	6·8	230	28	140	17
21	,,	—	4000	495	2000	250
22	Inorg. gelatin glycerol	—	0	0	0	0
23	Inorg. albumin glycerol	—	+	+	+	+
24	Inorg. amino-acids glycerol	—	0	0	0	0
25	Broth nitrate	—	0	0	0	0
26	Broth nitrate formate	6·8	240	30	0	0

* Exp. 20 was made with diluted broth.

Exps. 13–18 were all repeated several times. The only positive result—that of Exp. 18—was obtained by growing the culture for 40 hours. An 18-hour culture possessed no lyases.

In Exps. 12 and 18 glucose hydrogenlyase was investigated instead of the glycerol hydrogenlyase.

The media, broth and inorganic, were always diluted 1:3 from stock media. The medium in Exp. 20 contained 10 % of the undiluted broth and 33 % of the undiluted inorganic medium. That in Exp. 21 contained 33 % undiluted broth and 33 % undiluted inorganic medium; that is, it contained the normal amount of broth, as used in Exps. 1–12.

It will be seen that the values of the Q_{H_2} for bacteria grown on the same medium, and acting on the same substrate vary considerably. However, the ratio of the values for the different substrates acted upon by bacteria grown on the same medium is fairly constant. The ratio of the rates of action on glycerol and on formate has also been found in a number of cases where the Q_{H_2} is not known, the nitrogen value of the suspension not having been determined.

Bact. freundii grown on broth alone aerobically acts on glycerol at about one-quarter of the rate at which it acts on formate. Three determinations (Exps. 1 and 2, and another in which the nitrogen was not estimated) give 19, 28 and 25 %—average 24 %. Grown on broth anaerobically, the ratio is 15 %. When formate is present, the rate for glycerol remains about the same as on broth alone aerobically, whilst the rate for formate is approximately doubled. The ratios Q_{H_2} for glycerol/Q_{H_2} for formate in four experiments are 9, 7, 6 and 10 %—average 8 %. Grown on broth glycerol, suspensions give the ratios 63, 67 and 72 %—average 67 %. If both glycerol and formate are present, the ratio is 34 %.

We see then that the addition of formate to the medium increases the amount of formic hydrogenlyase produced, whilst the addition of glycerol increases both. A mixture of formate and glycerol gives a proportion of glycerol hydrogenlyase intermediate between those obtained in the presence of formate or glycerol alone.

Considering the results in Exps. 1–12 alone, one would have to say that, according to Karström's definition of constitutive and adaptive enzymes, the hydrogenlyases of both formic acid and glycerol are constitutive, since they are found when the organism is grown in the absence of these substrates. There are, it is true, small amounts of formic acid produced in the broth, and a similar suggestion may be made with regard to the presence of the hydrogenlyases in *Bact. freundii* when grown in broth alone as was made in the case of *Bact. coli* grown anaerobically on broth. One would then assume that *Bact. freundii* is more sensitive to the presence of formic acid than is *Bact. coli*.

The results of Exps. 13–24 show that only in very special conditions are the enzymes formed on a medium which does not contain broth. When the organism is grown on an inorganic medium, the enzymes are almost invariably absent. Small amounts of the formate and glucose hydrogenlyases are formed when the cells are grown in the presence of chalk, on a medium containing glucose. On a medium containing glycerol, aerobically or anaerobically, in the presence or absence of chalk, the hydrogenlyases are never produced. The non-formation of the enzymes when the culture is made in an inorganic

medium is not due to an inhibitory substance present in this. Exp. 21 shows that if the normal amount of broth is present, the amount of enzyme produced is the same as if no inorganic medium is present. If less broth is present (Exp. 20), a proportionately smaller amount of hydrogenlyase is formed.

It was thought that the absence of the hydrogenlyases when *Bact. freundii* is grown on an inorganic medium might be due to a destruction of the enzymes by the acid formed from the glycerol. However, when a large amount of phosphate buffer was added to the inorganic glycerol medium, no enzymes were formed. This was not due to a poisoning action of the buffer, since a similar amount of buffer added to broth medium resulted in no reduction of the hydrogenlyase activity.

Bact. freundii produces gas whilst growing on an inorganic medium + glucose.

Some experiments were performed in order to investigate the effect of formate in an inorganic medium. It was found, however, that the presence of formate entirely inhibited growth in several media in which growth otherwise occurs.

Unhydrolysed protein is not sufficient to ensure the production of any appreciable amount of enzyme (Exps. 22, 23). 0·5 % gelatin in the medium gives no hydrogenlyases at all. The presence of 0·5 % albumin gives a very small amount of enzyme. The Q_{H_2} was not measured since some of the protein was precipitated and consequently the nitrogen value did not give a true measure of the amount of bacteria. However, a very thick suspension was used, and the rate of hydrogen output was only just measurable.

A mixture of amino-acids was then tried. The medium contained about 1 % of a mixture of glycine, alanine, leucine, tyrosine, tryptophan, histidine, glutamic acid and aspartic acid, together with inorganic medium and 0·5 % glycerol. The suspension of bacteria which had been grown on this medium did not possess the hydrogenlyases.

It will be seen from Table V that nitrate entirely inhibits the production of the enzymes from broth alone. The addition of nitrate to a broth formate medium results in the formation of a small amount of the formic hydrogenlyase but of no glycerol hydrogenlyase.

Discussion.

Hydrogenlyases are formed by many species of bacteria in certain conditions. The factors influencing their formation are seen to vary somewhat with the organism studied, but the following general conclusions may be drawn.

Aeration has an inhibitory effect on the formation of the hydrogenlyases. This is not due to a destruction of the enzymes. Once the enzymes have been formed, aeration of a washed suspension of the bacteria, in which the enzymes are present, does not destroy them.

Suspensions of the two strains of *Bact. lactis aerogenes*, and *Bact. cloacae*, strain 259, were aerated for 24 hours at room temperature. For the same

period, portions of the same preparations were left in a vacuum, also at room temperature. The activities of the hydrogenlyases were determined before and after the experiment (Table VI).

Table VI. *Effect of aeration on activity of hydrogenlyases.*

	Q_{H_2} (formic hydrogenlyase)			Q_{H_2} (glucose hydrogenlyase)		
Bacterium	Initial	After vacuum 24 hrs.	After aeration 24 hrs.	Initial	After vacuum 24 hrs.	After aeration 24 hrs.
Bact. lactis aerogenes						
Strain 124	225	225	200	35	100	73
Strain 418	250	250	270	35	130	50
Bact. cloacae						
Strain 259	510	435	375	75	175	75

The formic hydrogenlyases are at most slightly decreased either by keeping in a vacuum or by aeration. The effect on the glucose enzyme of keeping in a vacuum is to increase it considerably. Aeration, on the other hand, results in rather less increase.

It may be concluded, therefore, that aeration of a growing culture does not result in the destruction of formed enzymes but actually prevents their formation. This may possibly be due to the oxidation of some substance in the medium necessary for the production of the enzymes.

A second factor is the presence of broth. Although all the organisms except *Bact. dispar* form hydrogen from glucose while growing in an inorganic medium, only *Bact. lactis aerogenes* and, in very special conditions, *Bact. freundii* give the enzymes in washed suspension of cultures from this medium. It is possible that the enzymes in these conditions are less stable, or formed in extremely small amounts, and are thus found only in some cases. The problem is being further investigated.

A third factor is the presence of the substrate. The influence of the substrate on the production of organisms possessing the hydrogenlyases may be pictured as either (1) a natural selection, or (2) a chemical adaptation. In the former case, one must suppose that there exists in all cultures a small but definite number of cells possessing the enzyme. Since at some period each of the organisms used has been grown from a single cell colony, one must imagine a biochemical variation or mutation of a definite though low frequency, in order to account for the existence of the cells containing the hydrogenlyases. Such variations have been known to occur. Neisser [1906] described the sudden appearance in a certain coliform bacterium of an ability to ferment lactose, and Massini [1907] showed that the new strain was immunologically identical with the old strain. Enzymes, then, arising by some such mutation, become, according to this hypothesis of natural selection, of biological value to the organism. Those members possessing them are therefore at an advantage and tend to multiply at the expense of the others. A strain is thus formed in which the majority of the members possess the enzyme in question.

Such an explanation presupposes that the reaction catalysed by the enzyme is of service to the organism. In the case of the formic hydrogenlyase this is difficult to demonstrate. The reaction $HCOOH = H_2 + CO_2$ can hardly be of any value to the cell. The heats of reaction show that it is only slightly exothermic, and although the data for the free energy change are not known, it is probable that little or no energy is available to the cell. Moreover, the end products cannot enter into the cell structure. It is of course possible that formate is detrimental to the organism and that the action of the hydrogenlyase permits of its removal anaerobically. Such an explanation is supported by the following facts: (1) the hydrogenlyases are not formed in highly aerobic conditions, where the removal of formate occurs by the action of formic dehydrogenase, and (2) in inorganic media where growth normally takes place, sodium formate is entirely inhibitory (see above *Bact. freundii*).

On the other hand, it is difficult to see how this applies to the cases of glucose and glycerol.

Against the selection hypothesis the following experiment may be quoted. *Bact. freundii* was grown for a month, with frequent sub-culturing, on broth glycerol in one case, and on broth formate in another. After this time, an inoculation was made from each of these into plain broth, and the Q_{H_2} values for formate and glycerol determined in each case after the usual 15 hours' growth. It was found that these were of exactly the same order as was obtained when the inoculation was made into plain broth from "untrained" *Bact. freundii*.

Hence, if selection had occurred during cultivation on the glycerol or formate medium, the character acquired was immediately lost on returning to plain broth. This must again involve natural selection of the few remaining unaltered cells. This presupposes that the loss of the hydrogenlyases is an advantage when the cells are growing in plain broth—a supposition difficult to support.

More plausible is the second suggestion, that of chemical adaptation. On this view, an adaptive enzyme arises as a response to its chemical environment. It would then partake of the nature of an acquired character in higher organisms. With the removal of the stimulus, the character is lost by the descendants of the organism. They still, of course, retain the power to develop that character in conditions similar to those in which the parent organism developed it. This explanation, though admittedly incomplete, is in accordance with the experimental facts so far determined.

In this way, one would distinguish between "training" and "adaptation" by considering the former to be an inheritable variation, whilst the latter is a specific response to a change in the environment and is hence of the nature of an uninheritable acquired character.

SUMMARY.

1. The conditions governing the formation of the hydrogenlyases in several species of bacteria have been studied.

2. A method for measuring the activity of the culture has been worked out. The unit employed is the Q_{H_2}, *i.e.* mm.^{3} of hydrogen liberated per mg. of dry bacteria per hour.

3. Aerobic conditions operating during growth prevent the formation of the enzymes in some cases and greatly decrease their production in others.

4. Aerating a washed suspension does not destroy the enzyme. It is therefore the actual formation of the enzyme which is inhibited by air.

5. In most cases, no hydrogenlyases can be demonstrated in organisms grown on a simple inorganic medium, whereas organisms grown in presence of broth contain them.

6. The presence in the growing culture of the substrates on which the enzymes act is either a necessary factor in their production or very greatly increases the amount formed.

7. The formation of a culture which possesses the hydrogenlyases is probably the result of a specific action on the growing organisms of certain substances in the medium in which they are grown. It is unlikely that a natural selection of pre-existing enzyme-containing organisms occurs.

8. A distinction is drawn between "training" and "adaptation." The former seems to be a selection of a variation, the latter a direct response to the stimulus of the chemical environment.

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