XLVI. THE EFFECT OF CARBOHYDRATES AND ALLIED SUBSTANCES ON UREASE PRODUCTION BY *PROTEUS VULGARIS*

By REGINALD PASSMORE AND JOHN YUDKIN

From the Department of Pathology, Cambridge

(Received 29 December 1936)

INTRODUCTION

KARSTRÖM [1930] has classified bacterial enzymes as adaptive and constitutive. The former are only produced if the substrate is present during growth. Examples of adaptive enzymes which have recently been studied in some detail are the hydrogenlyase of *Bact. coli* [Stephenson & Stickland, 1932; 1933; Yudkin, 1932] and the galactozymase of yeast [Stephenson & Yudkin, 1936]. The mode of production of these enzymes is of considerable theoretical interest, and some speculations as to its nature and biological implications have been put forward by one of us [Yudkin, 1932; 1937].

The classification of enzymes into the two groups, adaptive and constitutive, is however inadequate in view of the fact that enzyme production may be influenced by the presence of substances other than the enzyme substrate. In many cases these substances are chemically related to the substrate, a fact of theoretical interest discussed elsewhere [Yudkin, 1937]. In other cases, the inciting agent is a compound entirely unrelated to the enzyme substrate.

The most interesting example of this type of enzyme production is that described by Jacoby [1916-18] in the bacterial formation of urease. Jacoby suggested that the production of an enzyme by a cell may be limited by the presence of specific substances required by the cell for the building up of the enzyme molecule. His first results suggested that such "bricks" (Bausteine) could be obtained from lecithin (but not cholesterol) [1916, 1], the alcohol-soluble fraction of serum [1916, 2] and glucose [1916, 3].

Table I. *Jacoby's conclusions. Effect of carbohydrates and allied substances on urease production by bacteria*

Very active	Fairly active	Slightly active	Inactive
<i>d</i> -Glucose	<i>d</i> -Fructose	Ethyleneglycol	<i>d</i> -Sorbitose
<i>d</i> -Galactose	<i>d</i> - and <i>l</i> -Arabinose	Propyleneglycol	<i>d</i> -Mannose
Glycerol		Maltose	Rhamnose
Glyceraldehyde			Sucrose
Dihydroxyacetone			Lactose
Pyruvic acid			Raffinose
Lactic acid			Mannitol

Later Jacoby [1917, 1] stated that the carbohydrates and allied compounds could be classed into 4 groups according to their efficacy in urease production (Table I). From these results, Jacoby concluded that the group

was necessary for the formation of the enzyme. His first attempt to produce the enzyme by growing the bacteria in a synthetic medium did not succeed [1917, 2]. Later, however, it was found that the enzyme was formed if amino-acids, of which leucine must be one, were present in an inorganic medium to which glycerol was added [1917, 3, 4].

These experiments had been carried out with *Proteus vulgaris*. *Bact. coli* yielded similar results [Jacoby, 1918, 1].

The results obtained with these experiments are not so unequivocal as Jacoby assumed. From his several papers containing scanty experimental detail, it appears that the experiments were conducted along the following lines. The bacteria were usually grown on a broth medium. After 3 or 4 days' growth, 1 ml. of this growing culture was added to 20 ml. 2% urea solution, together with 10 ml. of water or a solution of the substance whose enzyme-forming power was to be investigated. The ammonia produced in 24 hours was measured, and the production of more ammonia in the experiments with the additional substance was held to be due to the production of more enzyme by the bacteria through the action of this substance.

Two criticisms can be raised regarding these experiments. First, the effect observed might be due to an increase of the rate of the enzymic reaction by the substance concerned rather than the production of fresh enzyme. Secondly, in none of Jacoby's experiments was it made clear that the increased ammonia production was not due to growth during the experiment, and that the supposed enzyme-forming substance was not a general growth-promoter. Of interest in this connexion are the results obtained from a study of catalase production by *Proteus vulgaris*. With an experimental technique similar to that used for urease, Jacoby [1918, 2] had claimed that catalase production in this organism was increased by the presence of lactate. Virtanen & Karström [1925], however, showed that under the experimental conditions employed by Jacoby, there was increased growth of the bacteria in the media containing lactate, which was sufficient to account for the results obtained.

The first criticism was partly met in one or two of Jacoby's papers. In one case, he compared the effect of lecithin on the bacterial enzyme with its effect on soya bean urease. He found that lecithin increased ammonia production with the former but had no effect on the latter. Arguing that the enzymes are identical since their behaviours are similar, Jacoby interpreted these results as showing that the action of the lecithin is not on preformed enzyme and must therefore be due to its stimulation of the bacteria to increased enzyme production. In a second case, the control experiments were more satisfactory in that Jacoby [1917, 5] showed directly on a dried bacterial enzyme preparation that lecithin did not result in the increased action observed with the living organisms.

The production of urease by micro-organisms has received some attention from other workers. Using a technique exactly similar to that of Jacoby and hence subject to the same criticisms, Lehr [1926] found that in cultures of *Proteus* small concentrations of beryllium produced an increased urease activity, whilst a control with a dried bacterial preparation showed no such increase. Although he mentioned that this result might just as well be explained by an increased growth of bacteria as by a specific increase in enzyme production, Lehr concluded that "die Wirkung des Berylliums besteht in der Anregung der Bakterienzelle zu vermehrter Fermentproduktion".

The effect of glucose on urease production by *Aspergillus niger* was studied by Iwanoff [1925]. It was found that when the mould was grown in the presence of small amounts of glucose and large amounts of nitrogen compounds, large quantities of urea were produced in the medium. If

more sugar were present, no urea was formed. These results were to be explained by the fact that urease was not found in the mould when little sugar was present, whereas it appeared when more sugar was added. Similar results were obtained with other moulds. Iwanoff suggested that the addition of sugar, which certainly increases the growth of the mould, allows it to build more protoplasm. For this, large amounts of nitrogen are used which would, in the absence of sugar, be excreted in the form of urea. However, he was unable to explain the formation of urease in the presence of sugar.

Similar results with bacteria were obtained by Iwanoff & Smirnowa [1927]. Certain bacteria, such as *B. subtilis* and *B. megatherium*, produce urea on a broth-peptone medium, whilst others, such as *Proteus*, do not. This is associated with the absence of urease from the former and its presence in the latter. The addition of glucose to the medium results in urease being produced by the organisms of the first group too, and urea is then not found in the medium in which they are grown.

The formation of urease by several strains of bacteria was stated by Rubentschik [1926], to be independent of the presence of urea in the medium—that is, urease is a “constitutive” enzyme. On a broth-peptone medium, containing in most cases ammonium carbonate, which was found to be necessary for growth, urease was produced, and growing for five generations in such urea-free media did not decrease the amount of enzyme formed. However, the possibility exists that urea is formed in the broth-peptone medium by bacterial breakdown.

The hypothesis of Jacoby that enzyme production may be dependent on the presence of substances required for the synthesis of the enzyme is of great theoretical interest and importance. In view of this and of the criticisms to which experiments with growing organisms are subject, it was considered desirable to reinvestigate the effect of carbohydrates on urease production, using washed suspensions of bacteria. Thus the substance to be investigated was present only during growth, and not during the estimation of the urease activity of the preparation.

Methods

Preparation of bacterial suspension. The organism used was *Proteus vulgaris* from the laboratory stock originally isolated from the large intestine of a rabbit. It was motile and fermented maltose, sucrose, glucose, galactose and salicin with production of acid and gas. Lactose, arabinose and mannitol were not fermented. For the preparation of suspensions, two tubes each containing 50 ml. of peptone broth, enriched with the substances studied, were inoculated from a Pasteur pipette with 7 drops of a 24-hour broth culture and incubated for 24 hours. The organisms were then centrifuged and washed twice with saline and finally resuspended in 5 ml. of saline.

Urease estimations. A 1 in 5 dilution of the suspension was made. 2 ml. were taken and added together with 4 drops of phenol red to 2 ml. of 2% urea in a test-tube. Tubes were set up in quadruplicate in a water-bath at room temperature. The indicator formed a convenient rough test of the strength of the suspension. It was found that if pH 7.6 had not been reached within 5 minutes,

Table II

2 ml. urea solution and 2 ml. of *Proteus* suspension diluted 1 in 5. ml. N/100 acid required to titrate to the neutral point

Time from mixing min.	Tube 1	Tube 2	Tube 3
68	0.92	—	—
122	0.42	1.32	—
180	0.48	0.48	1.88
Total acid required	1.82	1.80	1.88

the dilution was too great to give adequate readings, and a fresh dilution could at once be set up. The ammonia produced was titrated at 30 min. intervals directly with $N/100$ H_2SO_4 from a microburette calibrated to 0.01 ml. Table II shows that provided that not more than 1.5 ml. of acid are required, it is immaterial whether one, two or more titrations are done in a given period. Thus within the limits of our experiments, the enzyme activity is independent of pH changes. Table III shows that the activity is directly proportional to the quantity of suspension present.

Table III

Exp. 2. *Proteus* suspension 1 : 2.5

ml. suspension	ml. $N/100$ H_2SO_4 needed 3 hours at 20°	ml. acid/ml. suspension
2.00	3.38	1.69
1.50	2.47	1.65
1.00	1.74	1.74
0.80	1.44	1.80
0.60	1.24	(2.07)
0.50	0.90	1.80
0.40	0.68	1.70
0.30	0.48	1.60
0.20	0.32	1.60
0.15	0.26	1.71
0.10	0.19	1.9
0.05	0.085	1.7

It was originally planned to estimate the urease activity by direct determination of ammonia produced by the micro-method of Conway. In preliminary experiments, it was found that the range of urease activity was so great that the ammonia production frequently overshot the acid in the Conway unit. Direct titrations were found to be as reliable and much more simple. In one experiment parallel estimations were made by the Conway method, and it was found that this method gave values for ammonia production consistently 20% above the direct titration.

The total quantity of organisms in each suspension was determined by total nitrogen estimations by the micro-Kjeldahl method.

RESULTS AND CONCLUSIONS

Table IV shows a typical experiment. The urease activity is taken as ml. of $N/100$ H_2SO_4 required to neutralize the ammonia produced per mg. N in the

Table IV

Exp. 10. 2 ml. of urea solution and 2 ml. of *Proteus* suspension
in water-bath at 21°

Substance added during growth	mg. N in suspension	NH_3 produced $\equiv$ ml. $N/100$ acid	ml. acid/hour/mg. of N
Control	0.328	1.80	5.5
Arabinose	0.165	2.02	12.2
Galactose	0.169	1.46	8.7
Glucose	0.166	0.44	2.5
Glycerol	0.181	1.43	7.9
Lactate	0.214	0.37	1.7

bacterial suspension per hour. Different experiments can conveniently be compared by expressing the control value in each case as 100. Table V shows the results so expressed for our five experiments. It will be seen that with arabinose

Table V. *Urease activity*

Control = 100							
Exp.	Control	Arabinose	Fructose	Galactose	Glucose	Glycerol	Lactate
6	100	130	—	140	—	140	—
8	100	—	120	—	30	—	50
9	100	240	200	—	20	140	50
10	100	220	—	160	50	140	30
11	100	180	90	100	—	—	50

as substrate there is approximately 100 % increase in urease production, with glycerol 40 % increase, with fructose and galactose an inconstant increase and with glucose and lactate over 50 % decrease. In the experiment given in detail in Table IV, the N content of the control is approximately twice that of the remainder. A similar result was obtained in all experiments, and, although no precautions were taken to ensure no loss of organisms during the washing, it was obvious that the growth from all the tubes was less than that in the control.

A comparison of Table V with Table I shows that we have not been able to confirm Jacoby's results. We have shown that certain substances have definite effects on urease production, but that there is no obvious association between the molecular structures of the substances and their effects. No carbohydrate group is an essential "brick" for urease formation.

SUMMARY

1. Jacoby's conclusion that the group

is an essential "brick" for urease formation by *Proteus vulgaris* has not been confirmed.

2. With arabinose added to the culture medium there is a 100 % increase in urease production, with glycerol 40 % increase, with fructose and galactose an inconstant increase and with glucose and lactate 50 % decrease.

3. All these substances reduce the total growth below the level of the control.

REFERENCES

- Iwanoff (1925). *Biochem. Z.* **157**, 229.
 — & Smirnowa (1927). *Biochem. Z.* **181**, 8.
 Jacoby (1916, 1). *Biochem. Z.* **77**, 124.
 — (1916, 2). *Biochem. Z.* **77**, 402.
 — (1916, 3). *Biochem. Z.* **77**, 405.
 — (1917, 1). *Biochem. Z.* **79**, 35.
 — (1917, 2). *Biochem. Z.* **80**, 357.
 — (1917, 3). *Biochem. Z.* **81**, 332.
 — (1917, 4). *Biochem. Z.* **83**, 74.
 — (1917, 5). *Biochem. Z.* **84**, 358.
 — (1918, 1). *Biochem. Z.* **86**, 329.
 — (1918, 2). *Biochem. Z.* **88**, 35.
 Karström (1930). Dissertation. Helsingfors.
 Lehr (1926). *Biochem. Z.* **168**, 166.
 Rubentschik (1926). *Biochem. Z.* **175**, 482.
 Stephenson & Stickland (1932). *Biochem. J.* **26**, 712.
 — (1933). *Biochem. J.* **27**, 1528.
 — & Yudkin (1936). *Biochem. J.* **30**, 506.
 Virtanen & Karström (1925). *Biochem. Z.* **161**, 9.
 Yudkin (1932). *Biochem. J.* **26**, 1859.
 — (1937). *Biol. Rev.* (in the Press).