CLXVI. FACTORS INFLUENCING BACTERIAL DEAMINATION

I. THE DEAMINATION OF GLYCINE, *dl*-ALANINE AND *l*-GLUTAMIC ACID BY *BACTERIUM COLI*

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(*Received 24 June 1937*)

THE idea that in growing cultures of bacteria carbohydrate exerts a sparing action on the decomposition of protein and amino-acids has for long been current. In its crudest and most generalized form this notion was elaborated by Kendall [1922]; put briefly, he showed that in growing cultures of a number of different bacterial species the ammonia liberated from protein digests decreased or even disappeared when carbohydrate was present. He interpreted this as due to a sparing action exerted by carbohydrate on the deamination of proteins, believing that in the presence of a readily available source of carbon and energy the organism decomposed less nitrogenous material. He failed to take account of the fact that ammonia is not only a product in the decomposition of amino-acids but also a source of nitrogen for growth; in the protein digest medium this ammonia is in excess of what can be used for cell synthesis, the additional carbohydrate enables this excess to be used for increased cell production. This point was brought out by Raistrick & Clark [1921] in work actually designed for another purpose; various species of bacteria were grown in synthetic medium with (1) tryptophan and (2) tryptophan+glycerol as sole sources of nitrogen and carbon. A study of the nitrogen distribution at the end of the growth period showed that the presence of glycerol had had four results: (1) to decrease the ammonia-N from 19·15 to 0·47 mg.; (2) to increase the cell-N from 4·34 to 12·36 mg.; (3) to increase the amino-N from 0·40 to 3·21 mg.; and (4) to increase the non-amino-N (N of the indole nucleus) from 3·0 to 10·85; (2) is a direct result of (1), whilst (3) and (4) illustrate the "sparing" action of glycerol in inhibiting the decomposition of tryptophan in spite of the increased number of cells available.

Another instance of the influence of carbohydrate in inhibiting or deflecting the course of amino-acid breakdown is its well-known action in preventing indole formation from tryptophan. In recent studies on this subject, Woods [1935] showed that washed suspensions of *Bact. coli*, which in the absence of glucose produce indole from tryptophan in 90% yield, fail to produce any in presence of glucose. Happold & Hoyle [1935] have shown that in growing cultures glucose may act in another way, i.e. by preventing the formation of the enzyme system ("tryptophanase") responsible for the production of indole. It has also been shown that in organisms of the Bacteriaceae grown in glucose broth the deamination (and dephosphorylation) of adenosinetriphosphate is strongly inhibited, though no such effect is obtained with other adenine compounds [Lutwak-Mann, 1936]; and that a similar inhibition of 50 to 70% in urease production occurs with *Proteus vulgaris* [Passmore & Yudkin, 1937].

These and other examples show that carbohydrate exerts an influence on the decomposition of amino-acids by bacteria and it is proposed in this and subsequent papers to examine the nature and cause of this influence.

In the case of bacterial enzyme action interference may operate at two points: (1) by so altering the growth of the organism that the enzyme system may be increased or diminished; (2) by deflecting the course of enzyme action after growth is complete. In growing cultures it is impossible to distinguish these two actions; by the use of washed bacterial suspensions they may be separately studied.

Technique

Three amino-acids were studied, glycine, *dl*-alanine and *l*-aspartic acid. Two strains of *Bact. coli* were used, the stock strain in use in this department (I) and a strain given to us by the Department of Bacteriology of Sheffield University (II).

In order to determine the conditions affecting enzyme formation during growth, strain I was grown in seven different ways, viz. (1) on the surface of broth agar in Roux bottles; (2) the same as (1) but with the addition of 1 % of the amino-acid; (3) in liquid broth aerobically in Roux bottles; (4) in liquid broth anaerobically; (5) in liquid broth anaerobically with 1 or 2 % glucose and chalk; (6) in liquid broth aerobically with 1 or 2 % glucose and chalk ((5) was kept anaerobic and (6) aerobic by bubbling with nitrogen and oxygen respectively; this also served to stir the chalk and maintain a neutral reaction); (7) liquid broth with 0·5 % sodium formate. The broth used was a tryptic digest of casein.

The bacteria were grown, unless otherwise stated, for 18 hr.; centrifuged and washed, and suspended in water so as to contain 1–3 mg. dry wt./ml.; the adjustment was done by help of a photo-electric turbidimeter [Clifton *et al.* 1935] which was calibrated for the organism in question. The rate of aerobic deamination was estimated by shaking 1 ml. each of buffer, bacterial suspension and $3M/250$ amino-acid at 40° for various intervals; 2 ml. of the mixture were used for each ammonia determination, which was done by the method of Parnas. Anaerobic deamination was done in Thunberg tubes with similar amounts, which

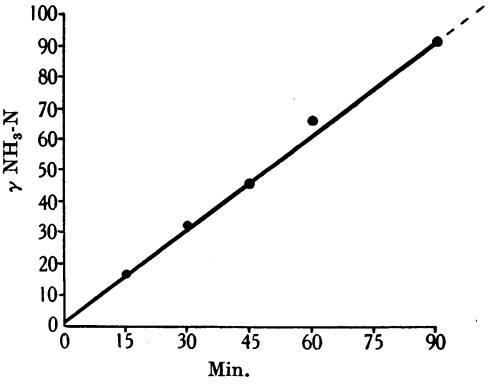

Fig. 1. γ NH_3-N/2·18 mg. bacteria/3 ml. $M/250$ *dl*-alanine.

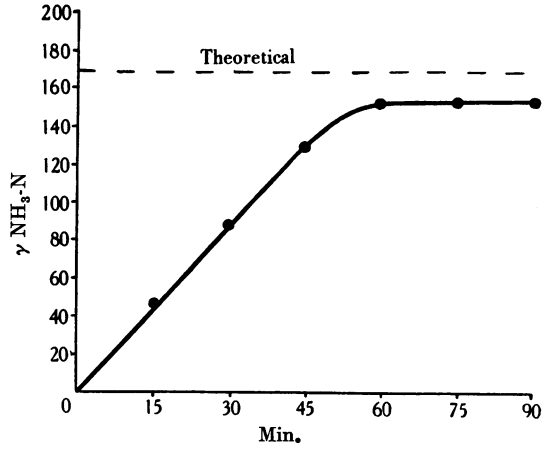

Fig. 2. γ NH_3-N/5·25 mg. bacteria/3 ml. $M/250$ *dl*-alanine.

were also used in Barcroft manometers in cases where oxidations were also measured. For convenience results are recorded as Q_N; this unit is comparable with the Q_{O_2} and is equal to γ N/mg. dry wt. bacteria/hr. $\gamma N = 1{\cdot}6\ \mu l\ NH_3$.

Fig. 1 shows that in these conditions with *dl*-alanine Q_N is linear with time; Fig. 2 shows that with somewhat increased concentration of bacteria the reaction is complete in 1 hr.

The deamination of glycine

Fig. 3 shows the optimum values of *p*H for this and other deaminations. Fig. 4 shows the influence of growth medium on the reaction. Here, as in all cases so far tried, the deaminase is unaffected by the presence of substrate added

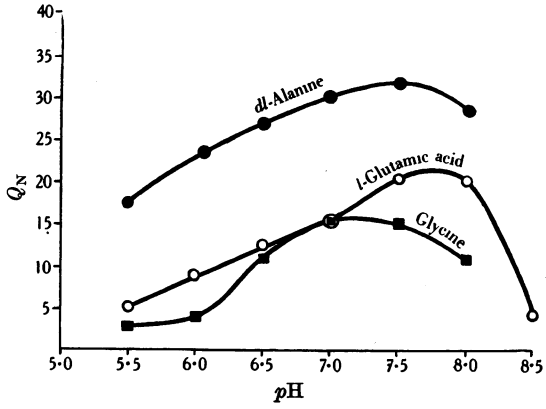

Fig. 3. Effect of *p*H on deamination by *Bact. coli* I.

to the growth medium. Compare (1) and (2) where organism I is grown on the surface of agar with (2) and without (1) the addition of 1 % glycine. (3) shows the very large decrease when passing from solid to liquid medium; we attribute this difference to the growth in liquid medium, even in Roux bottles, being less aerobic than on the surface of agar; it is not possible to be certain of this point. (4) shows a further decrease when conditions become strictly anaerobic; (5) the very remarkable further decrease when 2 % glucose is present in the medium. The specific effect of glucose over and above its effect in producing an anaerobiosis is seen by comparing (5) with (4) and (6) with (3). (7) shows that sodium formate has no effect which is not accounted for by the production of anaerobiosis (cf. (7) and (4)).

The presence of glucose during deamination produces no regular effect on the rate of deamination.

No anaerobic deamination of glycine occurs in the conditions of our experiments.

Strain II (Fig. 5) shows the main features of the picture produced by strain I, with variations due, we think, to the special characteristics of this organism. Strain II grows aerobically on broth about three times as fast, producing a total crop about three times as large as strain I. Anaerobically the growth curves follow each other closely. Strain II therefore appears to be a more efficient oxidizer than strain I though the particulars of the difference have not been examined. On turning to the effect of medium on the Q_N we see that the drop due to anaerobiosis occurs not between solid and liquid medium, (1) and (3), but between liquid medium in Roux bottles and the same anaerobically, (3) and (4). The depression due to glucose is still very marked (75 %) though not so great as in strain I (95 %).

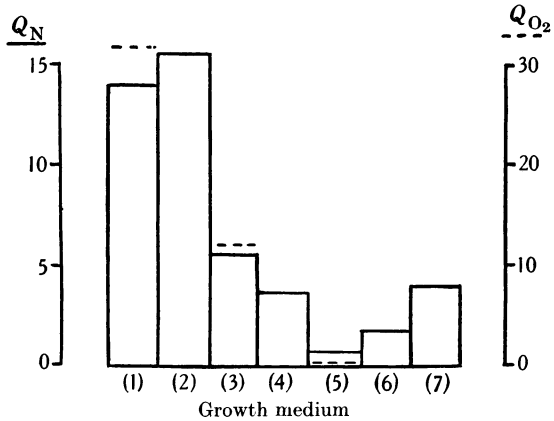

Fig. 4.

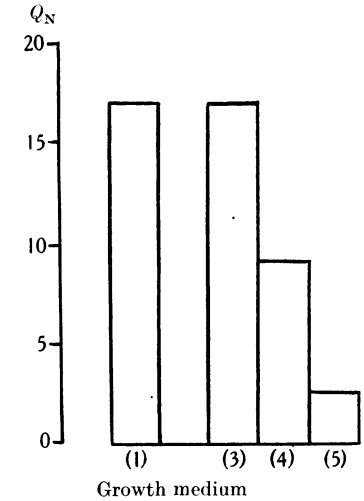

Fig. 5.

Fig. 4. Influence of growth medium on glycine deaminase *Bact. coli* I.
——— Q_N. – – – Q_{O_2}.

Fig. 5. Influence of growth medium on glycine deaminase *Bact. coli* II.

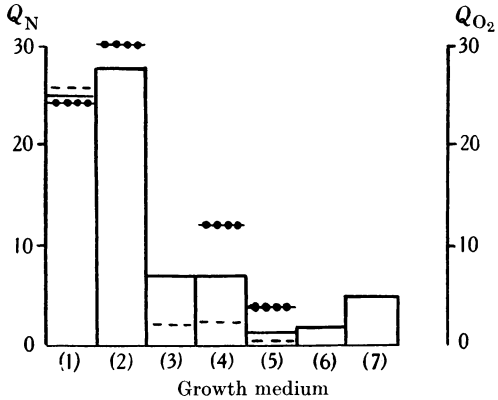

Fig. 6.

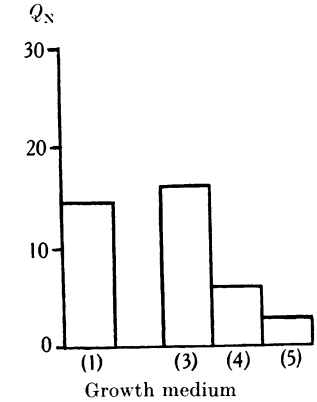

Fig. 7.

Fig. 6. Influence of growth medium on alanine deaminase *Bact. coli* I.
——— Q_N. ●●●● Q_N in presence of glucose. – – – Q_{O_2}.

Fig. 7. Influence of growth medium on alanine deaminase *Bact. coli* II.

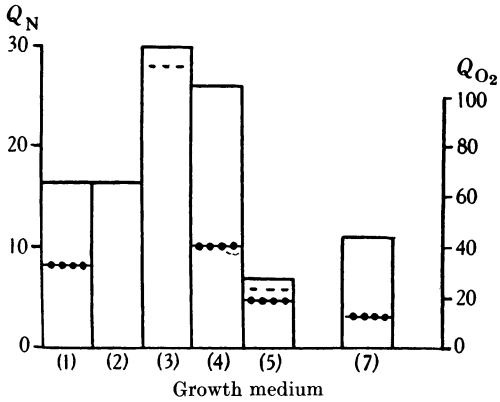

Fig. 8.

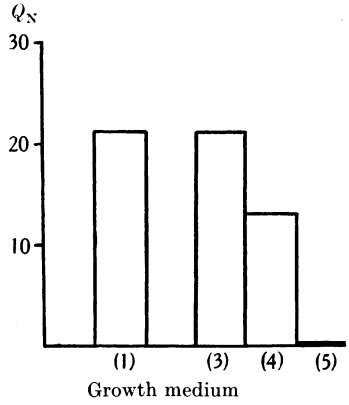

Fig. 9.

Fig. 8. Influence of growth medium on glutamic deaminase *Bact. coli* I.
——— Q_N. ●●●● Q_N in presence of glucose. – – – Q_{O_2}.

Fig. 9. Influence of growth medium on glutamic deaminase *Bact. coli* II.

The deamination of alanine

Generally speaking, the Q_N of alanine is about twice as great as that of glycine, Fig. 6; otherwise the two pictures are very similar, including the differences between strains I and II (Fig. 7). The presence of glucose in the medium during deamination is accelerative and the Q_{O_2} follows the Q_N.

The deamination of glutamic acid

This differs markedly from the two cases just described in that anaerobic conditions increase the formation of the enzyme (Fig. 8 (3) and (4)); glucose on the other hand has the same effect as before; where 2% glucose is used with strain II (Fig. 9) the effect is greater.

The effect of the age of the culture

Recent work [Wooldridge *et al.* 1936] has shown that certain dehydrogenases of *Bact. coli* differ in strength according to the time after sowing at which the organisms are removed from the growth medium. In case our results were being

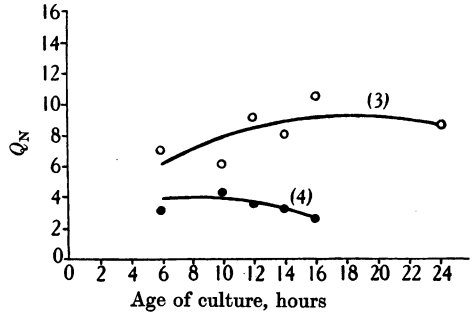

Fig. 10.

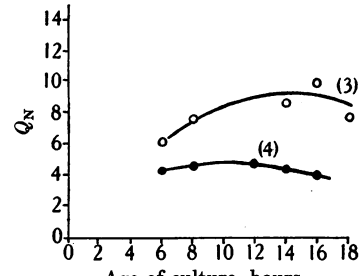

Fig. 11.

Fig. 10. Effect of age of culture on glycine deaminase on growth media (3) and (4).

Fig. 11. Effect of age of culture on alanine deaminase on growth media (3) and (4).

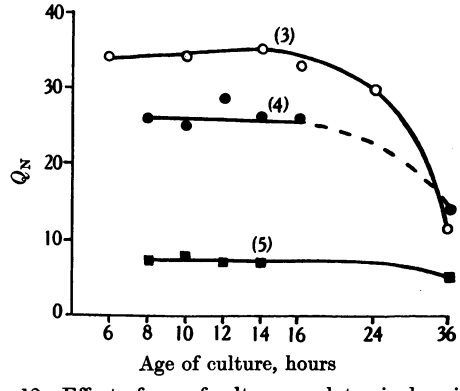

Fig. 12. Effect of age of culture on glutamic deaminase on growth media (3), (4) and (5).

vitiated through such a variation we have checked this point by removing organisms at intervals during the growth period and measuring the Q_N. From Figs. 10, 11 and 12 it is seen that no marked variation occurs before 20 hr., after which the activity falls off with age.

The effect of glucose (M/500) on the deamination process

This is less regular and important than the effect on enzyme formation. In the case of glycine the effect is irregular and does not exceed 20% either way. In the case of alanine the effect varies according to the growth medium, being negative in medium (1) and (2) and slightly accelerative in (4); the latter effect varies with the initial concentration of the glucose (Figs. 13 and 14). In the case of glutamic acid the effect is inhibitory throughout.

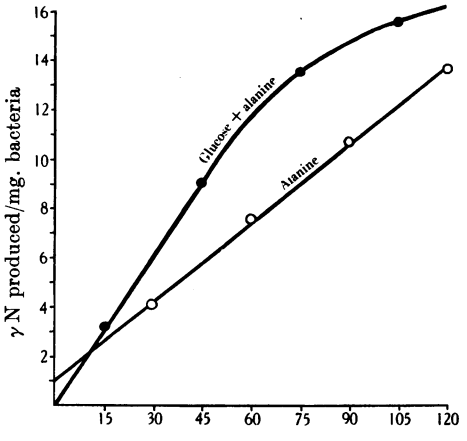

Fig. 13.

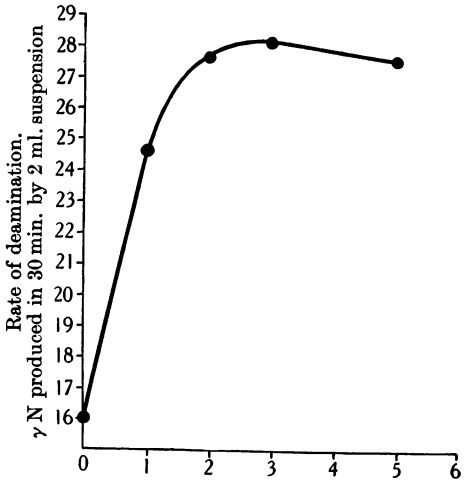

Fig. 14.

Fig. 13. Effect of glucose (initial conc. *M*/500) on deamination of *dl*-alanine. (Organism grown anaerobically.)

Fig. 14. Effect of varying concentrations of glucose on rate of deamination of *dl*-alanine. (Organism grown anaerobically.)

Nature of the deamination

The deamination of the three amino-acids is exclusively aerobic; in the conditions of our experiments no deamination occurs anaerobically. We conclude that we are here dealing with the oxidative deamination of the α-amino- to the α-keto-acid. Such a deamination has been described for *l*-alanine by an unidentified soil organism [Aubel & Egami, 1936] and for glycine by *Bact. coli*, *Bact. vulgare*, *Ps. fluorescens* and *Bac. mycoides* [Janke & Tayenthal, 1937]. The Q_{O_2} where given is shown by the dotted line on the diagram; this bears no strict relation to the Q_N; supposing oxidation and deamination to be proceeding at the same rate and oxidation to stop at the formation of the α-ketonic acid, 1 atom of nitrogen would be liberated in the form of ammonia during the uptake of 1 atom of oxygen, that is $Q_N = Q_{O_2} \times 0{\cdot}8$; actually, since no inhibitor was used oxidation proceeds further and this relationship is not realized but factors inhibiting deamination also inhibit oxidation and no deamination has been shown in the absence of a simultaneous oxidation.

SUMMARY

From our experiments it seems that the principal effect of glucose on the oxidative deamination of glycine, *dl*-alanine and *l*-glutamic acid is to inhibit the formation of the enzyme during growth. With strain I and 2% glucose this inhibition amounts to 95% for all three amino-acids. It is not attributable to anaerobiosis due to the evolution of fermentation gases or to the acidity produced by fermentation acids since these factors have been controlled. Anaerobic conditions during growth also inhibit the production of deaminase in the cases of glycine and alanine though less strongly than glucose but favour it in the case of glutamic acid. The presence of the specific amino-acid in the growth medium does not affect the formation of the enzyme; the age of the culture between 8 and 20 hr. causes a slight variation but not of an order to warrant further study in this connection.

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ERRATA

Vol. 31, page 1316 *delete* line 27: Woods (1935) *to* line 30: glucose.

„ „ „ „ line 31 *delete* "in another way, i.e."

„ „ „ „ line 30 *for* Happold & Hoyle [1935]
read Happold & Hoyle [1936]

„ „ page 1322, References *delete* Happold & Hoyle (1935).
Biochem J. **29**, 1918.

„ „ „ „ „ *delete* Woods (1935). *Biochem J.* **29**, 649.

„ „ „ „ „ *insert* Happold & Hoyle (1936).
Brit. J. Exp. Path. **17**, 136.