The Tryptophanase-tryptophan Reaction

6. CARBOHYDRATE-AMINO ACID RELATIONSHIPS CONCERNED IN THE INHIBITION OF INDOLE PRODUCTION BY GLUCOSE IN CULTURES OF *ESCHERICHIA COLI*

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The main facts which have been established previously by Happold & Hoyle [1936], and Evans, Handley & Happold [1941; 1942] are that *Escherichia coli* (*Bact. coli*), grown in a complex medium containing glucose and tryptophan, does not contain the tryptophanase enzyme system and that in a simple medium either phenylalanine or tyrosine is necessary to complete the glucose inhibition of the production of the tryptophanase system. A suggestion was made by Evans *et al.* [1942] that the inhibition of indole-production by cells grown in the presence of glucose and phenylalanine is maintained by the metabolism of stored carbohydrate. In this investigation we have attempted to substantiate this suggestion and also to determine the activity of the stereo-isomers of phenylalanine and tyrosine.

EXPERIMENTAL

The bacterial cultures. The Jebbs strain of *Escherichia coli* was used. The medium used for all but one of the investigations had the following composition: *l*-cystine, 1 mg.; glycine, 5 mg.; *dl*-valine, 10 mg.; *dl*-Na glutamate, 50 mg.; Na lactate, 150 mg.; NaCl, 50 mg.; $Na_2HPO_4.12H_2O$, 25 mg.; KH_2PO_4, 3·5 mg.; $MgSO_4.7H_2O$, 3 mg.; $FeSO_4.7H_2O$, 4 μg.; distilled water to 10 ml.; *p*H adjusted to 7·6. Glucose and tryptophan were added as sterile solutions to give a concentration of 1 and 0·003 % respectively, whilst phenylalanine, where used, was in a concentration of 0·01 %. The total volume of medium used in any particular experiment was 100 ml. Cultures were made in Roux bottles.

Estimation of polysaccharide. A washed suspension of the cells was prepared and its volume noted. A portion of this suspension was taken to obtain the total dry weight of organisms. The polysaccharide content of the remainder was determined by the method of Sahyun [1931]. The following are representative results for the estimation of known amounts of glycogen by this method in our hands:

Glycogen present mg.	Glycogen estimated mg.
8·5	7·4
4·4	3·5
2·2	1·6
1·0	1·1
0·4	0·4

These results indicated that the method was sensitive for small amounts of glycogen of the order of 1 mg. but for larger amounts the degree of recovery was only about 80 % and larger amounts of norite had to be used. Since the amount of glycogen estimated in the following experiments was of the order of 1 mg., the method was sensitive enough for our purposes.

Influence of phenylalanine on the metabolism of Esch. coli

(*a*) *Effect of added phenylalanine on oxygen uptake.* Evans *et al.* [1941], using the Van Slyke method for the estimation of amino-N, reported that phenylalanine was not metabolized by *Esch. coli*. This has now been examined with the Barcroft respirometer. 0·5 ml. of a washed suspension of *Esch. coli* grown on casein agar was added to 0·5 ml. of a 0·5 % solution of phenylalanine and 4·0 ml. K phosphate buffer (*p*H 7·6) contained in a Barcroft cup. The following results were obtained:

	μl./hr.			
O_2 uptake of cells with phenylalanine substrate	82	86	87	100
O_2 uptake of cells with no substrate	64		63	29

This difference in O_2 uptake, we suggest, corresponds either with the metabolism of phenylalanine or to the acceleration of the metabolism of some substance stored in the cell brought about by the phenylalanine.

(*b*) *Effect of phenylalanine on metabolism of glucose.* With phenylalanine and phenylalanine-free media, the glucose utilization of *Esch. coli* during a period of 24 hr. growth gave the following results:

Phenylalanine-free medium		*dl*-Phenylalanine (0·01 %) medium	
Total glucose utilization (mg.)	Glucose utilization (mg./mg. dry bact. wt.)	Total glucose utilization (mg.)	Glucose utilization (mg./mg. dry bact. wt.)
327	3·3	288	4·3
474	7·4	426	7·6
211	4·3	176	4·2

The total glucose metabolized was greater in the absence of phenylalanine and in general total growth was also greater, though this was less marked.

(*c*) *Effect of phenylalanine on polysaccharide storage.* The *dl* and the *l* forms of phenylalanine were used. The results obtained were as follows:

Cells grown in					
Phenylalanine-free medium		*dl*-Phenylalanine (0·01 %) medium		*l*-Phenylalanine (0·01 %) medium	
Polysacc. content (mg./mg. dry bact. wt.)	Total dry bact. wt. (mg.)	Polysacc. content (mg./mg. dry bact. wt.)	Total dry bact. wt. (mg.)	Polysacc. content (mg./mg. dry bact. wt.)	Total dry bact. wt. (mg.)
0·03	19	0·045	20	0·02	27
0·025	92	0·04	112	0·027	118
0·02	53	0·03	31	0·02	27
0·02	48	—	—	0·02	46
0·03	44	0·05	42	—	—
0·01	53	0·03	55	—	—

Thus *dl*-phenylalanine increased the polysaccharide storage of *Esch. coli*, but the naturally occurring *l*-form was inactive in this respect.

(*d*) *Effect of phenylalanine on the metabolism of stored polysaccharide.* Whilst the previous results indicated that there was a difference in the polysaccharide content of the two types of cells, they gave no indication that this polysaccharide was metabolizable. Cells were therefore grown on phenylalanine-free and phenylalanine-containing media, and their O_2 usage estimated in the absence of external substrate by means of a Barcroft respirometer. Typical results were as follows:

Oxygen uptake of cells grown on	
Phenylalanine-free medium μl./hr./mg. dry bact. wt.	*dl*-Phenylalanine medium μl./hr./mg. dry bact. wt.
16·7	41·7
11·0	42·3
29·0	78·0
22·5	36·0
22·5	44·0

A greater amount of some metabolizable substance is present in cells grown in the phenylalanine-glucose medium than in those grown in the phenylalanine-free medium. To confirm that the metabolism of stored polysaccharide did take place during the lag period before indole was produced, when cells grown in a glucose-phenylalanine medium were incubated with tryptophan, the following experiment was performed:

Washed cell suspensions of *Esch. coli* grown in phenylalanine-free and phenylalanine-containing media were prepared. Part of each of these suspensions was incubated with tryptophan and the oxygen consumption of the remainder was determined. The cells grown in the phenylalanine-free medium rapidly produced indole whilst the other cells had a lag period. The O_2 consumption of each of these two types of cells for this lag period was determined. The following are the results obtained:

Oxygen uptake of cells grown on	
Phenylalanine medium μl./hr./mg. dry bact. wt.	Phenylalanine-free medium μl./hr./mg. dry bact. wt.
36·0	18·8
44·0	18·8
40·0	20·0

The average difference between the O_2 uptakes of these two types of cell is 20·8 μl./hr./mg. dry bacterial wt. The amount of glycogen which would require this amount of O_2 for its complete combustion would be approx. 0·025 mg./mg. dry bacterial wt. This amount of glycogen is of the same order as the difference between the polysaccharide content of the cells grown in the two types of media. Therefore we can state that in all probability the increase in polysaccharide content of *Esch. coli* under the influence of phenylalanine corresponds with an increase in the metabolizable polysaccharide stored, and that this polysaccharide is metabolized before the development of a tryptophanase system can take place in the cell.

Influence of tryptophan and tyrosine

(*a*) *Effect of tryptophan on the polysaccharide content.* In this series of experiments the phenylalanine concentration was kept constant at 0·01 %, and the tryptophan content was varied. The following are the results obtained:

Indole production	Tryptophan present in medium %	Polysaccharide content (in duplicate) mg./mg. dry bact. wt.	
–	0·000	0·03	
–	0·003	0·035	0·03
Trace	0·01	0·03	0·025
+	0·02	0·01	0·01

Tryptophan exerts an action antagonistic to that of phenylalanine in stimulating polysaccharide storage in the organism, and evidently the stoichiometric relationship which exists between these two amino-acids in the production of indole in cultures containing glucose is due to this antagonism.

(*b*) *Effect of tyrosine on the polysaccharide content.* The same type of experiment as already described

for phenylalanine was repeated using *dl*- and *l*-tyrosine. The following results were obtained:

Cells grown in					
Tyrosine-free medium		*dl*-Tyrosine medium		*l*-Tyrosine medium	
Polysacc. content (mg./dry bact. wt.)	Total bact. wt. (mg.)	Polysacc. content (mg./dry bact. wt.)	Total bact. wt. (mg.)	Polysacc. content (mg./dry bact. wt.)	Total bact. wt. (mg.)
0·010	40	0·027	54	0·016	74
0·029	39	0·055	26	—	—
0·025	31	0·071	25	0·042	15

Thus results are similar to those obtained with phenylalanine but are less marked.

Influence of mannose

(*a*) *Effect of mannose on the polysaccharide content.* A comparison between the ability of mannose and glucose to allow storage of carbohydrate in *Esch. coli* has been made by determining the effect of replacing glucose in the medium by mannose. Indole was consistently produced in the mannose medium and not in the glucose medium when *dl*-phenylalanine was present. The following are the results obtained:

Polysaccharide content (mg./mg. dry bact. wt.) of cells grown in			
dl-Phenylalanine present		*dl*-Phenylalanine absent	
Glucose medium	Mannose medium	Glucose medium	Mannose medium
0·027	0·013	0·014	0·007
0·034	0·010	0·013	0·010

The range of bacterial dry wt. was from 32 to 82 mg., with the major part falling between 40 and 50 mg. *dl*-Phenylalanine appeared to be unable to increase polysaccharide storage with mannose to any marked extent. There was no evidence of storage with mannose alone.

(*b*) *Metabolism of mannose by* Esch. coli. Ashford & Holmes [1929] showed that in brain there were two mechanisms for glycolysis, one concerned with the breakdown of glycogen and requiring the presence of inorganic phosphate, whilst the other was concerned with the breakdown of glucose and did not require the presence of inorganic phosphate. Subsequently Ashford [1933] showed that whilst the anaerobic breakdown of glycogen and glucose could take place at the same time without any signs of mutual inhibition, mannose and glucose under similar conditions could not be metabolized simultaneously. Thus the conclusion was drawn that the same enzyme system was concerned in the metabolism of both glucose and mannose. In such circumstances both sugars were presumably metabolized by the non-phosphorylating route. In the previous experiments we have shown that glucose in the presence of *dl*-phenylalanine is able to increase the polysaccharide storage in *Esch. coli* and we wondered whether, in this case, the glucose was being metabolized by the phosphorylating route. On the other hand, since mannose is unable to effect such storage it might be metabolized by the non-phosphorylating route. To test this the oxygen consumption of washed suspensions of *Esch. coli* grown on phenylalanine-free glucose medium was determined, using glucose, mannose and a combination of the two as substrates. The concentration of the sugars was 0·5%. To a similar series phenylalanine was added to give a concentration of 0·01%. The results obtained were as follows:

Oxygen uptake of cells in the presence of					
Phenylalanine and			No phenylalanine		
Glucose Q_{O_2}	Mannose Q_{O_2}	Glucose + mannose Q_{O_2}	Glucose Q_{O_2}	Mannose Q_{O_2}	Glucose + mannose Q_{O_2}
11·3	12·1	12·5	12·1	9·8	11·7
7·3	7·6	11·4	11·0	9·0	10·0
10·3	12·3	12·4	14·3	14·5	13·3
5·6	5·9	5·9	—	—	—

Q_{O_2} = μl. oxygen/hr./mg. dry bact. wt.

No apparent summation of O_2 consumption takes place when the cells are acting upon the mixed substrate. Mannose is slightly more readily metabolized than glucose in the presence of phenylalanine and the reverse is true in its absence. The O_2 consumption of the cells metabolizing glucose is apparently greater when phenylalanine is absent from the medium. This conforms with its action in enhancing polysaccharide storage.

DISCUSSION

From the results presented there would appear to be no doubt that the mechanism of the glucose inhibition of the tryptophanase system in *Esch. coli* depends on the ability of the cell to store more polysaccharide than is present in the cell after culture in indole-producing media and that the metabolism of such stored polysaccharide may delay the onset of indole production when such cells are incubated with tryptophan.

Antagonistic actions on the storage of polysaccharide appear to be exerted by tryptophan and phenylalanine, the latter stimulating storage. Thus the suggestion can be made that a labile component of the enzyme systems concerned in the breakdown of tryptophan to indole and in the storage of carbohydrate is common to both and that competition occurs. The role which *dl*-phenylalanine plays in stabilizing the enzyme system so that storage of carbohydrate becomes its main function is obscure.

The 'natural' *l*(−) form appears to be inactive and therefore it is presumably the 'non-natural' *d*(+) form which exerts this action.

Butts, Dunn & Hallman [1938], working with rats, found that *dl*-phenylalanine was very active in the formation of liver glycogen in this animal, although *dl*-tyrosine was found to be inactive. Subsequently Butts, Sinnhuber & Dunn [1941] found that although *dl*-tyrosine was inactive in the formation of liver glycogen, the *l*(−) form caused some storage to occur. Our experiments may indicate that *l*(−)-tyrosine is more active in enhancing polysaccharide storage in *Esch. coli* than is *l*(−)-phenylalanine but in neither case does the *l*(−) form show an activity equal to the activity shown by the *dl* form. The similarities and dissimilarities of the action of phenylalanine and tyrosine on the carbohydrate metabolism of a mammal and of a bacterium open up some interesting questions.

dl-Phenylalanine inhibits to a slight extent the dissimilation of glucose by washed suspension of *Esch. coli*. If the amount of polysaccharide storage depends on a balance between the rate of storage and the rate of dissimilation, then if the latter rate is decreased we shall have increased storage. If we accept the scheme postulated by Hanes [1940] for the metabolism of glycogen by yeast cells, and the generally accepted scheme for the dissimilation of glucose, and apply them to the current problem, then phenylalanine may act either by influencing the equilibrium of the reaction glycogen ⇌ glucose-1-phosphate, or the dissimilation of the latter. Increase in glycogen storage may be sufficient to explain the decrease in the dissimilation of glucose, or the decrease in the dissimilation of glucose phosphate may be sufficient to explain the increased glycogen.

With regard to the failure of mannose to produce polysaccharide storage in *Esch. coli* under the influence of *dl*-phenylalanine, it would appear true to say that mannose is apparently metabolized by the same route as glucose, and since in this case glucose is capable of forming polysaccharide, it would appear most likely that this route is a phosphorylating one. It should, however, be pointed out that Cori, Schmidt & Cori [1939] prepared an eluate of yeast which together with the addition of adenylic acid and glucose-1-phosphate could produce a polysaccharide. Such a mixture could not, however, produce a polysaccharide from a similar mannose phosphate ester. Thus the conclusion to which we come is that although the mannose may be undergoing metabolism by the phosphorylating route, the enzyme system concerned in polysaccharide formation is unable to form polysaccharide from the mannose-1-phosphate which may be formed.

SUMMARY

1. *dl*-Phenylalanine and *dl*-tyrosine bring about an increased storage of metabolizable polysaccharide in *Esch. coli* when the latter is grown on a medium of known composition containing tryptophan and glucose.

2. The *l*(−) forms of these amino-acids are inactive in bringing about this increased polysaccharide storage.

3. Mannose is unable to increase the polysaccharide storage of *Esch. coli* in the presence of *dl*-phenylalanine, and suggestions have been given to explain this.

4. Tryptophan is antagonistic to *dl*-phenylalanine in its action on polysaccharide storage.

5. Suggestions have been put forward to localize the site of activity of *dl*-phenylalanine, and also to relate the tryptophanase system and the systems concerned in carbohydrate breakdown.

REFERENCES

Ashford, C. A. [1933]. *Biochem. J.* **27**, 903.
—— & Holmes, E. G. [1929]. *Biochem. J.* **23**, 748.
Butts, J. S., Dunn, M. S. & Hallman, L. F. [1938]. *J. biol. Chem.* **123**, 711.
—— Sinnhuber, R. O. & Dunn, M. S. [1941]. *Proc. Soc. exp. Biol., N.Y.*, **46**, 671.
Cori, G. T., Schmidt, G. & Cori, C. F. [1939]. *Science*, **89**, 464.
Evans, W. C., Handley, W. R. C. & Happold, F. C. [1941]. *Biochem. J.* **35**, 207.
—— —— —— [1942]. *Biochem. J.* **36**, 311.
Hanes, C. S. [1940]. *Proc. roy. Soc.* B, **128**, 421.
Happold, F. C. & Hoyle, L. [1936]. *Brit. J. exp. Path.* **17**, 136.
Sahyun, M. [1931]. *J. biol. Chem.* **93**, 227.