# 20. THE TRYPTOPHANASE-INDOLE REACTION

## 4. SOME OBSERVATIONS ON THE PRODUCTION OF TRYPTOPHANASE BY *ESCH. COLI*; IN PARTICULAR THE EFFECT OF THE PRESENCE OF GLUCOSE AND AMINO-ACIDS ON THE FORMATION OF TRYPTOPHANASE

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FILDES [1938], referring to the claims of Happold & Hoyle [1936] that 'the failure to produce indole by cultures of *E. coli* grown in the presence of glucose is due to the non-production of a tryptophanase system', suggests that this is but a partial truth and that the tryptophanase system in the cells consists of two parts, a 'constitutive' part and an 'adaptive' part, of which only the 'adaptive' part is not produced in the presence of glucose. Fildes further states that suspensions of *E. coli* are always capable of catalysing the oxidation of tryptophan to indole even when grown in the absence of tryptophan or in the presence of glucose and tryptophan.

The main points of difference in technique and findings between the two series of investigations are: Fildes obtained his cells from cultures grown on a synthetic medium containing glucose and tryptophan and reported that indole was formed in these cultures and that the suspensions derived therefrom when incubated with tryptophan produced indole. Happold & Hoyle, having sought to explain the absence of indole formation which occurs in glucose bouillon cultures, grew their cells in this medium; indole was not produced in such cultures nor was there enzyme formation, since when non-viable preparations from these cells were placed in contact with tryptophan, indole was not formed, though similar preparations from bouillon cultures were active. The latter technique does not allow neo-enzyme formation to occur during the course of the experiment.

Both series of investigations have been repeated and are experimentally correct. Fildes' results may be due to neo-enzyme formation by the viable cell suspension in the presence of tryptophan as substrate and the absence of glucose. Certainly there are as yet no grounds for postulating a separate 'constitutive' tryptophanase system.

The experimental evidence which supports the above thesis was obtained as follows. Use was made of the previously observed phenomenon that the viability of washed cells of *E. coli* can be destroyed by the addition of a small amount of toluene immediately after the addition of the substrate and that this procedure inactivates only a proportion of the tryptophanase already formed, provided that the relative concentration of the toluene to the cells does not become too high. Treatment of the cells with toluene destroys their ability to cause carbohydrate breakdown.

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## Experimental

The Jebbs strain of *E. coli* (previously supplied by Prof. McIntosh) was used in all experiments. It was inoculated into media from weak suspensions prepared by suspending an agar colony of *E. coli* in 10 ml. sterile saline. The suspensions used for the enzyme work were gathered from 18–24 hr. cultures at 37°. Those taken from solid media were washed off with a mixture of 0·5 % saline and *M*/60 phosphate buffer (*p*H 7·0) and the cells separated from the suspending fluid by a high-speed centrifuge. The cells were washed twice in the saline phosphate mixture and finally suspended in it. Those taken from fluid media were separated from the medium, washed twice with the saline phosphate mixture and also suspended in the same manner. Glucose, where used, was added to the medium as a sterile solution to give final concentrations of 0·5–1·0 %. The details for growth experiments are given separately.

### *The non-production of the tryptophanase system in glucose bouillon agar cultures of* E. coli

Washed suspensions from (*a*) two Roux bottles of bouillon agar and (*b*) two Roux bottles of bouillon agar +1 % glucose were used. Suspensions and substrate were incubated separately at 37° for 15 min. before mixing. The following

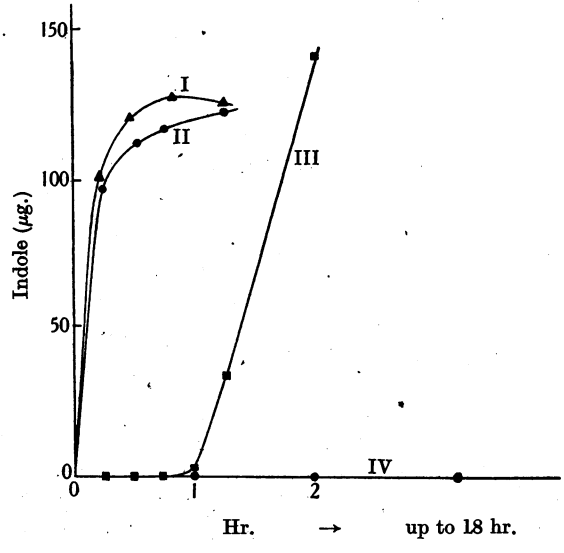


Fig. 1 Indole production from tryptophan by *E. coli*. I, *coli* cells from bouillon agar. II, *coli* cells from bouillon agar toluenated. III, *coli* cells from bouillon agar +1% glucose. IV, *coli* cells from bouillon agar +1% glucose toluenated.

series of flasks was then set up, each flask containing 250 μg. tryptophan in 10 ml. of buffer mixture:

Series 1. Substrate +5 ml. of cell suspension from bouillon agar culture.
Series 2. Substrate +5 ml. of cell suspension from bouillon agar culture +0·2 ml. toluene.
Series 3. Substrate +5 ml. of cell suspension from glucose bouillon agar culture.
Series 4. Substrate +5 ml. of cell suspension from glucose bouillon agar culture +0·2 ml. toluene.

The final turbidity of the suspensions was 2500 million cells per ml. The results are shown in Fig. 1.

The above series of results is characteristic of many similar experiments except that it represents one where there was no appreciable inactivation of the enzyme by the toluene. It will be observed that 87 % conversion of tryptophan into indole occurred as a result of the activity of cells grown in the absence of glucose, that in the glucose series there was practically no enzymic action after 1 hr. but that the reaction was 97 % complete after a further 2 hr. incubation, and finally that no indole was produced in the toluene-treated glucose agar suspensions. The only variations in results from many similar experiments of the above type are: (*a*) that inhibition of indole production in series (3) varies in lasting from 45 min. to 2 hr. after the addition of the substrate (this is probably due to the metabolism of stored carbohydrate); (*b*) that suspensions grown in the absence of glucose and treated with toluene are normally less active than their corresponding viable cells in series (1) by anything up to 60 %, depending on the cell concentration. This is shown in the following results, which are expressed in $\mu$g. units:

| | 30 min. | 60 min. | 120 min. | 180 min. |
|---|---|---|---|---|
| (1) With 250 $\mu$g. tryptophan, after: | | | | |
| Washed suspension | 20 | 30 | 50 | 60 |
| Washed suspension + 0·2 ml. toluene | + | 15 | 17 | 17 |
| (2) With 1000 $\mu$g. tryptophan: | | | | |
| Washed suspensions | Rapid rise to 119 | | — | 100 |
| Washed suspensions + 0·2 ml. toluene | Rapid rise to 55 | | — | 62 |

The greatest variation has been obtained with washed cells grown in Fildes' medium + glucose and tryptophan. Frequently, we have obtained washed cells which have not produced more than 1–2 $\mu$g. indole from 250 $\mu$g. tryptophan in 16 hr.; frequently we have had rapid indole production with the washed cells, but always after a lag period, never have we obtained any indole formation from washed cells which have had toluene added after the addition of the substrate. An example of the second type of result is given in the following table:

Cells grown in 200 ml. Fildes' medium + glucose + tryptophan

| | Indole in $\mu$g. | | | |
|---|---|---|---|---|
| | 20 min. | 30 min. | 40 min. | 60 min. |
| Washed cells + 250 $\mu$g. tryptophan | Nil | 10 | 75 | 125 |
| Washed cells + 250 $\mu$g. tryptophan + 0·2 ml. toluene | Nil | Nil | Nil | Nil |

It will be observed that the results with the washed cells obviously fall on an autocatalytic curve as do those with the glucose bouillon agar suspensions and that adaptation to the substrate is taking place. The metabolism of stored carbohydrate is also progressing in the suspensions which are free from toluene. Cells grown in Fildes' medium alone (no tryptophan) are devoid of tryptophanase activity.

It has again been confirmed that fermenting glucose does not stop the tryptophan-tryptophanase indole reaction when the tryptophanase is already present before the glucose is added, and further that the tryptophan content of a medium does not decrease when *E. coli* is grown in it in the presence of glucose, at least whilst other amino-acids are present.

The former point is demonstrated by the following experiment. Twice-washed suspensions of organisms grown on bouillon agar were incubated in four 250 ml. flasks each containing 1 % glucose and 2 mg. of tryptophan per 50 ml. Two of the flasks contained 1 ml. of toluene in each flask. Glucose determinations showed that glucose was disappearing from the non-toluenated flasks during the course of the experiment. The agreement using this technique is not so good as

when separate flasks are used for each determination, but the results are adequate for the purpose required:

| Period of incubation | Indole produced per 10 ml. in μg. | | | |
|---|---|---|---|---|
| | Without toluene | | With toluene | |
| | Flask (1) | Flask (2) | Flask (3) | Flask (4) |
| 30 min. | 51 | 41 | 21 | 22 |
| 1¼ hr. | 83 | 69 | 37 | 44 |
| 3½ hr. | 100 | 83 | 41 | 41 |
| 5¼ hr. | 101 | 95 | 50 | 44 |

The non-disappearance of tryptophan from the culture medium when *E. coli* is grown in the presence of glucose, tryptophan and other amino-acids, i.e. when tryptophanase is not formed, was demonstrated by means of an eight-amino-acid medium [Evans *et al.* 1939] as follows:

Four flasks of the above eight-amino-acid culture medium were set up: (1) Basal medium uninoculated. (2) Basal medium inoculated with *E. coli*. (3) Basal medium + 0·5 % glucose uninoculated. (4) Basal medium + 0·5 % glucose inoculated with *E. coli*.

After 23 hr. incubation at 37°, 10-ml. samples from the flasks showed no change in reaction except that from flask (4) which was acid (*p*H 5). Tryptophan estimations on samples (by the method of Lugg [1937]) gave the following results:

| | Tryptophan as g. per 100 ml. | | | |
|---|---|---|---|---|
| | Flask (1) | Flask (2) | Flask (3) | Flask (4) |
| After 23 hr. | 0·0112 | 0·0019 | 0·0117 | 0·0111 |

Although the inoculated flasks (2) and (4) had both yielded a good growth of *E. coli* tryptophan had only disappeared from flask (2); this was the only flask which gave any reaction for indole and in this case it was a very strong reaction.

From the above experimental evidence it seems certain that the presence of tryptophan is essential for the production of tryptophanase, that where there is no indole formation in cultures containing glucose and tryptophan there is no tryptophanase production. Further, since resting bacteria do not apparently rest (but are capable of giving rise to neo-enzyme formation) the washed viable cell technique is unsafe as a method of analysis for the presence or absence of an enzyme at a given point in time.

### *Specific inhibition of indole production by* E. coli *in the presence of phenylalanine or tyrosine and glucose in simple media*

There remains the fact, however, that under certain conditions, e.g. when grown in Fildes' medium + tryptophan, the inhibitory effect of glucose on the production of indole by cultures of *E. coli* is not complete, up to one-fifth of the indole produced in the absence of glucose being formed. This appears to be due to the peculiarities of the constitution of the medium.

It has been found that complete inhibition of indole formation in the presence of glucose and tryptophan in Fildes' medium can be obtained by adding 10 ml. of a 15 % gelatin solution per 50 ml. of Fildes' medium. Further, it was found that if the gelatin were replaced by a mixture of amino-acids representing those found in gelatin by analysis the same result was obtained. Each of the amino-acids found in gelatin hydrolysates was now added separately to Fildes' medium + glucose + tryptophan to a concentration of 0·01 %. Of the amino-acids used—

glycine, alanine, leucine, aspartic acid, glutamic acid, phenylalanine, cystine, lysine, arginine, histidine, proline and hydroxyproline—only phenylalanine and tyrosine (this was used later) completely inhibited indole production. At the concentration employed (0·01 %) the other amino-acids had no influence on the amount of indole formed. Further, it has been found that in the case of mono Na glutamate, leucine, valine, glycine, alanine, aspartic acid and histidine concentrations of 0·05, 0·10, 0·15 and 0·20 % have no effect on the production of indole in the presence of glucose. It has been demonstrated that phenylalanine and tyrosine do not affect the amount of indole produced from tryptophan in the absence of glucose. Numerous growth experiments have been carried out to determine the concentrations of phenylalanine and tyrosine necessary to complete the inhibition of indole production by glucose in Fildes' medium. The following relationships hold:

0·001 % tryptophan = 0·001–0·002 % tyrosine.
= 0·003–0·004 % phenylalanine.

0·002 % tryptophan = 0·003–0·004 % tyrosine.
= 0·005–0·006 % phenylalanine.

0·003 % tryptophan = 0·004–0·005 % tyrosine.
= 0·007 % phenylalanine.

There is evidence here of a possible stoichiometric relationship between the tryptophan and the tyrosine and a less exact relationship between the phenylalanine and the tryptophan (the phenylalanine is a synthetic amino-acid).

The action of glucose + phenylalanine or tyrosine on preformed tryptophanase has also been studied.

Washed suspensions of *E. coli* grown on bouillon agar were prepared and added to flasks containing buffer solution *p*H 7·6 and 0·01 % phenylalanine or tyrosine sterilized by autoclaving as basal substrate. The following experimental flasks were set up: 30 ml. buffer solution and 30 ml. of cell suspension were placed in all the flasks:

(1) 1·5 ml. 1/1000 sterile tryptophan.
(2) 1·5 ml. 1/1000 sterile tryptophan + 0·05 % phenylalanine.
(3) 1·5 ml. 1/1000 sterile tryptophan + 0·05 % phenylalanine + 1 ml. toluene.
(4) 1·5 ml. 1/1000 sterile tryptophan + 0·05 % phenylalanine + 1 ml. 50 % sterile glucose.
(5) 1·5 ml. 1/1000 sterile tryptophan + 0·05 % phenylalanine + 1 ml. 50 % sterile glucose + 1 ml. toluene.

The tryptophan was added last to all the flasks, which were then incubated at 37°. 10-ml. samples were taken from each flask, ½, 1 and 3 hr. after the start of incubation. The results were as follows:

| | Indole ½ hr. | Reaction | | |
|---|---|---|---|---|
| | | 1 hr. | 3 hr. | |
| Flask no. | μg. | μg. | μg. | Reaction |
| (1) | 76 | 119 | 124 | No change |
| (2) | 76 | 120 | 125 | ,, |
| (3) | 17 | 40 | 39 | ,, |
| (4) | 76 | 110 | 108 | Slightly acid |
| (5) | 17 | 45 | 44 | No change |

Similar results obtained with tyrosine show that in the presence of phenylalanine and tyrosine preformed tryptophanase produced almost theoretical yields of indole from tryptophan and also that in the presence of fermenting glucose this yield is not reduced. It should be added that experiments performed for us by Mr H. Anderson show that cells of *E. coli* do not deaminate phenylalanine.

It is clear to us from these and other findings that the effect of the phenylalanine and tyrosine is not exerted through a competitive action upon the enzyme system when formed, but that it must be exerted during the stage of cell division when metabolism is in its most active phase. At the same time the specific effect of these two amino-acids, which show a structural relationship to tryptophan, raises points of interest in view of the findings of Baker & Happold [1940], that the integrity of the alanine group attached to the heterocyclic indole ring is essential to indole formation by suspensions of *E. coli*. It will also be noted that phenylalanine and tyrosine are two of the small group of amino-acids which are regarded as ketogenic in the mammalian metabolism of amino-acids.

It should be unnecessary to add that the term 'tryptophanase' is used, not to imply a single enzyme but to describe a coupled oxidative deamination and decarboxylation in an enzymic system which is specific for the tryptophan-indole reaction.

## Summary

It is further confirmed that the presence of tryptophan in a medium is essential for the production of the tryptophanase system by cultures of *E. coli*, and that the enzyme system does not exist as such in cells which have been grown in glucose bouillon agar or glucose tryptic digest agar, but that such cells when freed from glucose by washing will re-develop the enzyme system when left in contact with tryptophan.

Cells grown in complex amino-acid media plus glucose and tryptophan will produce some indole if phenylalanine and tyrosine are absent from such media. There is evidence of a stoichiometric relationship between the amount of tyrosine (?phenylalanine) which inhibits indole production and the tryptophan in media containing an increasing amount of tryptophan. This action is restricted in its effect to the growing cell. Phenylalanine and tyrosine alone or with glucose do not affect the tryptophanase activity of washed cells.

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