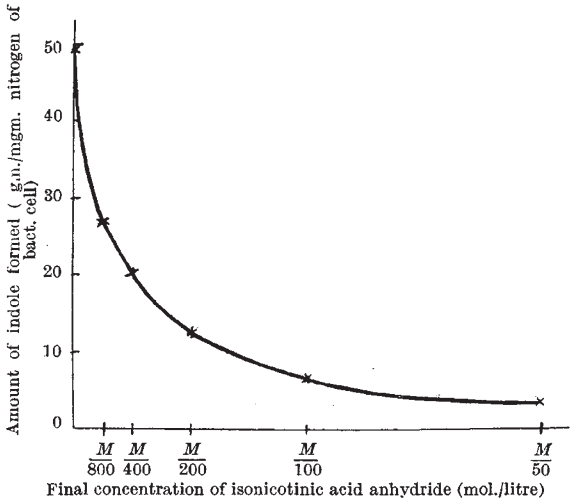
Competitive Action of Isonicotinic Acid Hydrazide and Vitamin B_6 in the Formation of Indole by *E. coli*

We have been seeking the mechanism to explain our observation that one of the substances which is contained in broth and is able to decrease the inhibitory effect of isonicotinic acid hydrazide on the growth of *E. coli* can, to some extent, be replaced by pyridoxine[1]. In further experiments, we have found that isonicotinic acid hydrazide considerably inhibits the formation by *E. coli* of indole, and that the activity of a suspension of *E. coli* treated with isonicotinic hydrazide recovers when the cells are in contact with pyridoxine.



Inhibitory activity of isonicotinic acid anhydride on the indole formation of *E. coli communior* (Exp. 1)

Typical results showing the inhibitory effect of isonicotinic acid hydrazide on the formation of indole by *E. coli communior* are given in the accompanying graph (Exp. 1), and the recovery effect due to pyridoxine is shown in Table 1 (Exp. 2). From the graph, it will be seen that the formation of indole by the washed suspension decreases considerably, generally in proportion to the amounts of isonicotinic acid hydrazide added ; the rate of inhibition of isonicotinic acid hydrazide calculated from the results is given in Table 2.

The results in Tables 1 and 2 show that pyridoxine has a remarkable antagonistic action against the inhibitory effect on a cell suspension of isonicotinic acid hydrazide ; for example, even 10^{-7} *M* (final concentration) of pyridoxine shows more than 50 per cent recovery effect.

In order to obtain the tryptophanase activity, the culture of *E. coli communior* (stock) was grown at

Table 1 (Exp. 2). Recovery Effect of Pyridoxine

Substrate : 0·4 ml. of *M*/160 DL-tryptophane in 3·4 ml. of reaction mixture. Reaction time : 10 minutes. Reaction temperature : 38° C.

Final concentration of INAH (*M*)	Final concentration of pyridoxine (*M*)	Indole formed (γ gm./mgm. nitrogen of bact. cell)	Recovery effect (per cent)
10^{-2}	10^{-4}	27·6	69·3
10^{-2}	10^{-5}	27·1	67·4
10^{-2}	10^{-6}	26·9	66·8
10^{-2}	10^{-7}	24·2	56·6
10^{-2}	10^{-8}	12·5	11·5
10^{-2}	10^{-9}	10·0	2·2
10^{-2}	—	9·5	0·0
—	—	35·7	—

Table 2

Final concentration of isonicotinic acid hydrazide (mol./lit.)	Inhibitory rate (per cent)
0·02	92
0·01	86
0·005	74
0·0025	58
0·00125	45
0·0	—

37° C. in broth containing 10 mgm./l. of DL-tryptophane for 18 hr. At the end of incubation, the organisms were centrifuged out of the growth medium, washed in saline three times and then made up into suspension in saline. The nitrogen content of the organisms per millimetre suspension was determined by the micro-Kjeldahl method as modified by Parnas.

As the preliminary experiments showed that contact for 30 min. was necessary for obtaining the complete action of isonicotinic acid hydrazide and of pyridoxine, suspensions of cells with the former or both of these substances respectively in phosphate buffer were incubated at 38° C. for 30 min. just before the determination of tryptophanase ; the following quantities were used : in each test-tube, 0·5 ml. of washed suspension, 0·3 ml. of isonicotinic acid hydrazide solution, 1·9 ml. of *M*/15 phosphate buffer (*p*H 8·0), and in Experiment 2, 0·3 ml. of pyridoxine hydrochloride solution. The quantity of the liquid in each test-tube was made up to a volume of 3·0 ml. with distilled water.

In order to test the tryptophanase activity of these suspensions, the method of Wood *et al.*[2] with modifications was used throughout this work. The procedure is as follows : at the end of the contact, 0·4 ml. of *M*/160 DL-tryptophane was added to each test-tube and the reaction mixture was incubated at 38° C. for 10 min. The reaction was stopped by the addition of 0·2 ml. of 100 per cent trichloracetic acid ; and 2–5 ml. of toluene containing the indole extracted from the reaction mixture by shaking was mixed thoroughly with 5·0 ml. of Ehrlich's reagent (2 gm. paradimethyl-amido benzaldehyde, 40 ml. concentrated hydrochloric acid, 190 ml. of ethanol). After 10 min. reaction, 5·0 ml. of ethanol was added to dissolve the toluene and the red-coloured solution was used immediately for electrophotometric determination.

Since it is generally agreed that the coenzyme of the tryptophanase of *E. coli* is identical with pyridoxal phosphate[3], it is thus apparent from our results that isonicotinic acid hydrazide may act on the tryptophanase as an antimetabolite against the coenzyme, and that the considerable recovery effect of pyridoxine is due to the competitive action of the pyridoxal phosphate, which may be produced in the cell from pyridoxine added. These results point in the same direction as our experiments on the inhibitory action of isonicotinic acid hydrazide on the decarboxylation of amino-acids by *E. coli*[1]. Further research is now in progress. This work will be published in detail later.

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