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Effect of Indole Compounds on Vitamin B_{12} Utilization

By JERRY DREXLER

A DEFINITE RELATIONSHIP has never been established between indole metabolism and the megoloblastic anemias. Rhoads and associates[1,2] were able to produce a hemolytic-like anemia with many features of pernicious anemia, in dogs on vitamin B deficient diets, by the oral administration of pure indole. Interestingly, the relief of this anemia by oral feeding of liver extract was accompanied by a reticulocytosis.[2] In dogs on well balanced diets, the administration of a similar amount of pure indole caused a well defined increase in bilirubin excretion, and massive doses of indole caused a severe anemia. Tönnis and Horster, cited by Rhoads and Miller,[2] reported the occurrence of indicanuria and anemia in dogs with surgically formed, inactive, open jejunal segments, and the relief of the anemia after the administration of liver extract. These investigations suggested the possibility that indole compounds might produce anemia by affecting the utilization of some hematopoietic factor.

The purpose of the present study is to describe the effect of indole compounds on the utilization of vitamin B_{12} by a vitamin-dependent microorganism.

MATERIALS AND METHODS

The indole compounds were selected from the serotonin (5-hydroxytryptamine) pathway of tryptophan metabolism, since these compounds may be present in the gastrointestinal tract where vitamin B_{12} normally is absorbed. A mutant strain of *Escherichia coli*, which is dependent upon vitamin B_{12} for growth, was used in the microbiologic assays. The inherent dangers of such an organism for assay of impure vitamin B_{12} preparations have been stressed,[3] but for assay of purified preparations, the *E. coli* tube method, as modified by Glick,[4] is reliable and easily and rapidly performed.

The assay medium deficient in vitamin B_{12} was prepared according to the method of Burkholder.[5] One ml. of this double strength medium was used in each tube of the eight series of assays. The volume of the contents of each tube was increased to 2 ml. by the addition of distilled water (series 1 and 2), vitamin B_{12} and distilled water (series 3), or vitamin B_{12} and indole compound (series 4 through 8) (table 1). The distilled water and vitamin B_{12} were added to the assay medium in cotton-plugged test tubes which were then autoclaved at 115 C. for five minutes. The standard vitamin B_{12} solution contained 0.5 millimicrogram B_{12} per milliliter. The indole compounds were dissolved in sterile water by heating; after they had cooled, they were added to the cooled assay solutions immediately before inoculation. The indoles were prepared in a concentration of 1 mg. per milliliter.

All experiments were performed in triplicate. Except for series 1, each tube was inoculated with one drop of a freshly prepared saline suspension of washed *E. coli*,

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TABLE 1.—*Ingredients of Each Tube in Eight Series of Assay Tubes*

Series no.	Media (ml.)	B_{12} solution (ml.)	5-Hydroxy-tryptophan (ml.)	5-Hydroxy-tryptamine* (ml.)	5-Hydroxy-indoleacetic acid† (ml.)	3-Indoleacetic acid (ml.)	Indole (ml.)	Water (ml.)
1	1.0	—	—	—	—	—	—	1.0
2	1.0	—	—	—	—	—	—	1.0
3	1.0	0.5	—	—	—	—	—	0.5
4	1.0	0.5	0.5	—	—	—	—	—
5	1.0	0.5	—	0.5	—	—	—	—
6	1.0	0.5	—	—	0.5	—	—	—
7	1.0	0.5	—	—	—	0.5	—	—
8	1.0	0.5	—	—	—	—	0.5	—

* 5-hydroxytryptamine supplied by Abbott Laboratories.

† 5-hydroxyindoleacetic acid supplied by Dr. M. Speeter, Research Laboratories of the Upjohn Company, Kalamazoo, Michigan.

strain 113-3 (A.T.C.C. 11505).* The tubes were shaken for 18 hours; the ingredients were then diluted to 5 ml. with distilled water, and the final turbidity, indicating the growth of the microorganism, was measured by a Klett immersion colorimeter.

Two of the tested indole compounds were found to inhibit the growth of the microorganism. Consequently, experiments using procedures similar to those described were performed in order to elucidate the mechanism of this inhibition. The results of these latter experiments are reported later.

RESULTS

The results (table 2) of these experiments demonstrate the ability of indoleacetic acid and pure indole to inhibit the growth of the vitamin B_{12} dependent microorganism.

The standard growth curve for *E. coli*, strain 113-3, was depressed by the addition of indole (fig. 1). The inhibitory effect on the growth of the microorganism, which resulted from increasing the concentration of indoleacetic acid, is shown in figure 2. Although concentrations of the indole compound may appear high, a definite inhibitory effect can be seen after the addition of only 50 micrograms of indoleacetic acid.

Since an excess of vitamin B_{12} was found to overcome the inhibitory action of the indole (fig. 3) and since several non-B_{12} dependent *E. coli* organisms were not affected by a similar amount (0.5 mg.) of indole or indoleacetic acid, the indole compounds appeared to act by preventing utilization of vitamin B_{12}. As measured by radioactive B_{12} studies,† the uptake of vitamin B_{12} from the medium was actually only slightly impaired by indoleacetic acid. The addition of excess tryptophan, or the removal of tryptophan from the Burkholder medium, did not affect the inhibition. Cobalt, copper, and iron

*Supplied by Dr. E. L. R. Stokstad, American Cyanamid Co., Research Division, Lederle Laboratories Pearl River, N. Y.

†The radioactive material used in this study was supplied by Abbott Laboratories on authorization of the Isotopes Division, U. S. Atomic Energy Commission, Oak Ridge, Tennessee.

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TABLE 2.—*Turbidity Readings*° *in Eight Experiments, Each Performed in Triplicate*

Series no.	Assay solution	Turbidity readings 1	2	3	Average of turbidity readings
1	Uninoculated media	1	0	0	0.3
2	Inoculated media	6	4	4	4.7
3	B_{12} control	106	96	96	99.3
4	B_{12} plus 5-hydroxytryptophan	97	91	105	97.7
5	B_{12} plus 5-hydroxytryptamine	112	94	84	96.7
6	B_{12} plus 5-hydroxyindoleacetic acid	107	105	94	102.0
7	B_{12} plus indoleacetic acid	56	34	47	45.7
8	B_{12} plus pure indole	23	27	25	25.0

° Measured by Klett immersion colorimeter.

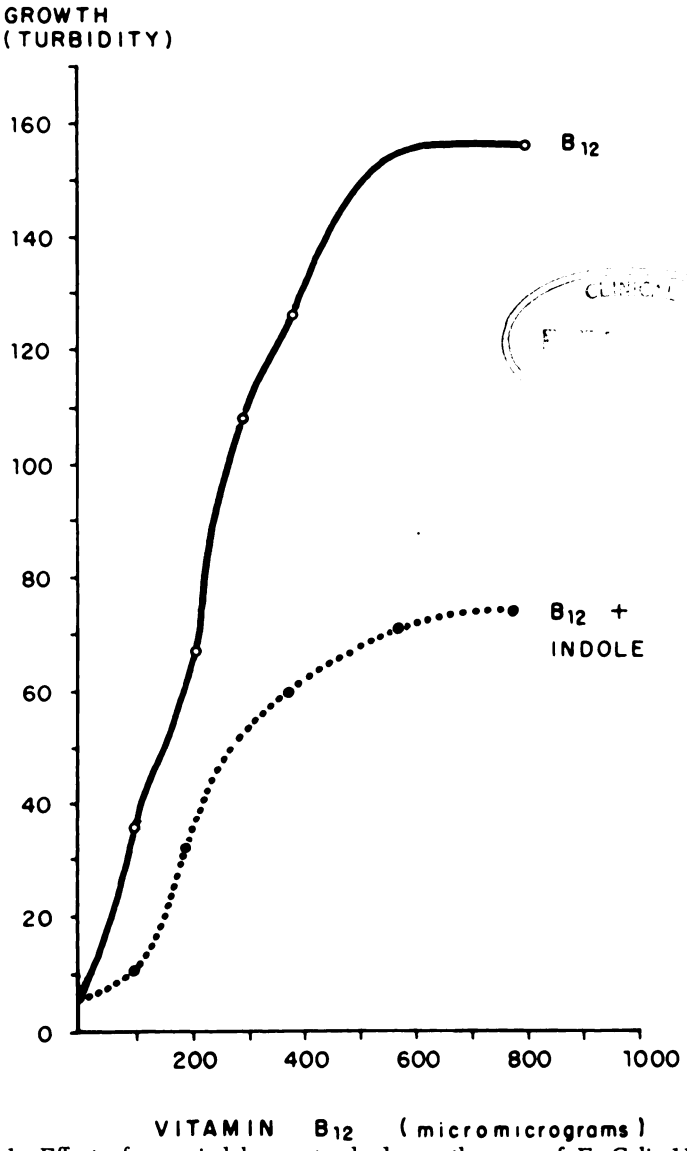

FIG. 1.—Effect of pure indole on standard growth-curve of E. Coli, 113–3.

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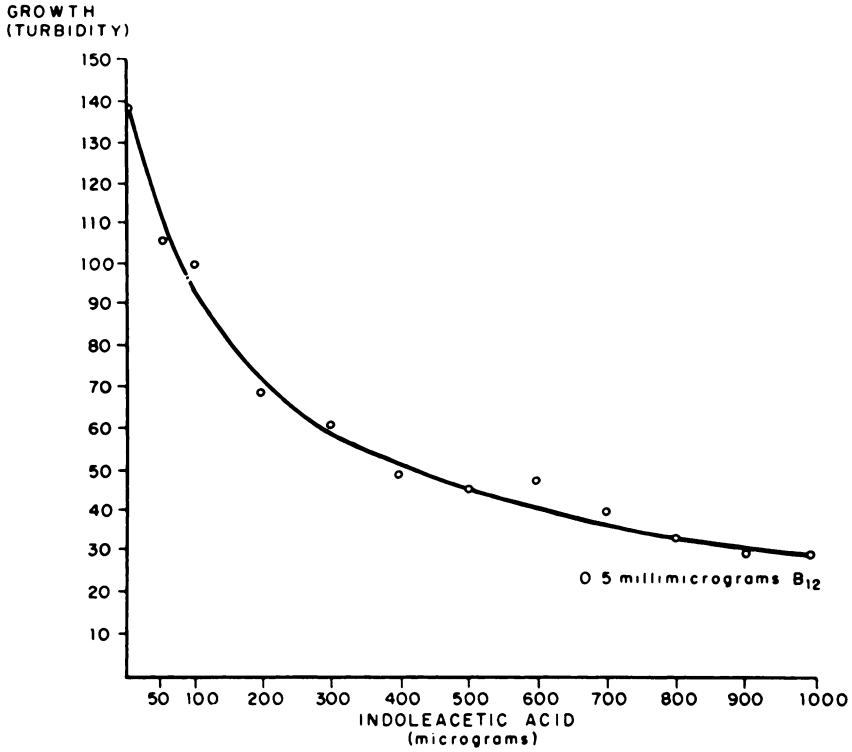

FIG. 2.—Effect of indoleacetic acid on maximum growth of E. Coli, 113–3.

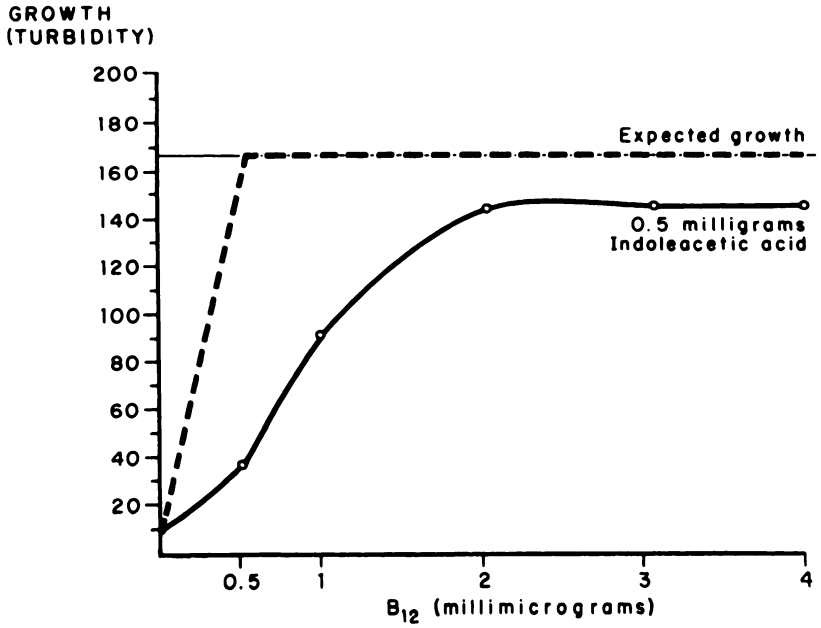

FIG. 3.—Effect of excess vitamin B_{12} on indoleacetic acid inhibition.

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also did not check the inhibitory effect. Autoclaving the indole compounds with the vitamin B_{12}, which usually frees vitamin B_{12} from binding complexes, or adding potassium cyanide which breaks peptide linkages with B_{12}, did not alter the inhibition. Incidentally, hydroxylamine, a known decarboxylase inhibitor, caused complete inhibition of the vitamin B_{12} dependent microorganism, but did not affect the growth of a non-B_{12} dependent organism.

Only a fresh preparation of indole was effective, whereas indoleacetic acid maintained its inhibitory powers for weeks. The failure of the 5-hydroxyindoleacetic acid to inhibit the growth of *E. coli* in the present experiment may be due in part to the lability of the indole compound to oxidation. On the other hand, two 5-benzyl serotonin-like compounds* in a concentration of 1 mg. per millimeter also inhibited the growth of the *E. coli* mutant by 90 per cent, but tryptamine hydrochloride failed to do so. Skatole in a similar concentration produced complete inhibition of growth. Concomitant studies revealed no significant change in pH to account for any of the results.

Discussion

The present study confirms the ability of several indole compounds to prevent the utilization of vitamin B_{12} by the test microorganism. That this is a specific effect on the vitamin B_{12} mechanism and not a general effect on microorganisms is established by the reversal of the inhibition with added B_{12}, and the inability of the indole compounds to affect the growth of several other *E. coli* microorganisms. Whether the indole compounds act by binding the vitamin, by blocking some enzymatic system, or by some other mechanism, is not known. Unsaturated indoles such as indole acrylic acid have been reported to inhibit the growth of *E. coli,* but this process is reversed by tryptophan.[6] Since tryptophan failed to affect the inhibition in the present study, a similar mechanism does not appear to be involved. Also, radioactive vitamin B_{12} dialysis studies, electrophoretic and chromatographic procedures failed to demonstrate the formation of a vitamin B_{12} indole complex. Since the inhibition of B_{12} utilization was reversed by excess B_{12}, the indole compounds would appear to act by a competitive inhibition of some enzymatic system necessary for the utilization of vitamin B_{12}. Saxena and Agarwala[7] have shown that phenylhydrazine and heavy metals like molybdenum and tungsten inhibit the *E. coli* mutant, and vitamin B_{12} reverses this inhibition. The particular enzymatic system affected is unknown. Perhaps a similar mechanism is involved in the present experiments.

Watson and Witts[8] attribute the macrocytic anemia associated with intestinal strictures and anastomoses to a change in the bacterial flora of the small intestine which interferes with the formation or utilization of hematopoietic material. Coliform microorganisms are known to produce indole; in the present experiment, indoles have been shown to inhibit the utilization of vitamin B_{12}, an essential hematopoietic material. Thus, an indole product of gastrointestinal microorganisms could conceivably be the toxic factor in the mega-

*5-benzyloxy-3-β-N-benzyl-N-methylaminoethyl indole hydrochloride and 5-benzyloxy-3-β-dimethylaminoethyl-indole hydrochloride.

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loblastic anemia of intestinal blind loops. Antibiotics prevent the formation of this factor and the development of anemia. Perhaps a similar compound in the human intestine, eliminated by sterilization of the gastrointestinal flora, might be the cause of the slight hematologic response to orally administered aureomycin noted by Lichtman, Ginsberg, and Watson[9] in several patients with pernicious anemia. Similarly, the anemia produced by Rhoads and associates,[1,2,10] with indole feeding in dogs, is likely due to interference with vitamin B_{12} utilization. The role of tryptophan and indole metabolism in normal absorption and utilization of vitamin B_{12} needs further elucidation.

Summary

Indole and indoleacetic acid are shown to inhibit the growth of a vitamin B_{12} dependent microorganism, a mutant strain of *Escherichia coli*. It is suggested that the mechanism of that inhibition may be a competitive inhibition of some enzymatic system necessary for the utilization of vitamin B_{12}.

Summario in Interlingua

Es monstrate que indol e acido indolo-acetic inhibi le crescentia de un microorganismo, un racia mutante de *Escherichia coli* que depende de vitamina B_{12}. Es formulate le these que le mechanismo de ille inhibition es possibilemente indirecte e consiste in le inhibition de un systema enzymatic que es indispensabile in le utilisation de vitamina B_{12}.

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