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Formation of Indoleacetic Acid by Intestinal Anaerobes

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Indoleacetic acid was produced from tryptophan by only three of 23 intestinal anaerobes studied. Evidence is presented to show that the formation of indoleacetic acid proceeds through the intermediate, indolepyruvic acid, via transamination with α-ketoglutarate rather than by the tryptamine pathway.

Indoleacetic acid, which was isolated from human urine, was identified as a plant growth factor (7). In addition, the elevation of indoleacetic acid in the urine has been associated with clinical conditions such as phenylketonuria (2) and various other syndromes (3). Indoleacetic acid can also induce pulmonary edema and emphysema (4) in cattle.

Rauschenbakh demonstrated that malignant tumors of the lymphoreticular system or leukemia were observed in mice given indoleacetic acid (8). Dunning and Curtis also showed that the addition of 1.0% indoleacetic acid to a synthetic diet containing 26% casein and 0.06% 2-acetylaminofluorene increased the incidence of bladder cancer in Fischer line female rats beyond the incidence obtained with the same ration without indoleacetic acid (6). Therefore, the formation of indoleacetic acid may be important in the etiology of cancer.

It has been reported that fecal bacteria produce indoleacetic acid (9), but the bacterial species responsible for this were not determined. In this study we have attempted to determine which of the intestinal anaerobes might be responsible for the production of indoleacetic acid and the pathway of its formation.

The conversion of tryptophan to indoleacetic acid might occur through one of two pathways (9) (Fig. 1): (i) the formation of indolepyruvic acid by transamination with α-ketoglutarate, followed by decarboxylation, or (ii) decarboxylation to tryptamine followed by deamination and oxidation of this amine, which would be inhibited by iproniazid, a monoamine oxidase inhibitor.

Bacterial cultures (10 ml) were grown in basal medium supplemented with 0.5% tryptophan and incubated at 37 C (5). One-milliliter samples were taken at 60 to 66 h, filtered through Acropor membranes (0.45-μm pore size), and then screened for the presence of indoleacetic acid by thin-layer chromatography (1). Of the 23 strains of intestinal anaerobes tested, including the genera of peptostreptococci, bifidobacteria, lactobacilli, eubacteria, fusobacteria, bacteroides, and citrobacter, only three, *Bacteroides fragilis* subsp. *thetaiotaomicron* (Virginia Polytechnic Institute [VPI] C20-6 and VPI C20-22) and *Citrobacter* sp. (VPI C20-36), showed production of indoleacetic acid ($\gtrsim$5 μg/ml) (Table 1) (5).

Further experiments were conducted to determine the pathway of indoleacetic acid formation by these anaerobes. Basal tryptophan-free casein hydrolysate-yeast extract medium (600 ml) containing 0.1% maltose and 0.5% tryptophan was prepared anaerobically according to a previously described method (5), inoculated with bacterial cultures or fresh rat fecal pellets (1 g wet weight), and incubated overnight at 37 C. The cultures were transferred to air-tight tubes under anaerobic conditions (under CO_2) and centrifuged at 20,000 × *g* for 15 min. The cells were recovered, washed with 80 ml of oxygen-free 0.1 M phosphate buffer at pH 6.0, and resuspended into 50 ml of the same buffer. Each reaction tube contained 6 ml of cell suspension and 4 ml of the phosphate buffer containing one of the following reagents or reaction mixtures: 100 mg of L-tryptophan; 100 mg of L-tryptophan and 50 mg of α-ketoglutarate; 100 mg of L-tryptophan, 50 mg of α-ketoglutarate, and 35 mg of iproniazid; 100 mg of L-tryptophan and 35 mg of iproniazid. A control tube contained 6 ml of cell suspension and 4 ml of buffer only. Each tube was incubated anaerobically (under CO_2) at 37 C for 5 h.

At the end of the incubation period, the cell suspensions were immediately centrifuged at 20,000 × *g* for 20 min, and the supernatants were used for the determination of indoleacetic acid and indolepyruvic acids by gas chromatography.

A 9.0-ml aliquot of each supernatant was

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transferred to a 30-ml screw-top test tube, and 1.0 ml of phosphate buffer was added. The combined sample was adjusted to pH 2.0 to 2.5 with 0.20 ml of 6 M phosphoric acid. After stirring, 10.0 ml of ethyl ether was added to the tube, which was quickly capped and shaken for 3 min. After separation of the phases, 8.0 ml of the ether layer was transferred to a 20-ml test tube and dried under a stream of nitrogen at 30 to 35 C.

After drying for approximately 30 min, trimethylsilylation of the residue was effected by adding 100 μl of *N,O*-bis-trimethylsilyl-acetamide (BSA) and 50 μl of pyridine to the tube. The tube was stoppered, vortex mixed for 5 s, and then held upright for 3 to 4 s. The sample was then transferred to a 1-dram (ca. 1.2-g) vial with a disposable pipette. After approximately 18 h at ~22 C in the dark, the samples were assayed for the indoleacetic and indolepyruvic trimethylsilyl derivatives.

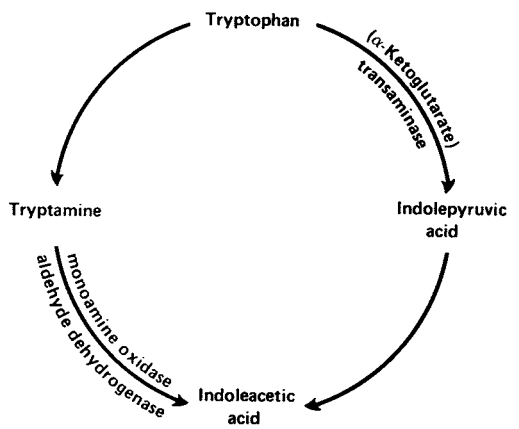

FIG. 1. *Pathways for the conversion of tryptophan to indoleacetic acid.*

The gas chromatography was performed using a Shimadzu model GC-4B MPF with a 2-m, 3-mm ID column and a packing of 1% SE30 on Gas Chrom Q (80 to 100 mesh). The column oven was programmed from 70 to 225 C at 10 C/min, and the injector and flame ionization dectector temperatures were set at 285 C. Helium was used as the carrier gas at a flow rate of 50 ml/min.

Identification of peaks in the sample was accomplished by co-injection of trimethylsilyl standards and examination of the enhancement patterns. When considered necessary for positive identification, co-injection was repeated using a 3% OV-17 column under similar chromatographic conditions. Quantitation of positively identified peaks was accomplished by measuring the area via triangulation. Standards were prepared by adding known amounts of indoleacetic acid and indolepyruvic acid in ether (0.1 mg/ml) to 10 ml of 0.1 M phosphate buffer, pH 6.0 (previously adjusted to pH 2.0 to 2.5 with phosphoric acid). The ether volume was made up to 10.0 ml, and the standards were then extracted and derivatized in the same manner as the sample. The retention times for the trimethylsilyl derivatives of indoleacetic and indolepyruvic acids were 12:40 and 17:05 min, respectively.

The results of experiments are shown in Table 1; α-ketoglutarate stimulated the formation of indoleacetic and indolepyruvic acids (B versus A) in pure or mixed fecal cultures, and ipronia-zid, the monoamine oxidase inhibitor, did not

TABLE 1. *Indoleacetic acid (IAA) and indolepyruvic acid (IPVA) formation by intestinal anaerobes*

Reaction tubes	Compounds added (mg)	Products (μg/mg [dry weight])[a]							
		B. fragilis (C20-6)		subsp. *thetaiotaomicron* (C20-22)		*Citrobacter* sp. (C20-36)		Rat feces	
		IAA	IPVA	IAA	IPVA	IAA	IPVA		
A	L-Tryptophan (100)	0.33	ND[b]	0.98	ND	1.43	3.92	0.52	ND
B	L-Tryptophan (100) + α-ketoglutarate (50)	1.08	ND	1.96	1.86	1.60	9.46	2.72	4.78
C	L-Tryptophan (100) + α-ketoglutarate (50) + iproniazid (35)	1.08	ND	2.65	ND	2.50	21.8	2.78	5.76
D	L-Tryptophan (100) + iproniazid (35)	0.33	ND	1.18	ND	1.25	4.82	0.58	ND

[a] Dried weight of cells: C20-6, 1.2 mg/ml; C20-22, 1.02 mg/ml; C20-36, 0.56 mg/ml; rat feces, 4.6 mg/ml.
[b] ND, Not detectable (less than 0.15 μg/ml).

inhibit the indoleacetic acid formation from tryptophan (A versus D; B versus C). Pure or mixed fecal cultures did not synthesize indoleacetic acid from tryptamine or tryptamine with iproniazid. Our results indicate that the microorganisms used in this study transaminate tryptophan with α-ketoglutarate to indolepyruvic acid, and the latter compound is decarboxylated to form indoleacetic acid. Such a pathway may be important in the biosynthesis of indoleacetic acid in the large bowel and in the etiology of colon cancer.

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