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Indoleacetic Acid and Growth of Bacteria with Varying Requirements for Nicotinic Acid and Tryptophane.

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An antagonism between indole-3-acetic acid, and nicotinic acid and/or tryptophane, was suggested by the data of Kodicek, Carpenter, and Harris[1] as a possible explanation for the role of corn in the etiology of pellagra. It was thought of interest to study the relationship between these compounds in certain bacteria whose nutritional requirements are relatively well known, and which can be grown in chemically defined media.

The following organisms were selected (numbers in parentheses refer to American Type Culture Collection listings): Requiring both nicotinic acid and tryptophane: *Lactobacillus casei* (7469), *Streptococococcus faecalis* R (8043), and *Lactobacillus arabinosus* 17-5 (8014). Shankman *et al.*, state[2] that the nicotinic acid requirement of the latter organism is abolished upon long incubation. Requiring nicotinic acid alone: *Staphylococcus aureus*; requiring neither: *Escherichia coli* and *Aerobacter aerogenes*.

After this work was initiated, Dubos[3] reported that indoleacetic acid in concentrations of 0.01 to 0.10 mg/ml inhibited *Mycobacterium tuberculosis* (Human strain), certain streptococci, and *Shigella paradysenteria* (Sonne) grown in enzymatic casein hydrolyzate-yeast extract medium at pH 6.0. This inhibition was reported to be relieved by concentrations of added tryptophane ten times that of the inhibitor.

Experimental. The lactic acid bacteria and *S. aureus* were grown on either the amino acid medium of Stokes *et al.*,[4] with sodium citrate substituted for sodium acetate, or, in most cases, on the same medium with acid-hydrolyzed casein substituted for the amino acid medium. These variations produced no significant alteration in results. *E. coli* and *Aerobacter aerogenes* were grown on MacLeod's[5] medium of glucose, ammonium sulfate, asparagine, and salts. Following the appearance of Dubos' report, a non-pathogenic strain of Mycobacterium tuberculosis (American Type Culture Collection No. 607) was tested, using Long's synthetic medium.

In all cases, indoleacetic acid, nicotinic acid, and tryptophane were adjusted to the pH of the respective media, and added as required. The medium was autoclaved following these additions. Except in the case of *M. tuberculosis*, where a small fragment of mycelium was employed for inoculation, the inoculum consisted of one drop of a saline suspension of organisms which had been washed with saline and then suspended in 2.5 times the original culture volume.

For lactic acid bacteria, growth was measured after 48 to 72 hours incubation at 37°C by titration with 0.1 N NaOH to pH 6.8 on a Berkman pH meter. Growth of *M. tuberculosis* was measured, after either 3 or 6 days incubation, by filtering, washing, drying, and weighing the mycelium. Growth of the other organisms was measured as turbidity (expressed as optical density) in the Evelyn Photoelectric Colorimeter after 24 to 48 hours incubation. In none of these cases did variations in incubation time significantly alter results.

Results. The results of these experiments are indicated in Table I. Each indicated value

1 Kodicek, E., Carpenter, K. J., and Harris, L. J., *The Lancet*, 1946, **251**, 491.

2 Shankman, S., Camien, M. N., Block, H., Merrifield, R. B., and Dunn, M. S., *J. Biol. Chem.*, 1947, **168**, 23.

3 Dubos, R. J., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 317.

4 Stokes, J. L., Gunness, M., Dwyer, I. M., and Caswell, M. C., *J. Biol. Chem.*, 1945, **160**, 35.

5 MacLeod, C. M., *J. Exp. Med.*, 1940, **72**, 217.

TABLE I.
Effect of Indoleacetic Acid, Nicotinic Acid, and Tryptophane upon Certain Bacteria.

Organism	Exp. No.	IAc (mg/ml)	Growth with indicated concentrations of NA and T (mg/ml) T=0.3 NA=0.01	T=0.3 NA=10	T=30 NA=0.01	T=30 NA=10
Streptococcus fæcalis R	1	0	3.8	5.5	3.8	5.4
Growth measured as ml of		0.1	3.4	5.3	3.4	5.2
0.1 N NaOH		1.0	2.5	—	2.5	3.8
	2	0	4.8	5.2	3.3	5.5
		0.1	4.4	5.0	3.4	4.9
		1.0	3.6	—	—	4.7
	3	0	2.7	3.0	3.5	7.8
		0.1	2.5	2.8	3.3	5.7
		1.0	2.4	3.1	3.1	5.6
	4	0	2.4	2.9	3.5	7.0
		0.1	1.7	2.6	3.4	6.4
		1.0	2.0	2.0	2.7	4.9
Lactobacillus arabinosus 17-5 (8014)	1	0	2.2	2.7	6.0	8.2
Growth measured as ml of		0.1	2.6	3.2	5.8	8.4
0.1 N NaOH		1.0	2.7	5.1	4.2	3.1
	2	0	2.0	2.9	3.2	9.9
		0.1	1.8	3.0	3.2	10.1
		1.0	2.4	2.8	1.4	4.4
Lactobacillus casei (7469)	1	0	2.9	2.9	6.6	—
Growth measured as ml of		0.1	3.1	3.3	6.3	7.8
0.1 N NaOH		1.0	—	4.7	4.0	6.1
	2	0	2.6	3.8	5.1	7.3
		0.1	3.1	3.8	3.0	7.5
		1.0	2.8	—	1.0	3.5
Staphylococcus aureus	1	0	0.31	0.30	—	0.31
Growth measured as optical density		0.1	0.35	0.39	—	0.41
		1.0	0.31	—	—	0.33
	2	0	0.28	0.36	0.33	0.42
		0.1	0.34	0.37	0.32	0.40
		1.0	0.30	0.39	0.32	0.43
Escherichia coli	1	0	0.18	0.17	0.15	0.17
Growth measured as optical density		0.1	0.18	0.17	0.17	0.17
		1.0	0.13	0.10	0.11	0.12
Aerobacter aerogenes	1	0	0.52	0.52	—	0.51
Growth measured as optical density		0.1	0.40	0.53	—	0.50
		1.0	0.13	0.14	—	0.14
	2	0	0.18	0.16	—	0.23
		0.1	0.17	0.14	—	0.17
		1.0	0.10	0.11	—	0.10

IAc = Indole-3-acetic acid.
T = *l*-tryptophane.
NA = Nicotinic acid.

for growth is based upon duplicate measurements.

In addition to the data given in Table I, *L. arabinosus* was grown on the basal medium supplemented with 1.0 mg/ml of nicotinic acid, and 1.0 mg/ml of indoleacetic acid. Varying amounts of tryptophane, from 1 γ to 1 mg/ml, were added, with no relief of inhibition.

In the case of *M. tuberculosis*, the results shown in Table II were obtained after 6 days incubation. Similar results, at lower levels of growth, obtained after 3 days incubation.

Discussion. From the results of these experiments, it can be seen that although indoleacetic acid may act as an inhibitor at relatively high concentrations (1 mg/ml), and, sometimes, to a smaller extent, at concentrations of 0.1 mg/ml, this inhibition appears to bear

TABLE II.
Effect of Indoleacetic Acid on Growth of *M. tuberculosis.*

IAc mg/ml	T mg/ml	NA mg/ml	Growth mg dried myceliun
0	0	0	.167
0	0	2	.144
0	2	0	.157
.2	0	0	.151
.2	0	2	.167
.2	2	0	.137

no relationship to the nicotinic acid or tryptophane requirements of the organism, and is not relieved, in the organisms studied, by either of the latter two substances. In fact, the organism most strongly inhibited by indoleacetic acid, *Aerobacter aerogenes,* requires neither tryptophane nor nicotinic acid for growth. It is interesting to note that *E. coli,* for which stimulation by indoleacetic acid at levels up to one γ/ml in a medium containing 0.1 mg/ml of tryptophane has been reported,[6] was unaffected by the lower concentration of indoleacetic acid used in this study, and was inhibited by the higher concentration. *S. aureus* showed no inhibition at the highest concentration of indoleacetic acid used.

In certain cases, particularly *L. arabinosus* and *L. casei,* a stimulatory effect at 0.1 mg/ml, and sometimes at 1 mg/ml, levels of indoleacetic acid was observed when the concentration of tryptophane was limiting; this stimulation disappeared at adequate levels of tryptophane regardless of nicotinic acid content. The latter organisms are not specific in their response to tryptophane,[7,8] since indole and anthranilic acid have been reported to have tryptophane activity. However, indoleacetic acid was stated to be inactive. It should be noted, however, that the latter studies[7,8] measured the response of these organisms to tryptophane derivatives in media completely devoid of tryptophane; it is possible that indoleacetic acid may stimulate only in the presence of small amounts of the amino acid.

It is realized that Dubos obtained relief of inhibition at concentrations of added tryptophane 10 times that of inhibitor, and that this ratio was not used in the present experiments. Further, Dubos used a basal medium already containing tryptophane in the enzymatic casein hydrolyzate and yeast extract. Because of these facts, our results and those of Dubos are not strictly comparable. Since our inhibition was generally obtained with one mg/ml of indoleacetic acid, using Dubos' ratio would not only have led to difficulties due to solubility, but would have produced such a high concentration of tryptophane as to make interpretation of results difficult. If a true metabolite-antagonist relationship exists between indoleacetic acid and nicotinic acid-tryptophane, it is a relatively unusual phenomenon that far greater concentrations of metabolite than of the antagonist are required to relieve inhibition. This fact, as well as the lack of relationship between nicotinic acid and tryptophane requirements of organisms and their susceptibility to indoleacetic acid inhibition, tend to detract from the probability of a true metabolite-antagonist relationship. A recent report by Krehl, Carvalho, and Cowgill,[9] failing to confirm the results of Kodicek *et al.*,[1] should be noted. It would appear that a metabolite-antagonist relationship between indoleacetic acid and nicotinic acid or tryptophane is not an invariably occurring biological phenomenon, nor is there any evidence indicating the mechanism of this reaction where it does occur.

Summary and Conclusions. 1. A number of organisms whose tryptophane and nicotinic acid requirements are known were tested for possible inhibition by indoleacetic acid and reversal by nicotinic acid and tryptophane in chemically defined media.

2. *Escherichia coli, Lactobacillus arabinosus* 17-5, *L. casei, Streptococcus faecalis R,* and *Aerobacter aerogenes* were inhibited by concentrations of indoleacetic acid of one mg/ml Occasionally slight inhibitions were noted at 0.1 mg/ml levels.

6 Ball, E., *J. Bact.,* 1938, **36**, 559.

7 Snell, E. E., *Arch. Biochem.,* 1943, **2**, 389.

8 Greene, R. D., and Black, A., *J. Biol. Chem.,* 1944, **155**, 1.

9 Krehl, W. A., Carvalho, A., and Cowgill, G. R., *Fed. Proc.,* 1947, **6**, 413.

3. No significant relief of inhibition was obtained with either nicotinic acid or tryptophane.

4. *Staphylococcus aureus* and *Mycobacterium tuberculosis* (607) were not inhibited by indoleacetic acid at the concentrations studied.

5. Fairly consistent stimulation at 0.1 mg/-ml levels of indoleacetic acid were obtained with *L. arabinosus* and *L. casei* in the presence of limiting concentrations of tryptophane.

6. The significance of these results in relation to possible metabolite-antagonist relationship between indoleacetic acid and tryptophane or nicotinic acid is discussed.

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Treatment of Experimental Intestinal Trichinosis with 1-Diethylcarbamyl-4-Methylpiperazine Hydrochloride (Hetrazan*).

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The drug 1-Diethylcarbamyl-4-methylpiperazine Hydrochloride (Hetrazan), when administered orally, causes the rapid disappearance of the microfilariae of *Wuchereria bancrofti* from the blood of infected individuals.[1] The marked effect of this drug on the circulating forms of *W. bancrofti* suggested its use in other parasitic infections in which treatment should be directed against such migrating forms. In view of the need for a drug in trichinosis which would destroy the migrating larvae as well as the intestinal forms, Hetrazan was tested on white rats infected with *Trichinella spiralis*. The effect of this drug on the intestinal trichinae is reported below.

Methods and Results. White rats weighing from 150 to 175 g were fed by stomach tube with 1,000 to 1,300 infective trichinae larvae. The larvae were obtained from rat muscle digested in a pepsin-hydrochloric acid mixture. Twenty-four hours after feeding the animals were started on Hetrazan. The drug was given on the basis of 200 mg per kg weight 3 times daily by stomach tube, for periods of 5 to 10 days. For the recovery of adult trichinae the treated animals and suitable controls were killed 24 hours after the last dose of the drug was administered. The small and large intestine were removed, slit open and cut into small pieces about 1 cm long. The sliced intestine was put in a Baermann apparatus with normal salt solution heated to 37°C. The adult worms settled rapidly to the tip of the funnel and were removed one hour after the apparatus was set up. The number of worms recovered from the intestines was determined directly by counting.

For the recovery of the muscle stages the rats were killed on or about the 30th day after feeding infective larvae. The rat muscles were ground and digested in a pepsin-hydrochloric acid mixture. The larvae were recovered from the mixture and their number estimated by counting aliquot portions of the total suspension.

The average number of adult worms recovered from the rats treated with Hetrazan was considerably less than the number recovered from the untreated animals (Table I). Thus the average number of worms

* The drug was supplied by the Lederle Laboratories Division, American Cyanamid Co., Pearl River, N.Y.

1 Santiago, D., Oliver-González, J., and Hewitt, R. I., in press.