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Inhibition of Growth of Microorganisms by Benzimidazoles.* (24362)

HELEN B. FUNK† AND HELENE A. NATHAN (Introduced by Phoebe J. Crittenden)
Department of Botany, Barnard College, Columbia University and Haskins Laboratories, N. Y. City

In microorganisms utilization of adenine or guanine is competitively antagonized by benzimidazole(1). The nucleotide moiety of Vit. B_{12} contains 5,6-dimethyl benzimidazole, and analogs of B_{12} may contain other benzimidazoles, purines, or triazoles(2). Certain substituted benzimidazoles inhibit growth of bacteria that require Vit. B_{12}, *e.g., Lactobacillus lactis*(3), *L. leichmannii,* and mutants of *Escherichia coli*(4). Indeed, inhibition of a mutant of *E. coli* by 4-nitrobenzimidazole or mercaptobenzimidazole can be reversed by Vit. B_{12}(4). The relation between benzimidazole inhibition and B_{12} metabolism should be studied with organisms whose specificity toward B_{12} is more rigid than that of these bacteria in which the B_{12} requirement can be by-passed with desoxyribosides or methionine. This paper is concerned with benzimidazole inhibition of growth of 2 algae which have the more specific B_{12} requirements and also of some gram negative bacteria that synthesize B_{12}.

Methods. For *Euglena gracilis,* strain Z, the media used for maintenance, vitamin depletion, and experimental studies were those used by Hutner *et al.*(5) for assay of B_{12}. *Ochromonas malhamensis* was maintained in the medium of Hutner *et al.*(6) and grown for experiments in a B_{12} assay medium(6). Algal cultures were incubated in the light at 25-26°C. *Rhizobium* species were maintained on yeast extract mannitol agar [medium 79 in (7)] in which Difco yeast extract, 0.5%, replaced yeast water; experimental cultures were grown aerobically at 30°C in mannitol, 1.0; Difco yeast extract, 0.5; K_2HPO_4, 0.05; $MgSO_4 \cdot 7H_2O$, 0.02; NaCl, 0.01; $CaCl_2 \cdot 2H_2O$, 0.0025; $FeCl_3 \cdot 6H_2O$, 0.001 (all g/100 ml medium). The 2-ethyl-5-methyl benzimidazole,‡ 5-methyl benzimidazole and benzimidazole were dissolved in absolute ethanol or acetone and added to media before autoclaving. Growth of cultures is expressed as optical density.

Results. Benzimidazole at 5×10^{-3}M concentration inhibited growth of *E. gracilis* (Fig 1) and *O. malhamensis* (Table I), both of which require B_{12}, and of 4 *Rhizobium* species (Table I), all of which synthesize B_{12} (8,9). This inhibition may, of course, be explained as due in part to interference with the utilization of purines though the results of Scott *et al.*(4) with *Escherichia coli* mutants imply that the purine site may be only of secondary importance.

The effects of 2-ethyl-5-methyl benzimidazole on growth of *E. gracilis* (Fig. 2) and *O. malhamensis* (Table I) appeared to be the same as those of benzimidazole. However, the rhizobia were more sensitive to 2-ethyl-5-methyl benzimidazole; they were inhibited by this drug at concentrations similar to those that inhibit synthesis of heme (Table I). The sensitivity of these bacteria to this drug is of interest because(11) the rhizobia are involved somehow in production of leghemoglobin in the root nodules of leguminous plants. *O. malhamensis* was more sensitive to a third compound, 5-methyl benzimidazole, than to either benzimidazole or the 2-ethyl-5-methyl derivative (Table I).

E. gracilis can utilize as a substitute for Vit.

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† Present address: Dept. of Biological Sciences, Goucher College, Baltimore, Md.

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TABLE I. Inhibitory Concentrations of Benzimidazoles in Some Biological Systems.

Compound	System	Conc. of compound ($\times 10^{-3}$M)	% inhibition of system	Ref.
Benzimidazole	Growth			
	Euglena gracilis	5	100	
	Ochromonas malhamensis	5	"	
	Rhizobium meliloti	5	"	
	" *trifolii*	5	"	
	" *leguminosarum*	5	"	
	" *japonicum*	5	"	
	Escherichia coli	3*	50	14
	Streptococcus lactis R	6*	"	14
	Saccharomyces cerevisiae	5*	100	14
	Endomyces vernalis	2.5*	50	14
	Vaccinia virus	8.5*	†	15
	Poliomyelitis virus	4.2*	†	16
	Influenza virus	3.5	75	17
	Heme synthesis			
	Avian red blood cells	8.5	100	10
2-ethyl-5-methyl benzimidazole	Growth			
	Euglena gracilis	5	100	
	Ochromonas malhamensis	5	"	
	Rhizobium meliloti	1	"	
	" *trifolii*	1	"	
	" *leguminosarum*	.5	"	
	" *japonicum*	.5	"	
	Influenza virus	.18	75	17
	Heme synthesis			
	Avian red blood cells	.6–.9	100	10
5-methyl benzimidazole	Growth			
	Ochromonas malhamensis	.45	100	

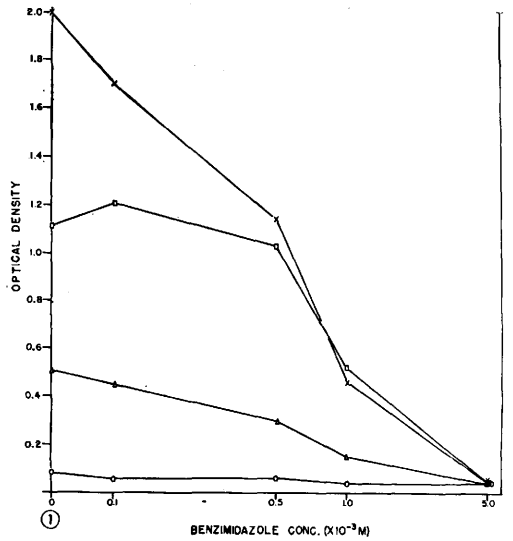
* Calculated from data given in references cited.
† The % inhibition is not clear from the data given in the reference.

B_{12} a variety of B_{12} analogs which differ with respect to the benzimidazole moiety(12), but benzimidazoles alone are toxic (Fig. 2). *O. malhamensis* utilizes, as do chicks, B_{12} analogs in which benzimidazole or 5-methyl benzimidazole constitute the benzimidazole moiety(12), but, here too, these benzimidazoles by themselves are toxic (Table I). Neither *O. malhamensis* (Table II) nor *E. gracilis* was able to substitute factor B plus benzimidazole or 5-methyl benzimidazole for Vit. B_{12}: the organisms were not able to manufacture a B_{12} analog which they could utilize even if given all the parts.

Apparently a benzimidazole can attack a site which can be influenced by Vit. B_{12}. Evidence for such a relationship was obtained in growth experiments with *E. gracilis* in which concentrations of Vit. B_{12} and benzimidazole were varied. Inhibition by benzimidazole was not competitive (see *e.g.* Fig. 1). Rather, when the ratio of inhibitor: vitamin was 10^8: 1 there was a relation of growth to amount of vitamin in the medium which could be expressed as a straight line (Fig. 3). This relation maintained only when the amount of the vitamin in the medium was limiting and when the growth response to the vitamin was linear.

The similarity of inhibitory levels of the benzimidazoles in a variety of biological systems (Table I) points to some basic sites common to these diverse systems. Abbott and Dodson(13) suggested that nucleic acid or nucleoprotein is involved. That the mechanisms are complex is apparent from the relations expressed in Fig. 3.

Summary. Benzimidazole and 2-ethyl-5-methyl benzimidazole inhibited the growth of 2 B_{12}-requiring algae, *Euglena gracilis*, strain Z, and *Ochromonas malhamensis*, and of 4 B_{12}-synthesizing bacteria, *Rhizobium meli-*



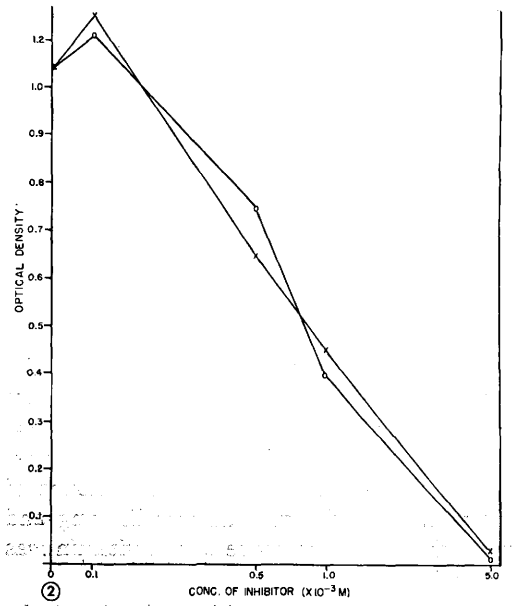


FIG. 1. Inhibition of growth of *Euglena gracilis* by benzimidazole in the presence of the following concentrations of vit. B_{12}: ×—×, 1×10^{-10} M; □—□, 1×10^{-11} M; △—△, 5×10^{-12} M; ○—○, 1×10^{-12} M.

FIG. 2. Inhibition of growth of *E. gracilis* by 2 benzimidazoles in presence of 7.4×10^{-11} M vit. B_{12} (100 mμg/l). ○—○, benzimidazole; ×—×, 2-ethyl-5-methyl benzimidazole.

loti, R. trifolii, R. leguminosarum, and *R. japonicum.* Benzimidazole inhibition was complete at 5 x 10^{-3}M concentration. The 2-ethyl-5-methyl compound inhibited the algae at 5 x 10^{-3}M concentration; rhizobia were more sensitive. Although benzimidazole inhibition of *E. gracilis* was not competitive, there was, at a 10^8:1 ratio of inhibitor to vitamin, a relation to amount of growth which could be expressed graphically as a straight line.

TABLE II. Inability of Factor B plus Benzimidazoles to Satisfy the B_{12} Requirement of *Ochromonas malhamensis.*

		No additions	B_{12}, 10.0 mμg %
Factor B (1.0 mg %)			
No additions		.09*	1.07
Benzimidazole	.1 μg %	.09	1.76
	1.0 "	.12	1.28
	10.0 "	.11	1.22
	100.0 "	.10	1.42
	1.0 mg %	.09	1.43
5-methyl benzimidazole	.1 μg %	.08	1.01
	1.0 "	"	.84
	10.0 "	"	.92
	100.0 "	"	1.00
	1.0 mg %	"	1.16

* Growth expressed as optical density units.

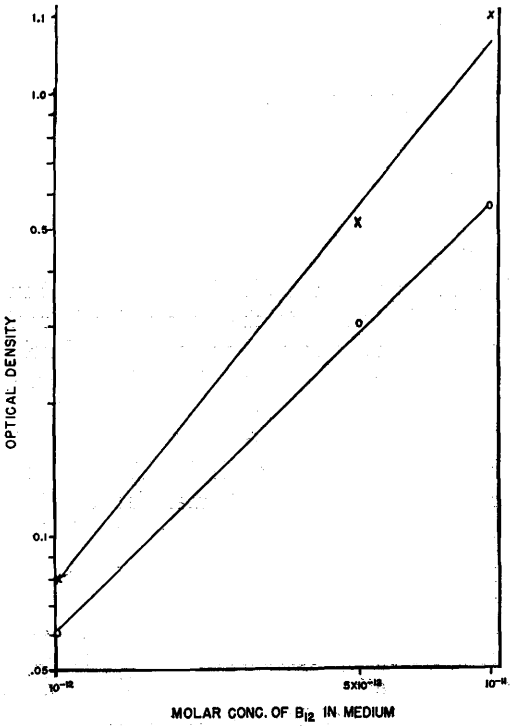


FIG. 3. Growth response of *Euglena gracilis* to vit. B_{12} (×—×) and when the ratio of benzimidazole to vit. B_{12} is 10^8:1 (○—○).

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Thyroid-Specific Autoantibodies Studied by Passive Cutaneous Anaphylaxis of Guinea Pig.* (24363)

ZOLTAN OVARY, HARRY RANDALL,† ERNEST WITEBSKY, NOEL R. ROSE, SIDNEY SHULMAN, AND RICHARD METZGAR‡

Dept. of Microbiology, Johns Hopkins University and of Bacteriology and Immunology, University of Buffalo School of Medicine

Thyroid-specific autoantibodies in animals have been demonstrated serologically by tanned cell hemagglutination, complement fixation, and precipitation technics(1,2,3.) Subsequently, circulating thyroid-specific autoantibodies in sera of patients suffering from chronic thyroiditis have been demonstrated by tanned cell hemagglutination(3,4) and precipitation(4,5,6) technics. Additional discussions have appeared(7,8,9,10). The present study reports detection and specificity of thyroid-specific autoantibodies by the method of passive cutaneous anaphylaxis of the guinea pig (PCA).

Materials and methods. Antisera. The following sera were studied by the PCA technic: rabbit anti-rabbit thyroid, guinea pig anti-guinea pig thyroid, and sera from patients with chronic thyroiditis. All these sera contained circulating antibody of high titer against the homologous antigen, as demonstrated by tanned cell hemagglutination. The procedure for animal immunization has been described(1). *Antigens.* Crude saline extracts were prepared by procedures previously described(11,12). The extracted organs (and extract concentrations in mg N/ml) included rabbit thyroid (2.5),§ guinea pig thyroid (0.92),§ human thyroid (7.8),§ beef thyroid (12.1), hog thyroid (10.4), human pancreas (3.6), human kidney (4.8), and beef muscle (1.7) Partially purified by human thyroid material (5.0 mg N/ml) was prepared by precipitation with sodium sulfate in a procedure similar to that of Heidelberger and Palmer(13), but omitting the preliminary acidification. This material was used only as

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† Research Fellow of P.H.S.

‡ Public Health Service Research Fellow, Nat. Cancer Inst.

§ Based on determination of protein by light absorption and an arbitrary conversion factor for nitrogen of 6.0.