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Inhibition of *in vitro* Heme Synthesis from N^{15}-Glycine by 2-Ethyl-5-methylbenzimidazole.*† (21138)

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Avian erythrocytes incorporate N^{15} into heme when incubated with N^{15}-glycine and provide an *in vitro* biological system for the study of heme synthesis(1). Because of the chemical relationships of methyl-substituted benzimidazoles to the benzimidazole moiety of vit. B_{12} and to the purines, we investigated the effect of their addition to this system(2).

2,5-Dimethylbenzimidazole previously had been noted to act as a growth depressant when fed to rats in tests of compounds for vit. B_{12}-like activity(3). It inhibited red blood cell formation in mice made anemic by phenylhydrazine injection(4) and inhibited influenza virus multiplication(5).

We found that 2,5-dimethylbenzimidazole and 5,6-dimethylbenzimidazole prevented incorporation of N^{15} from N^{15}-glycine into heme. Benzimidazole and 2-methylbenzimidazole at the same concentration had slight but not comparable activity. 5-Methylbenzimidazole had an inhibitory effect intermediate between that of benzimidazole and 2,5-dimethylbenzimidazole(2).

These compounds, in approximately the same concentration and in precisely the same relative order of inhibitory activity, were found by Tamm *et al.*(6) to inhibit multiplication of influenza A or B virus. The striking coincidence of relative inhibitory activities of the methyl-substituted benzimidazoles for 2 widely different systems, *in vitro* hemoglobin synthesis by avian erythrocytes and virus duplication, indicated to us that some fundamental mechanism underlying both processes might be involved.

Tamm *et al.*(6) found 2-ethyl-5-methylbenzimidazole to be a much more potent antiviral agent than any of the benzimidazoles studied by us in the avian erythrocyte system(2). It was considered to be approximately 7 times more effective than 2,5-dimethylbenzimidazole and 19 times more so than benzimidazole. It is thus of importance to know whether 2-ethyl-5-methylbenzimidazole likewise is a more effective inhibitor than 2,5-dimethylbenzimidazole for the synthesis of heme by the avian erythrocyte. This has been determined to be so in the present study and supplies additional support to the concept that the same fundamental mechanism underlies both processes.

Experimental. Fresh chicken blood was obtained for each experiment. Penicillin and streptomycin were added to inhibit microbial action during incubation(1). Twenty ml blood samples were incubated with the experimental compound and 25 mg N^{15}-glycine (31 atom % N^{15}) in 50 ml Erlenmeyer flasks with constant shaking for 24 hours in a water bath at 37°C. In each experiment the effect on

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TABLE I. Effect of 2-Ethyl-5-methylbenzimidazole and 2,5-Dimethylbenzimidazole on Incorporation of N^{15} from N^{15}-Glycine into Heme by Erythrocytes of Chicken Blood during *In Vitro* Incubation.

Exp.	N^{15} conc. in hemin* Control	Test		Supplement†
	Atom % excess		Molar conc.	
1	.066	.054	.0017	5 mg 2,5-dimethylbenzimidazole
		.022	.0016	5 " 2-ethyl-5-methylbenzimidazole
2	.080	.034	.0034	10 " 2,5-dimethylbenzimidazole
		.003	.0031	10 " 2-ethyl-5-methylbenzimidazole
3	.081	.008	.0068	20 " 2,5-dimethylbenzimidazole
		.051	.0009	3 " 2-ethyl-5-methylbenzimidazole
4	.078	.046	.0006	2 " 2-ethyl-5-methylbenzimidazole
		.042	.0085	20 " benzimidazole

* Hemin isolated from chicken blood has slightly increased N^{15} concentration over tank nitrogen similar to that of hemin from normal dog and human blood(9). Values on the order of .002 to .006 atom % excess thus indicate complete inhibition of N^{15} incorporation into heme.

† These were prepared by refluxing the appropriate diamine and acid according to the general procedure of Wagner and Millett(10). The compounds were recrystallized several times, and melting points and nitrogen analyses were checked before use. Benzimidazole and 2,5-dimethylbenzimidazole have been described previously. 2-Ethyl-5-methylbenzimidazole melted at 168°-169°C, and gave the following nitrogen analyses: 17.5%, 17.4%, 17.4% (theoretical 17.5%).

heme synthesis of the substance studied was determined by comparison with a control sample of the same blood incubated with N^{15}-glycine alone at the same time under identical conditions. Three samples of the same blood were incubated at the same time, 2 test samples with one control.

Hemin was isolated as described by Shemin and Rittenberg(7) and recrystallized according to Fischer(8). The N^{15} atom percent excess over tank nitrogen was determined on the nitrogen gas from duplicate samples of hemin isolated from each incubated blood. Previous experiments had demonstrated that the same blood incubated with N^{15}-glycine under identical conditions yielded hemin with the same N^{15}-content(2).

Results. Table I shows that 2-ethyl-5-methylbenzimidazole was considerably more inhibitory than 2,5-dimethylbenzimidazole. Thus 5 mg (.0017 M), which produced minimal (18%) inhibition with 2,5-dimethylbenzimidazole, gave marked (67%) inhibition with the 2-ethyl-5-methyl derivative (Exp. 1). Ten mg 2-ethyl-5-methylbenzimidazole (.003 M) gave complete inhibition, whereas this required 20 mg 2,5-dimethylbenzimidazole (Exp. 2 and 3). Two mg 2-ethyl-5-methylbenzimidazole (.0006 M) produced approximately the same degree of inhibition as 20 mg benzimidazole (.0085 M). Thus, in Exp. 4, .0006 M 2-ethyl-5-methylbenzimidazole was as effective as 14 times the concentration of benzimidazole, and at the .0016 M level the percentage decrease in N^{15} uptake from the control experiment was about 4 times greater with the 2-ethyl-5-methyl derivative than with 2,5-dimethylbenzimidazole. Complete inhibition occurred with half the amount required with 2,5-dimethylbenzimidazole (Exp. 2).

Discussion. Demonstration that 2-ethyl-5-methylbenzimidazole is considerably more inhibitory than 2,5-dimethylbenzimidazole for synthesis of heme by chicken erythrocytes incubated *in vitro,* similar to findings of Tamm *et al.*(6) in studies on the inhibition of influenza virus multiplication, adds further support to our belief that a fundamental mechanism underlying both processes is involved. This might be the result of selective inhibition of a basic step in the mechanisms whereby nucleic acid (or nucleoprotein) plays some very important role in biosynthesis. It is of interest to note that the 5 (or 6) position of benzimidazole is analogous to the 2 position of the purines. Recent discovery of 2-methyladenine in biological material(11) brings attention for the first time to the existence of analogous methyl-substituted purines in nature.

Summary. 2-Ethyl-5-methylbenzimidazole was found to be considerably more inhibitory than 2,5-dimethylbenzimidazole, or benzimidazole, for the synthesis of heme by chicken erythrocytes incubated *in vitro*. This was in the same relative order of inhibitory activities as was noted for multiplication of influenza A or B virus, and is considered to add further support to the concept that the same basic mechanism, possibly involving a fundamental role of nucleic acid in biosynthesis, underlies both processes.

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Progesterone-Like Activity in the Plasma of Ovoviviparous Snakes.*† (21139)

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Studies on the natural occurrence of progestin have been carried out primarily on mammals. This hormone has been found in a large number of mammalian species and seems to be ubiquitous in this class. The available evidence indicates that the hormone is produced in the mammal by the corpus luteum (and to a lesser extent, the pre-ovulatory follicle), the placenta, the adrenal cortex and possibly the testis. Fraps, Hooker and Forbes(1,2) reported that substances with progesterone-like activity were found in the plasma of ovulating hens, non-ovulating hens, and cocks, but not in the plasma of capons. Porto(3) reported the presence of a substance in an extract of 158 corpora lutea of two species of ovoviviparous South American snakes, *Bothrops jararaca* and *Crotalus terrificus*, that gave a 2+ endometrial reaction in the McPhail assay for progesterone(4). This is the only evidence thus far for the presence of substances with progestational activity in reptiles. It was therefore felt worthwhile to examine the blood plasma of snakes by using the sensitive bioassay method for small amounts of progesterone described by Hooker and Forbes(5). Such information might conceivably lead to a better understanding of the evolutionary significance of this hormone and its role in the lower vertebrates.

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