Toxicity of a Homologous Series of 2-Alkyl-5-methylbenzimidazoles in Chick Embryos.* (27614)

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The effects of benzimidazole and its derivatives on virus multiplication(1), embryonic membrane(2), heme synthesis(3), liver homogenate(4), newt larva(5), chick embryos (6), spermatozoa(7), and bacteria(9) have been studied by a number of investigators. These studies have demonstrated that degree of inhibition increases considerably with addition of an alkyl carbon atom to 2,5-dimethylbenzimidazole in the 2 position (2-ethyl-5-methylbenzimidazole)(1-7,9), while further carbon additions in the 2 position appear to increase or decrease inhibitory effectiveness, depending upon the organism under investigation(1,2,6,7). However, information available on chain lengths higher than ethyl in this homologous series is limited to butyl(1,2) and heptyl(6,7). The purpose of our paper is to demonstrate the effect of 2-alkyl-5-methylbenzimidazoles with chain lengths varying from 2 to 16 carbons on the mortality of 6-day chick embryos.

Materials and methods. White eggs, from commercial Leghorn-type chickens mated *enter se,* were incubated in Jamesway incubators. After 5 days of incubation the eggs were candled and the site for injection of compounds was marked on eggs containing living embryos. On the 6th day eggs were swabbed with 70% ethanol and a small hole was made in the shell with an egg punch. An absolute ethanol solution of 0.05 ml of the homologues was injected with a 0.25 ml syringe into the yolk sac of each egg. The hole in the shell was sealed with collodin. Control groups were injected with absolute ethanol. Yolk sac rather than air cell injection into 6-day embryonated eggs was used because absolute ethanol was highly toxic when injected into the air cell. In addition, embryos younger than 6 days were sensitive to absolute ethanol regardless of route of administration. Dead embryos were determined by candling on the 8th, 10th, and 12th day of incubation. Results are presented as total number dead on the 12th day of incubation since after this time no significant mortality occurred. Each dose of all homologues was injected into 15 eggs in each experiment which was repeated at least 2 times giving a total of 45 embryos per dose. A linear regression for each homologue was calculated from the data.

Results. The calculated regression lines shown in Fig. 1 were found to fit the data at

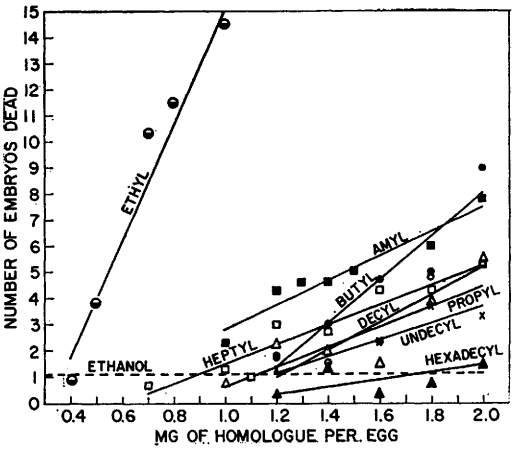

FIG. 1. Linear regressions of lethal dose response of 6-day chick embryos to 2-alkyl-5-methylbenzimidazole homologues. A total of 15 embryos were treated with each dose in at least 3 experiments. ◒ ethyl, △ propyl, ● butyl, ■ amyl, □ heptyl, ○ decyl, × undecyl, ▲ hexadecyl.

the 5% level of significance. The ethyl homologue was approximately 5 times more lethal than the other homologues. The higher homologues, with the exception of propyl, decreased in toxicity as the chain length increased. The highest mortality observed with 2 mg of amyl and of butyl was approximately 50%. Remaining homologues which followed closely in decreasing order of effectiveness were heptyl, propyl, decyl, and undecyl. Hexadecyl was essentially non-lethal at the dosages tested. Because of the difficulty of dissolving the longer chain compounds, no dose

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larger than 2 mg was studied. Therefore, the effectiveness of the compounds could not be compared on a molar basis.

A small percentage of chicks from the various treatments had completely or partially bald heads. The fact that this phenomenon had not been observed in untreated chicks hatched from eggs from the same source indicates that the cause is not of genetic or nutritional origin.

Discussion. The lethal effect of the ethyl homologue found in this study was similar to that previously reported by Blackwood(8) indicating that neither the solvent (absolute ethanol or dilute HCl) nor the route of injection (yolk sac or air cell) alters the lethal effect of this compound in the 6-day embryo.

The present study along with previous work(1,2,6,7) supports the indication that the order of toxicity of 2-alkyl-5-methylbenzimidazoles with chains longer than ethyl differs in different organisms. Hendlin and Soars(9) using *lactobacillus lactis* as a test organism for a closely related homologous series, 2-ethyl, 2-propyl, and 2-butyl-5, 6-dimethylbenzimidazole, found that as the chain length increased toxicity increased. Similarly, Blackwood and Harris(7) found that the heptyl homologue inhibited motility and fructolysis in chicken spermatozoa to a much greater extent than the ethyl homologue.

The difference in toxicity may depend upon whether the organism is multicellular or unicellular(7). It is possible that a multicellular organism can detoxify the larger homologues because of more specialized cell function, while unicellular organisms are not as effective in this respect. Another possible cause for the selective toxicity between unicellular and multicellular organisms may be the difference in solubility of the various homologues. As the chain length increased the homologues became more lipid soluble. The solubility factor may be important in penetration of the homologues into the cell or cells of the organism.

Summary. The effect of eight 2-alkyl-5-methylbenzimidazole homologues with chain lengths ranging from 2 to 16 carbons on the mortality of 6-day chick embryos was investigated. Comparing compounds on a weight basis, it was found that the toxicity of the ethyl homologue was approximately 5 times greater than that of any of the other homologues and that, with the exception of propyl, as the chain length increased, the toxicity decreased. The propyl homologue was less toxic than the butyl, amyl, or heptyl homologues. A linear relationship between dose level and toxicity for each of the compounds was demonstrated.

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