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INHIBITION OF INFLUENZA VIRUS MULTIPLICATION BY ALKYL DERIVATIVES OF BENZIMIDAZOLE

III. Relationship between Inhibitory Activity and Chemical Structure

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When it was found that 2,5-dimethylbenzimidazole inhibits the multiplication of influenza viruses (1–3), a systematic investigation of other alkyl derivatives of benzimidazole was undertaken. It seemed probable that studies on the relationship between the chemical structure of such derivatives and their inhibitory activity relative to influenza virus multiplication would yield information of theoretical importance which might have practical usefulness. Also, it appeared possible that such studies could throw some light on problems regarding the nature and mechanism of biological specificity as exemplified in the process of virus reproduction.

The literature contains few quantitative data on the inhibition of influenza virus multiplication by groups of compounds of known chemical structure. It is difficult to compare directly the data reported in the literature with the results which were obtained in the present investigations because of important differences in procedure. However, it is possible to correlate the results obtained by other workers who used membrane culture (4–6) or tissue culture (7–9) with some obtained in these studies. In those cases in which the effect of the amount of virus in the inoculum was studied, the degree of inhibition was found to be markedly dependent on this variable (1, 7, 8). When virus titer-time curves were studied, it was apparent that the degree of inhibition changed with time, particularly during the early phases of multiplication (2, 3). These relationships are of importance not only when the results obtained by different workers are compared, but also when an attempt is made to compare the activity of one compound against several viruses. In a surviving membrane system, the inhibitory activity of *dl*-methoxinine (4) or malonate (5) against PR8 virus was of a degree much lower than that of the more active alkyl derivatives of benzimidazole used in this investigation against Lee virus. α-Amino sulfonic acids (6) caused inhibition of PR8 virus multiplication at the same molar concentrations as the more active alkyl derivatives. However, in these earlier studies, much less virus was used as inoculum and shorter periods of incubation were employed. In a tissue culture system, acridines (7) and diamidines (8) inhibited influenza virus multiplication when low concentrations of the compounds were used. Relatively high concentrations

of desoxypyridoxine or oxythiamine inhibited PR8 virus multiplication in tissue culture (9). Because of the different host-cell systems employed, it is not possible to compare quantitatively the activity of these compounds with that of alkyl derivatives of benzimidazole described in this communication.

Obviously the implications of the finding that a substance has inhibitory activity may be far more important than the molar fraction or weight required to cause inhibition of virus multiplication under defined experimental conditions. However, not enough experimental data are available and not enough is known about the mechanism of action of different inhibitory substances to permit an evaluation of the biological aspects of the inhibition they cause.

This report deals with the inhibitory activity of various alkyl derivatives of benzimidazole relative to the reproduction of influenza virus. Procedures for the quantitative analysis of the inhibitory activity of compounds affecting influenza virus multiplication in membrane culture have been developed and are described in detail in the accompanying report (3). The experimental conditions utilized made it possible to compare the activity of different compounds with a high degree of precision. It will be shown that the inhibitory activity of benzimidazole derivatives upon the multiplication of influenza B virus is strikingly dependent on the nature and position of substituent groups in the molecule and that certain regularities were observed in regard to the inhibitory effects.

Materials and Methods

Virus.—The Lee strain of influenza B virus, contained in infected allantoic fluid, was used throughout this study. The procedures for maintaining and storing the virus were identical with those described in the accompanying papers (2, 3).

Membrane Cultures.—The procedures used in the preparation of membrane cultures are described in the accompanying communication (3). The mean surface area of pieces of chorioallantoic membrane used was 5.75 $cm.^2$. The final volume of the fluid medium was 1 ml., and this contained $10^{5.5}$ EID_{50} of Lee virus. A 36 hour period of incubation with continuous shaking at 35°C. was employed. The experimental conditions defined in the preceding study (3) were adhered to rigidly.

Benzimidazole Derivatives.—After the solubility of each compound in the culture medium was determined, preliminary experiments were carried out to define the range of concentration which caused 60 to 90 per cent inhibition of multiplication of the virus in membrane cultures. Definitive experiments were performed with a series of concentrations of each compound in the range thus determined. The concentrations used differed only by small amounts, *e.g.* 1.25-fold, because it was found that the degree of inhibition depended markedly on the concentration of the compound. A group of 6 membrane cultures was used for each concentration employed. In most experiments, two or three similar groups of 6 cultures without any added compound were included as controls. In some experiments, one, in others four, groups of control cultures were used. The reproducibility of the virus titer of control cultures is set forth in the preceding paper (3).

Hemagglutination Titrations.—The amount of virus present in the medium of each culture after 36 hours of incubation was determined by hemagglutination titration. The titrations were performed as described in the accompanying paper (3). The geometric mean titer of each group of 6 cultures was computed.

Inhibitory Activity of Compounds.—The degree of inhibition of multiplication was expressed as the percentage value of the control titer and was plotted against the molar concentration of the benzimidazole derivative used. A straight line which intercepted the zero concentration axis at 100 per cent was fitted to the experimental points. From this line, the concentration of the compound which held the virus titer to 25 per cent of the control value, *i.e.* 75 per cent inhibition of multiplication, was determined. Compounds were compared in terms of the molar concentration which caused 75 per cent inhibition. For this purpose, the mean activity value determined in different experiments with the same compound was used. The standard deviation of the results obtained in different experiments with each compound was computed. The significance of differences in the inhibitory activity of various compounds was estimated by means of the *t* test (10). With few exceptions, a 1.5-fold difference in the inhibitory activity of two compounds was found to be significant at the 0.95 probability level. With some pairs of compounds, even smaller differences in activity were found to be significant.

EXPERIMENTAL

Relationship between Concentration of Benzimidazole Derivatives and Degree of Inhibition.—The results of each experiment on inhibitory activity of benzimidazole derivatives relative to Lee virus multiplication were analyzed in order to determine whether a relationship existed between the concentration of the compound used and the extent of inhibition obtained. That there is a direct relationship between these two variables was indicated by the results secured with each of the 16 compounds studied.

In Figs. 1 to 4, the degree of inhibition, expressed as the percental difference in hemagglutination titer compared to the controls, is plotted against the molar concentration of the compound. The slope of the line drawn was determined by the mean activity computed from different experiments with the same compound. Examination of the data obtained with each compound reveals that, in the range between 60 and 90 per cent inhibition, *i.e.* 40 to 10 per cent of the control value, there appears to be a straight line relationship between the two parameters. As indicated above, for convenience in determining the concentration which yielded 75 per cent inhibition, the origin of the line was arbitrarily taken to be equivalent to no inhibition; *i.e.*, 100 per cent of the control titer.

Effect of Substitution of Methyl Groups in the Benzene Ring.—The inhibitory activity of alkyl derivatives of benzimidazole which contain methyl groups substituted in the benzene ring was compared with that of unsubstituted benzimidazole which, for the purposes of this study, was considered as a reference compound.

Table I shows the results of a number of experiments with such compounds. As can be seen, the substitution of a single methyl group at position 5 increased the inhibitory activity of the compound approximately twofold relative to the reference compound. The addition of a second methyl group at position 4 resulted in a further increase of activity, *i.e.* to 3.2-fold, whereas substitution of the second methyl group at position 6 had no similar effect. The 4,5,6,7-

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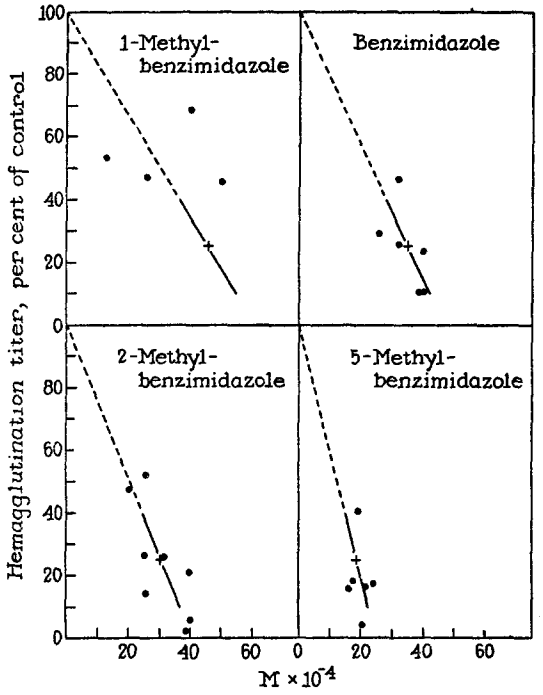

FIG. 1

FIGS. 1 to 4. Relationship between concentration of selected alkyl derivatives of benzimidazole and degree of inhibition of Lee virus multiplication. Each point represents the difference between the geometric mean titer of a group of 6 membranes and that of the controls. The crosses represent inhibitory activities obtained by computing the mean of the values determined in different experiments.

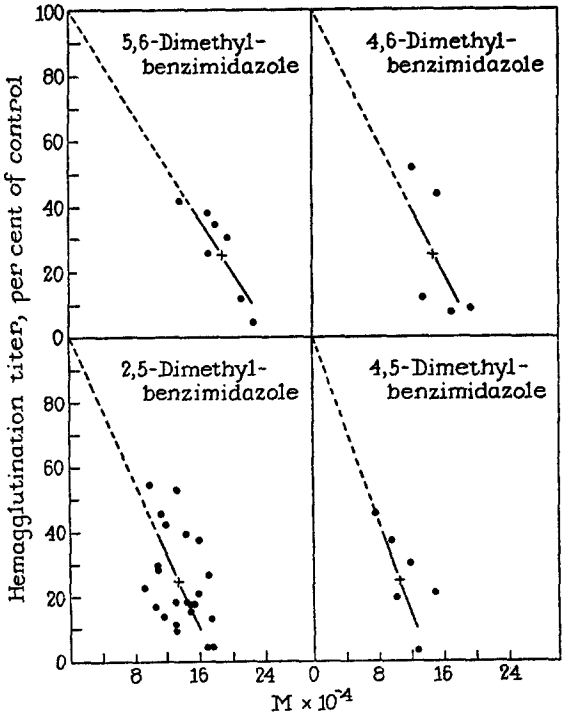

FIG. 2

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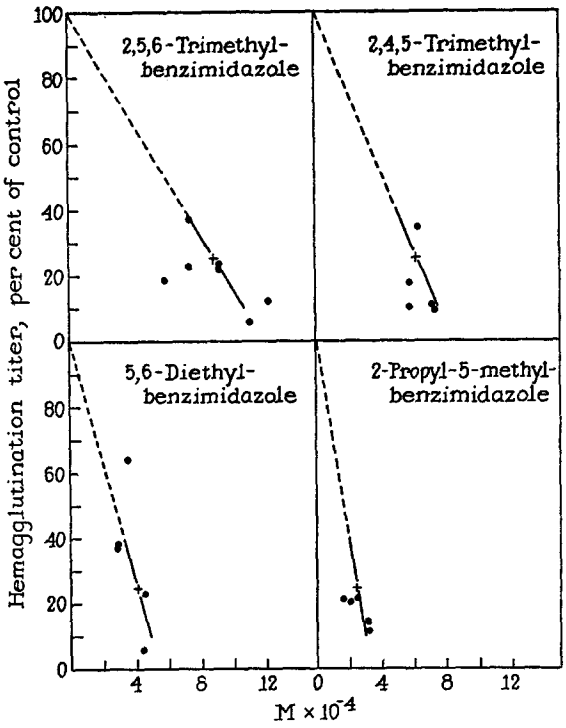

FIG. 3

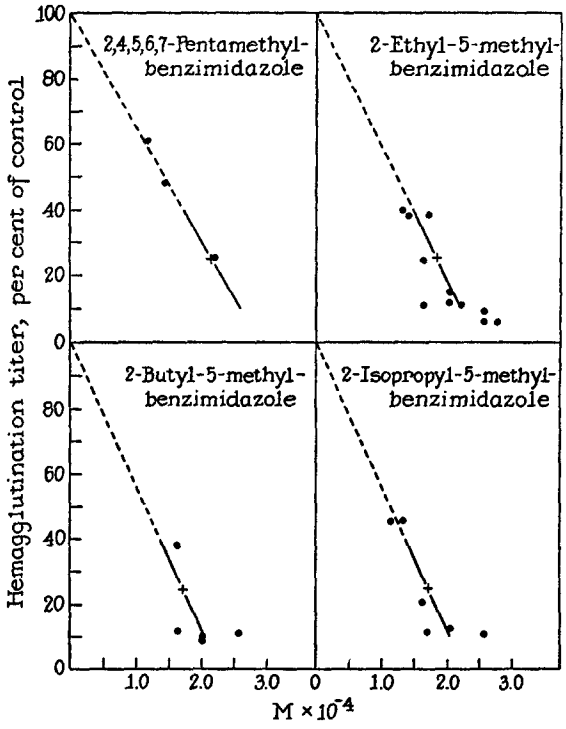

FIG. 4

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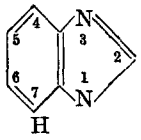
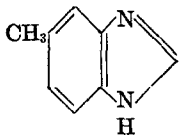
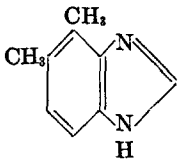
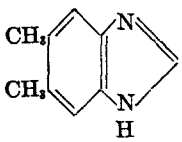
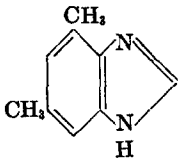
TABLE I

Inhibition of Influenza B Virus Multiplication by Benzimidazole Derivatives

Benzimidazole derivative	No. of experiments	No. of determinations	Inhibitory concentration*	Standard deviation		Inhibitory activity relative to benzimidazole
			$M \times 10^{-4}$	$M \times 10^{-4}$	per cent	
 Benzimidazole	3	6	35	6.1	17	1.0
 5-Methyl	3	6	19	3.5	18	1.8
 4,5-Dimethyl	2	6	11	1.3	12	3.2
 5,6-Dimethyl	3	7	19	1.2	6.3	1.8
 4,6-Dimethyl	2	5	15	2.9	19	2.3

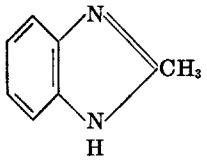
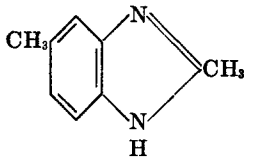
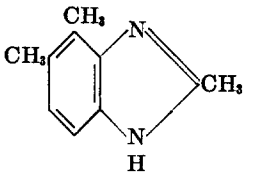
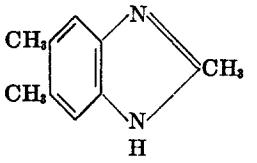
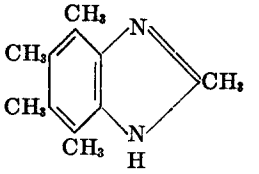
* Concentration giving 75 per cent inhibition of multiplication.

tetramethyl compound also was tested; however, no significant degree of inhibition was obtained with the low concentrations used. Unfortunately, this compound was very poorly soluble in the medium utilized.

These results indicate that the substitution of methyl groups at positions 4 and 5 of the benzene ring cumulatively increased the inhibitory activity of benzimidazole derivatives. In contrast, substitution of a methyl group at position 6 did not cause an increase in such activity.

TABLE II

Inhibition of Influenza B Virus Multiplication by Benzimidazole Derivatives

Benzimidazole derivative	No. of experiments	No. of determinations	Inhibitory concentration*	Standard deviation		Inhibitory activity relative to benzimidazole
			M × 10^{-4}	M × 10^{-4}	*per cent*	
 2-Methyl	4	11	31	3.6	12	1.1
 2,5-Dimethyl	7	18	13	1.6	12	2.7
 2,4,5-Trimethyl	2	5	6.3	0.99	16	5.6
 2,5,6-Trimethyl	3	7	8.9	1.3	15	3.9
 2,4,5,6,7-Pentamethyl	2	3	2.2	0.71	3.2	16

* Concentration giving 75 per cent inhibition of multiplication.

Effect of Substitution of a Methyl Group at Position 2 of the Imidazole Ring.— As can be seen from the results shown in Table II, substitution of a methyl group at position 2 of the imidazole ring did not cause an increase in inhibitory activity relative to that of benzimidazole. However, a comparison of the

results obtained with the following pairs of compounds (*cf.* Table I): 5-methyl and 2,5-dimethyl; 4,5-dimethyl and 2,4,5-trimethyl; and 5,6-dimethyl and 2,5,6-trimethyl, shows that when methyl groups were present at certain positions of the benzene ring, substitution of a methyl group at position 2 in the imidazole ring caused a definite increase in inhibitory activity. It will be noted that, although the 5-methyl and 5,6-dimethyl derivatives showed identical activities, the 2,5-dimethyl and 2,5,6-trimethyl compounds showed a significant difference in activity. The latter compound was 1.5 times more active than the former. Thus, it appears that certain alterations in the structure of the benzimidazole molecule produce different effects on the inhibitory activity depending on what other chemical groups are present or absent. The substitution of methyl groups in the benzene ring affects the biological potentialities of similar groups substituted in the imidazole ring and *vice versa*.

The results presented in Table II also show that substitution of methyl groups at all available carbon atoms in the benzene ring, when there was also a methyl group at position 2, yielded a highly active compound (the 2,4,5,6,7-pentamethyl derivative). This change was anticipated on the basis of the increase in activity shown by the 2,5-dimethyl as compared with the 2-methyl compound as well as by the 2,5,6-trimethyl and 2,4,5-trimethyl compounds as compared with the 2,5-dimethyl derivative. It is noteworthy that the 2,4,5,6,7-pentamethyl derivative caused 75 per cent inhibition of multiplication of Lee virus at a concentration of 41 μg. per ml.

Effect of Substitution at Position 2 of the Imidazole Ring with Alkyl Groups Other than Methyl.—In view of the enhancing effect of a methyl group at position 2 when the benzene ring also contained methyl substituents, it was desirable to investigate the effects of other alkyl radicals at position 2 using 5-methylbenzimidazole as the nucleus. As is shown in Table III, a striking increase in inhibitory activity was observed when an ethyl group was introduced into the molecule at position 2. The 2-ethyl-5-methyl compound was 7.3 times more active than the 2,5-dimethyl derivative (*cf.* Table II) and caused 75 per cent inhibition of Lee virus multiplication at a concentration of 29 μg. per ml. However, the 2-propyl-5-methyl compound, although considerably more active than the 2,5-dimethyl compound, was only 0.7 times as active as the 2-ethyl-5-methyl derivative. The 2-isopropyl-5-methyl and 2-butyl-5-methyl compounds had almost the same inhibitory activity as the 2-ethyl-5-methyl derivative.

The 2-ethyl-4,5,6,7-tetramethyl compound also was tested. As in the case of the 4,5,6,7-tetramethyl compound, no inhibition was demonstrable with the low concentrations used. This substance also was very poorly soluble in the culture medium.

Effect of Substitution of Ethyl Groups in the Benzene Ring.—Because of the striking increase in activity which occurred when an ethyl group rather than

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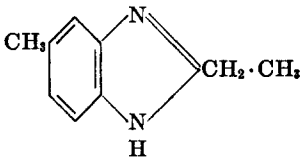
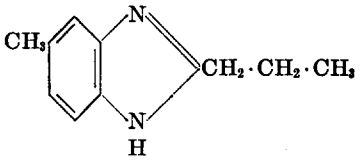
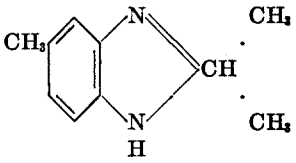
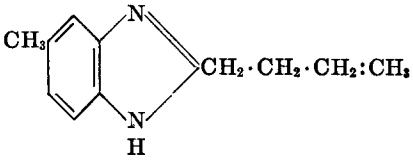
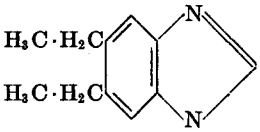
TABLE III

Inhibition of Influenza B Virus Multiplication by Benzimidazole Derivatives

Benzimidazole derivative		No. of experiments	No. of determinations	Inhibitory concentration*	Standard deviation		Inhibitory activity relative to benzimidazole
				M × 10^{-4}	M × 10^{-4}	*per cent*	
	2-Ethyl-5-methyl	3	11	1.8	0.1	5.6	19
	2-Propyl-5-methyl	2	4	2.5	0.077	3.1	14
	2-Isopropyl-5-methyl	2	6	1.7	0.1	5.9	21
	2-Butyl-5-methyl	2	5	1.7	0.32	19	21
	5,6-Diethyl	2	5	4.1	0.21	5.1	8.5

* Concentration giving 75 per cent inhibition of multiplication.

a methyl group was substituted at position 2 of the imidazole ring, it was important to explore the effect of substitution of ethyl radicals in the benzene

ring. As can be seen in Table III, the 5,6-diethyl compound was 4.7 times more active than the 5,6-dimethyl derivative (*cf.* Table I). This increase in activity is similar to that observed when the 2,5-dimethyl and 2-ethyl-5-methyl compounds are compared.

Effect of Substitution at Position 1 of the Imidazole Ring.—Experiments with 1-methylbenzimidazole indicated that substitution of a methyl group at position 1 of the imidazole ring resulted in a definite loss in inhibitory activity. Five determinations of activity were carried out in three experiments. The concentration which caused 75 per cent inhibition of multiplication of Lee

TABLE IV

Activities of Benzimidazole Derivatives as Inhibitors of Influenza B Virus Multiplication

Benzimidazole derivative	Inhibitory concentration*		Inhibitory activity relative to benzimidazole
	$M \times 10^{-4}$	mg./ml.	
1-Methyl	72	0.95	0.49
Benzimidazole	35	0.41	1.0
2-Methyl	31	0.41	1.1
5-Methyl	19	0.25	1.8
5,6-Dimethyl	19	0.28	1.8
4,6-Dimethyl	15	0.22	2.3
2,5-Dimethyl	13	0.19	2.7
4,5-Dimethyl	11	0.16	3.2
2,5,6-Trimethyl	8.9	0.14	3.9
2,4,5-Trimethyl	6.3	0.10	5.5
5,6-Diethyl	4.1	0.072	8.5
2-Propyl-5-methyl	2.5	0.044	14
2,4,5,6,7-Pentamethyl	2.2	0.041	16
2-Ethyl-5-methyl	1.8	0.029	19
2-Butyl-5-methyl	1.7	0.032	21
2-Isopropyl-5-methyl	1.7	0.030	21

* Concentration giving 75 per cent inhibition of multiplication.

virus was found to be 72×10^{-4} M. The standard deviation was 3.8×10^{-4} M or 5.3 per cent. Thus, the inhibitory activity relative to unsubstituted benzimidazole was 0.49.

A summary of the results obtained with the 16 compounds is shown in Table IV. Relative to activity as inhibitors of Lee virus multiplication, the derivatives can be divided into two groups: (*a*) those which were less than 10 times as active as benzimidazole itself; and (*b*) those which were more than 10 times as active as the reference compound. The first group contains all of the mono-, di-, and trimethyl derivatives. Thus, on the basis of the compounds studied, it appears that minor differences in activity result when one to three methyl groups are introduced in certain positions in the benzimidazole molecule

and that the position of the substituent group is of great importance in determining whether an added methyl radical will alter the activity of the molecule. The second group contains the derivatives with larger or more numerous substituents. The striking feature of this group is the finding that more extensive substitution in either the benzene or the imidazole ring results in marked increase in inhibitory activity.

Attempts to Block the Inhibitory Effect of Benzimidazole Derivatives.—Attempts were made to block the inhibitory activity of unsubstituted benzimidazole or of 2,5-dimethylbenzimidazole on the multiplication of Lee virus. Selected compounds, which, it was thought, might serve as metabolites in the process of virus reproduction, were used, but none proved capable of blocking the inhibitory effect of the substances employed.

With benzimidazole (0.0055 M) as inhibitor, the following compounds failed to block the inhibitory effect when given simultaneously: vitamin B_{12}, 0.079 mg. per ml.; a mixture of adenine, guanine, and uracil; a mixture of adenine and adenosine; adenylic acid or desoxyguanosine. These compounds were used at a concentration of 0.00033 to 0.00045 M of each. The following compounds failed to show blocking activity with 2,5-dimethylbenzimidazole which was employed at concentrations ranging from 0.0015 to 0.0026 M: thymine or adenine (0.0026 M); a mixture of adenine, guanine, and uracil (0.00033 to 0.00045 M of each); desoxyguanosine or desoxycytidine picrate (0.0002 M); *l*-serine or *l*-methionine (0.002 M); and a mixture of *dl*-methionine, choline, and *l*-tryptophane (0.001 M of each).

Reports in the literature indicate that blocking of the inhibitory effect of benzimidazole on microbial growth depends both on the species used and the metabolite employed (11–15). Adenine and guanine blocked the effect of benzimidazole on *Saccharomyces cerevisiae*, whereas hypoxanthine, xanthine, and uracil, as well as yeast and muscle adenylic acids, failed to do so (11). Vitamin B_{12}, adenine, and adenosine were effective in blocking the action of benzimidazole on *Lactobacillus leichmannii* (13) but ineffective in blocking the effect of 5,6-dimethylbenzimidazole on *Lactobacillus lactis* (14). Partial blocking of the inhibitory effect of benzimidazole on poliomyelitis virus multiplication was achieved with a mixture of adenine, guanine, and uracil, but not with either adenine or guanine alone (15).

DISCUSSION

That the structural configuration of benzimidazole derivatives bears on their inhibitory activity relative to the multiplication of influenza virus appears clear from the results of this investigation. By means of fairly extensive substitution of alkyl radicals in either of the two rings of the bicyclic skeleton of benzimidazole, derivatives with a high degree of inhibitory activity can be obtained. Under the strictly defined (3) experimental conditions employed, the more potent compounds caused 75 per cent inhibition of multiplication of Lee virus in membrane cultures at concentrations of approximately 0.0002 M.

Several workers have used unsubstituted benzimidazole as an inhibitor of microbial growth (11–14). With *Saccharomyces cerevisiae*, Woolley employed certain substituted benzimidazoles as well (11). Hendlin and Soars (14) used a number of alkyl derivatives of benzimidazole as inhibitors of *L. lactis* Dorner. The results with substituted benzimidazoles are discussed below. Along with numerous other compounds, benzimidazole has been tested against vaccinia (16), Theiler's GDVII (17) and poliomyelitis (15) viruses and has been found to be inhibitory in each case. Benzimidazole also inhibits psittacosis virus multiplication (18). From these reports, however, it seems apparent that the activity of unsubstituted benzimidazole as an inhibitor of bacterial or virus multiplication is low.

There are several remarkable features of the effects of structural modifications of benzimidazole by substitution of alkyl radicals which deserve comment. The effects on inhibitory activity with respect to influenza virus multiplication are quantitatively diverse and, depending upon the nature of the alteration of the molecule, may be negligible or marked. Both the nature and the position of substituent groups affect inhibitory activity. Substitution of a single methyl group at either position 1 or 2 of the imidazole ring did not increase activity. Substitution of one or two methyl groups at certain positions in the benzene ring resulted in an increase in activity of minor degree. The increase was greater if there was also a methyl group at position 2. Under the same condition, substitution in the benzene ring of four methyl groups yielded a substance which was much more active than unsubstituted benzimidazole. Another more radical change of the benzene ring which yielded a highly active compound was substitution of ethyl instead of methyl groups at positions 5 and 6. Lengthening the alkyl side chain from methyl to ethyl at position 2 of the imidazole ring also caused a marked increase in activity. Further extension of the side chain to propyl or butyl radicals at position 2 did not lead to a further increase in activity.

The fact that extensive alteration in either the benzene or imidazole ring of the benzimidazole molecule yields compounds with a high degree of activity indicates that, in this case, the relationship between structure and activity probably has a basis different from that proposed for methyl derivatives of indole in the growth of *Salmonella typhosa* (19, 20). In this system, the evidence suggested that certain methyl derivatives of indole interfere with the enzymatic conversion of indole into tryptophane. Compounds containing methyl groups in the pyrrole ring lacked inhibitory activity, whereas derivatives containing the same radical in the benzene ring were active as inhibitors of multiplication of *S. typhosa*. With *Saccharomyces cerevisiae*, Woolley (11) found that substitution of a methyl or hydroxyl group in position 2 of the imidazole ring of benzimidazole reduced or eliminated the inhibitory activity against yeast. However, substitution of nitro or amino groups in the benzene ring did not greatly alter activity.

Any attempt to interpret the significance of the effects of structural modifications of the benzimidazole molecule on inhibitory activity relative to influenza virus multiplication is seriously hampered by present lack of knowledge regarding the metabolic processes involved in reproduction of the virus. Even if the metabolites concerned with the reproductive process were known, it might be difficult to determine which of their functions were interfered with by inhibitory compounds. For example, adenine, which is an important structural unit of nucleic acids, also is present in several coenzymes and, through its presence in ATP and ADP, is directly involved in energy-yielding and phosphorylating mechanisms. Vitamin B_{12} contains a benzimidazole moiety, 5,6-dimethylbenzimidazole (21–24). If the metabolism of 5,6-dimethylbenzimidazole were interfered with, abnormalities in several of the functions of B_{12} might result. Among the reactions which could be affected are: synthesis of nucleosides, utilization of glycine in the synthesis of serine, and transfer of the labile methyl group to a precursor of methionine (25, 26).

Support for the idea that benzimidazole interferes with reactions in the area of nucleic acid metabolism can be derived from findings on blockade by purines or by vitamin B_{12} of its inhibitory effect on certain microbial species (11–13, 15). However, in the present study, attempts to block the inhibitory effect of benzimidazole or 2,5-dimethylbenzimidazole on Lee virus multiplication with the same substances were unsuccessful.

Comparison of the results secured in this investigation with Lee virus and those obtained with alkyl derivatives of benzimidazole against *L. lactis* Dorner (14) has led to the following observations: (*a*) The activity of benzimidazole and its mono- and dimethyl derivatives in the Lee virus system appeared to be correlated in most instances with the activity of such compounds against *L. lactis* Dorner. However, the 2,5-dimethyl compound failed to inhibit growth of the bacterium (14). (*b*) The activity of 2-alkyl substituted 5,6-dimethyl derivatives against *L. lactis* Dorner varied directly with the length of the side chain; each addition produced an increase of moderate degree. This is in sharp contrast to the effect that lengthening of the alkyl side chain at position 2 of the 5-methyl compound had on inhibitory activity relative to Lee virus multiplication: The ethyl derivative, although much more active than the methyl, was not less active than propyl or butyl derivatives. These observations suggest that, although alkyl derivatives of benzimidazole may be operative in the same biochemical area in the two systems, features specific to each system are present.

SUMMARY

The degree of inhibition of multiplication of influenza B virus, Lee strain, in membrane cultures *in vitro* appears to be directly related to the concentration of the inhibitory compounds used in this investigation. With each of the alkyl derivatives of benzimidazole, evidence for such a relationship was ob-

tained in the range between 60 and 90 per cent inhibition of virus multiplication.

Alteration of the structure of benzimidazole by substitution of alkyl radicals at various positions in either the benzene or the imidazole ring resulted in diverse differences in the capacity to inhibit influenza virus multiplication *in vitro*. Minor increases in inhibitory activity resulted when one to three methyl groups were introduced at certain positions in the molecule. Marked increases in inhibitory activity were achieved by more extensive substitution in either the benzene or the imidazole ring. The position and nature of substituent groups appeared to be of decisive importance.

Among the more highly active compounds were 2,4,5,6,7-pentamethylbenzimidazole, 5,6-diethylbenzimidazole, and 2-ethyl-5-methylbenzimidazole. Further extension of the alkyl chain at position 2 caused no significant change in the inhibitory activity of the derivative. The most active compounds studied caused 75 per cent inhibition of Lee virus multiplication in membrane cultures *in vitro* at concentrations of approximately 0.0002 M. Some of the implications of these findings are discussed.

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