Effect of Benzimidazole on Experimental Poliomyelitis in Mice and Monkeys.* (20374)

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As components of nucleo proteins, the purines and pyrimidines are intimately involved in biological systems. Their analogues, which interfere with the synthesis of nucleic acids, are known to affect the metabolic activity of certain bacteria(1-6) and viruses (7-10). One of these, benzimidazole, in which the pyrimidine ring has been replaced by benzene, was found to be active in tissue cultures against the viruses of vaccinia(11), tobacco mosaic(12), and psittacosis(13). More recently, the author(14) has demonstrated that this compound, as well as other inhibitors of nucleic acid synthesis, effectively suppresses the growth of poliomyelitis virus in tissue culture. The present report describes the effect of benzimidazole on poliomyelitic infection in animals.

Materials and methods. Swiss Albino mice weighing 17 to 20 g were individually weighed to insure exactitude in the drug administration. Toxicity tests revealed that mice of this weight could tolerate without ill effect 250 mg of commercial benzimidazole† per kilo of body weight when administered subcutaneously for as long as 21 days. The type 2 rodent adapted viruses (Lansing and MEF-1) used in tests with mice had been prepared in large quantities and stored as pools at —70°C in sealed ampoules until used. Mice were infected by intracerebral injections of 0.03 ml of the appropriate dilutions of virus and were observed daily for at least 21 days for signs of paralysis. The virus used in tests with monkeys was a pool of Mahoney strain, type 1, poliomyelitis virus from infected monkey central nervous tissue which was infective either intracerebrally, subcutaneously, or intraperitoneally in dilutions of at least 10^{-3}. The monkeys were observed for 21 days, and if not previously paralyzed, were posted at that time and sections of the nervous system were examined for histological evidence of poliomyelitis.

Experimental tests in mice. Lansing strain. Six separate tests were performed in mice with the Lansing strain of Type 2 poliomyelitis virus which had a PD_{50} titer of 10^{-4} or higher. Four dilutions of virus, 10^{-1} through 10^{-4}, were employed in groups of either 20 or 50 mice each for both the drug treated and the virus control mice. The animals received 250 mg/kg benzimidazole either once on the day of virus inoculation (acute administration) or daily for the duration of the experiment (chronic). No difference in morbidity or mortality was observed between those mice receiving drug on the acute schedule or those on chronic administration. Consequently, the results of all tests are considered irrespective of the schedule of drug administration. Table I shows the cumulative results of 6 separate experiments in mice employing 4 dilutions of the Lansing strain of poliomyelitis virus and 250 mg of benzimidazole per kilo of body weight. The only dilution of virus which killed more control than benzimidazole treated mice was 10^{-4}. The probability of chance occurrence of this difference, however, is $P = .01$, and therefore the 13% decrease in mortality seen in the drug treated groups represents statistical significance. It will be seen that the arithmetic mean of the incubation period in mice receiving drug increased 3 or 4 days with each 10-fold dilution of virus. A corresponding but not as marked an increase was seen in the control mice. However, the incubation period was almost as great in the 10^{-4} controls as in the drug treated mice suggesting that the major effect of benzimidazole was on the incidence of infection rather than in prolonging the incubation period. The cumulative mortalities of both control and drug treated mice receiving the 10^{-4} dilution of Lansing virus in 6 experiments are presented graphically in Fig. 1.

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TABLE I. Cumulative Results of 6 Experiments in Mice with Subcutaneously Administered Benzimidazole, 250 mg/kg and Lansing Strain Poliomyelitis Virus.

	Dilutions of virus							
	10^{-1}		10^{-2}		10^{-3}		10^{-4}	
	Drug (149)*	Con. (165)	Drug (229)	Con. (169)	Drug (225)	Con. (169)	Drug (232)	Con. (172)
A.M.†	4.6	5.3	7.57	5.93	10.75	9.5	14.5	13.0
Range	2–15	2–20	2–19	2–20	2–28	2–29	5–28	2–28
% mort.	99	98	98	98	86	82	46	59
Range mort., %	96–100	95–100	94–100	94–100	70–100	57–100	36–60	45–85

* Total No. of mice in all tests.
† Arithmetic mean of incubation period in days.

MEF-1 strain. Since the only dilution of the Lansing strain at which benzimidazole appeared to have an effect in mice was 10^{-4}, the approximate PD_{50} of that virus, similar tests at or near the end point of virus titer were performed using the MEF-1 strain of Type 2 poliomyelitis virus which usually infected mice when diluted as much as 10^{-5} and occasionally in a 10^{-6} dilution. The same concentration of benzimidazole (250 mg/kg) was inoculated and except for greater numbers of mice per dilution the technics were identical to those used in the above-described tests. Benzimidazole, however, was not effective in decreasing the mortality caused by this strain of poliomyelitis virus. In fact, more of the treated mice than the controls developed the disease with all 3 dilutions of this agent. Since the intracerebral inoculation of virus represents an extremely severe challenge to the efficacy of any drug which might influence infection, suckling mice between the ages of one and 3 days were inoculated intraperitoneally with 0.1 ml of a 10% suspension of the MEF-1 strain of virus. Some of these were treated subcutaneously with 250 mg/kg benzimidazole and some with 100 mg/kg. Thirty-three of the animals received the virus alone and 16 developed the disease. Of the mice receiving 100 mg/kg benzimidazole 23 of 30 became paralyzed as did all 6 who received 250 mg/kg. This indicates that far from preventing infection in suckling mice inoculated with virus intraperitoneally, the drug apparently enhanced their susceptibility to poliomyelitis.

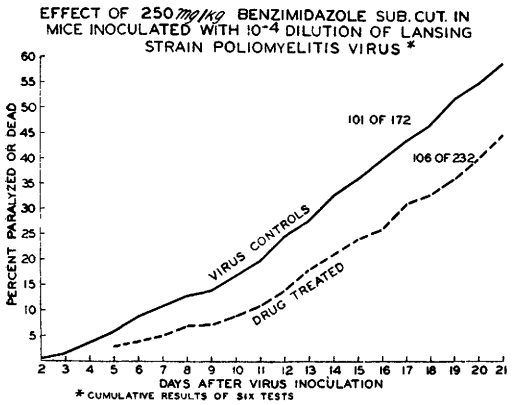

FIG. 1.

Tests in monkeys. The effect of benzimidazole on infection in monkeys was investigated. Ten rhesus monkeys (*Macaca mulatta*) were inoculated intracerebrally with 0.5 ml of a 10^{-2} dilution of the Mahoney strain of Type 1 virus. One-half of the animals received 250 mg of benzimidazole per kilo of body weight subcutaneously for 3 days beginning on the day of virus inoculation. In another test 20 cynomolgus monkeys (*Macaca iris*) were inoculated intraperitoneally with 1.0 ml of a 10^{-2} dilution of the same pool of virus and half of these were treated for 3 days with 200 mg of benzimidazole per kilo. The results of these tests are shown in Table II. All the drug treated animals as well as the controls developed paralysis regardless of the route of inoculation. The only difference between the 2 groups of monkeys was evidenced by a slightly longer and more irregular incubation period in the drug treated groups. This slight difference, however, was emphasized by the regularity with which the virus controls developed the paralytic disease, most of them within 24 hours of each other. In a third test 10 cynomolgus monkeys were treated with 250 mg of the drug per kilo daily for 5 days prior to and for 2 days after the subcutaneous

TABLE II. Effect of Benzimidazole on Infection with Mahoney Strain of Poliomyelitis Virus in Monkeys.

Monkey	Virus: Route	s.c. drug, mg/kg	Day of paralysis: Control	Day of paralysis: Benzimidazole
Rhesus (10)	10^{-2} i.c.	250	7, 7, 8, 8, 8	7, 8, 9, 10, 10
		$3 \times \bar{p}$	Avg = 7.6	Avg = 8.8
Cyno. (20)	10^{-2} i.p.	200	6, 7, 7, 7, 7, 7,	7, 7, 8, 8, 8, 8,
		$3 \times \bar{p}$	7, 7, 8, 11	9, 10, 11, 12
			Avg = 7.4	Avg = 8.8
Cyno. (20)	10^{-3} s.c.	250	9, 12, 12, 12, 14,	8, 9, 12, 13, 14,
		$5 \times \bar{a}$	14, 16, 17, —, —	14, 21, —, —, X
		$2 \times \bar{p}$	Avg = 13.2	Avg = 13.0

inoculation of 1.0 ml of a 10^{-3} dilution of Mahoney virus. Seven of these animals developed paralysis as compared with 8 of the 10 controls, and the average incubation periods of the 2 groups were almost identical. It was apparent that benzimidazole, under the conditions tested, had little or no effect upon the course of poliomyelitic infection in monkeys.

Discussion. Few compounds which show antiviral activity in tissue culture are similarly effective in animals. This is probably due to the inability to exhaust or replace completely a given metabolite from the tissues of the intact host. Furthermore, the quantity necessary to accomplish this state of deprivation is usually far in excess of the toxic levels which are tolerated by the animal. The results of experiments with benzimidazole, therefore, are singular in this regard. Although the decrease in mortality is moderate and is observed only with minimal infective quantities of Lansing strain poliomyelitis virus, the influence of this compound on the process of disease is evident in the rather consistently prolonged incubation period even with larger quantities of virus. The drug itself is well tolerated by the animal without ill effect for the duration of these experiments. It is possible, therefore, that this represents an example of reducing the availability of a metabolite essential for the growth of virus without adversely affecting the host itself.

It would be far too optimistic to expect to find a drug or chemical which would completely prevent the occurrence of poliomyelitis in experimentally infected animals. However, when many compounds are tested without observing the slightest effect on either the incubation period or the mortality, as is the case in this laboratory, the results obtained in the present study appear highly suggestive that the investigation of this general class of compounds offers a reasonable approach to the chemotherapy of this disease.

Summary. 1. The influence of benzimidazole on experimental poliomyelitis in mice and monkeys was investigated. The subcutaneous administration of 250 mg/kilo prolonged the incubation period of infection with the Lansing strain of Type 2 poliomyelitis in mice and reduced the mortality caused by minimal quantities of this virus. 2. Infection of adult mice with intracerebrally inoculated MEF-1 strain of Type 2 virus was not affected by this compound which even appeared to enhance infection in suckling mice inoculated intraperitoneally with this same strain. 3. In monkeys the incubation period of infection with the Mahoney strain of Type 1 virus was prolonged slightly by limited treatment with benzimidazole, but the mortality was not reduced. More intensive treatment was without effect. 4. The advisability of further work with this and structurally similar compounds is discussed.

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Protection Against Radiation Lethality: Effect of β-Mercaptoethylamine.* (20375)

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The search for some agent which will protect man and animals against, or alleviate them from, the effects of accidental or overexposure of ionizing radiations has been the incentive for many investigations. The most promising of the many chemical compounds used has been *l*-cysteine hydrochloride. Patt and his coworkers(1) found that if mice were injected with cysteine before exposure to x-irradiations, their tolerance would be greatly increased and many would survive doses which would normally prove fatal to 100% of the untreated controls. This led others to investigate the amines, particularly those containing the —SH radical because of the suggestion that an extra supply of this radical might temporarily neutralize certain enzymes with respect to their normally high radiosensitivity(2). Recently, Bacq and his coworkers in Belgium investigated many such compounds. One of these was related to Patt's cysteine, namely cysteinamine or β-mercaptoethylamine. By injecting this compound into C_{57} black mice, they raised the minimum 100% lethal dose of x-rays from 700 r to 1300 r(3). They also found that this compound gave complete protection to mice exposed to 100% lethal dose of x-rays. In this paper the term "cysteinamine" is used instead of β-mercaptoethylamine in order to emphasize its similarity to cysteine which has been so effective in radiation protection studies.

The reasons for the present further investigation of cysteinamine as a protective agent against ionizing radiations are: (a) Variations in sensitivity make it impossible to interpolate results with C_{57} mice for the mice more commonly used in this country, namely the Swiss albino and CF_1 strains, and (b) Radiation studies using male mice exclusively are more reliable than those using females, or both sexes.

Materials and method. The Swiss albino mice were raised in our laboratories and were 6 months of age. The CF_1 were 2 months of age when purchased from the Carworth Farms. They were brought to a uniform weight of approximately 25 g at the time of irradiation. Some 659 mice were used in all. The cysteinamine was acquired in ampoules, labelled 1573 L and produced by the Labaz Laboratory in Belgium. Each ampoule consisted of 10 cc of a 10% solution. This was refrigerated until opened, and was used within a few minutes after dilution with physiological saline. The dilution was such that each cubic centimeter contained 3 mg of cysteinamine. Several parallel experiments were run with

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