Reversal of Nitrogen Mustard Intoxication by Anti-Histamines.* (29116)

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This laboratory previously reported that serotonin antagonists possessed an unusual ability to reduce the toxic effects of nitrogen mustard in mice(1,2). This effect was more evident when the serotonin antagonist was given after rather than before the nitrogen mustard, and it was most apparent in reduced lethality and leukopenia. The similarity in biological effects of serotonin antagonists to histamine antagonists(3) soon made an investigation of the latter necessary. The unique effects described with serotonin antagonists(1,2) have been essentially duplicated with two representative anti-histamines. The results of these studies are reported here.

Method. Mechlorethamine (nitrogen mustard) (NH_2)† was freshly prepared in saline solution and given to rested adult White Swiss (Simonsen Laboratories, Inc., Gilroy, Calif.) female mice 19-24 g in weight in a single intraperitoneal dose of 5 mg/kg. This amount produced a virtually uniform 85-100% mortality by the sixth day in several hundred tests involving thousands of mice. The anti-histamines tripelennamine (Pyribenzamine hydrochloride) and dimethypyrindene maleate (Forhistal)‡ were dissolved in physiological saline solution. They were given in a single dose of 37.5 mg/kg for the former and 50 mg/kg for the latter. They were administered intraperitoneally to groups of mice, 10 mice per group, at intervals of 4 hours before and 4, 8, 24 or 48 hours after the HN_2 had been given. White cell counts were done on daily blood samples taken from the tip of the tail and daily body weights were recorded for all mice. Control groups of mice received only the HN_2 or the anti-histamines. When physiological saline solution was given intraperitoneally it had no effect on the incidence of surviving mice.

Results. The survival data are given in Tables I and II. It is apparent that both compounds produced a significant reduction of the lethal effect of the HN_2 when given 4 hours before the HN_2 and substantially the same or greater protection when given after it. There was a survival of 53 to 83% when either of the drugs was given 4 to 8 hours after the HN_2. This was consistently reproducible in 15 separate tests. When the drugs were administered 24 hours after the HN_2, the survival fell to 33 or 37%. At 48 hours, administration of the tripelennamine had only a slight protective effect (30% survival) in the HN_2-treated mice while dimethypyrindene maleate had virtually no effect. The weight of all mice initially fell but in those which had received the anti-histamine a plateau occurred by the fifth or sixth day, while the HN_2-treated controls continued to deteriorate (Table I).

White blood cell counts are given in Table III. The cell count of control HN_2-treated mice fell to levels of 2000-3000/cmm and remained at that level until death at the fifth to seventh days, or recovered slowly in the survivors. When anti-histamines were given just before or within 24 hours after the HN_2, a considerable leukopenia was observed; this appeared to be less severe than that of the controls, and there was a fairly rapid recovery to normal levels. The white cell count was almost always at a level above 5000/cmm about 6 days after the anti-histamines had been given. Anti-histamine-treated mice surviving the administration of HN_2 were observed for several months. Delayed

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† Supplied by Dr. Elmer Alpert, Merck & Co., Inc., Rahway, N. J.

‡ Supplied by Dr. S. S. Barkulis, CIBA Pharmaceutical Co., Summit, N. J.

TABLE I. Effect of HN_2 Alone or HN_2 with Pyribenzamine Hydrochloride on Survival and Body Weight of Mice.

	Control mice	Pyribenzamine-treated mice (HN_2 plus pyribenzamine)				
Surviving mice	(HN_2 alone) 11/80 (14%)	4 hr before 19/30 (63%)	4 hr after 26/40 (65%)	8 hr after 33/40 (83%)	24 hr after 11/30 (37%)	48 hr after 6/20 (30%)
Probability factor (P)		<.01	<.01	<.01	<.05	>.05
Day				Avg body wt (g)		
1	21.6	21.7	21.0	21.8	21.8	21.5
2	20.8	21.2	20.1	21.0	21.3	21.0
3	18.9	19.3	18.9	19.6	19.1	19.2
4	18.0	18.5	17.9	19.0	18.1	18.1
6	16.4	18.0	17.8	18.8	19.0	17.6
8	16.3	18.7	17.9	19.6	19.8	18.7
10	17.5	20.3	19.2	20.6	20.8	20.1
12		22.1	20.9	22.0	22.6	21.8

TABLE II. Effect of HN_2 Alone or HN_2 with Dimethypyrindene Maleate on Survival of Mice.

	Controls	Dimethypyrindene-treated mice (HN_2 plus dimethypyrindene)				
Surviving mice	HN_2 alone	4 hr before	4 hr after	8 hr after	24 hr after	48 hr after
	11/18 (14%)	8/20 (40%)	16/30 (53%)	29/40 (73%)	13/40 (33%)	3/20 (15%)
Probability factor (P)		<.05	<.01	<.01	<.05	>.05

TABLE III. Effect of HN_2 Alone or HN_2 with Pyribenzamine Hydrochloride on Average Leukocyte Count of Mice.

	Control mice	Pyribenzamine-treated mice (HN_2 plus pyribenzamine)				
Day	(HN_2 alone)	4 hr before	4 hr after	8 hr after	24 hr after	48 hr after
				White cell count per mm^3		
1	3646	4230	4429	3979	3984	3407
2	3176	4222	3904	3688	4292	3094
3	3016	3605	3419	3598	3205	3038
4	2596	2950	3413	3579	3150	3276
6	3108	4838	5310	5313	4800	5451
8	3414	5866	5217	5263	4788	3982
10	4518	5841	5777	5204	4950	4525
12		6923	6151	4741	6032	5850

deaths which could be attributed to the HN_2 were not seen and their white blood cell count remained virtually constant.

Discussion. The present report indicates that in the mouse the lethal, and to a lesser extent, the leukopenic effects of the alkylating agent, HN_2 can be consistently and significantly reduced by the anti-histamines, tripelennamine hydrochloride and dimethypyrindene maleate. This reaction is very similar in degree to the protective effects described for a serotonin antagonist(1,2) with survival 8 hours after treatment with either agent being alike. However, while the survival of mice receiving the serotonin antagonist 24 hours after HN_2 remained substantial (79%), that of the anti-histamine-treated mice was less (33 to 37%) as it was also less than the survival 8 hours after the HN_2 (73 to 83%).

It is a matter of considerable interest that the reduction of nitrogen mustard toxicity afforded by anti-serotonin drugs could also be accomplished by other chemical agents which have a similar biological function, the anti-histamine drugs. That there is a fundamental enzyme process affected by nitrogen mustard which is reversed by either the serotonin or histamine antagonists is now more likely since both types of agents produced similar effects(3). It would seem likely that serotonin or histamine antagonists reverse the same interaction of nitrogen mustard through an as yet unknown mechanism.

The practical implication of the above findings necessitate a preliminary statement of

work in progress. Serotonin or histamine antagonists do not interfere with the measurable inhibition of Sarcoma 180 or Ehrlich Ascites in mice, induced by HN_2. The antagonists afforded a measure of protection for the HN_2-treated tumor-bearing mice, similar to that reported here.

Summary. Intraperitoneal administration of either of 2 anti-histamine compounds before and after a single intraperitoneal nearly-lethal dose of nitrogen mustard had been given to mice produced a marked reduction of the mortality expected from the nitrogen mustard. The leukopenia resulting from the nitrogen mustard in combination with the anti-histamines was less in extent and duration.

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Assay of Mouse Adapted Dengue Viruses in Mammalian Cell Cultures by An Interference Method. (29117)

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Several authors have reported growth of dengue viruses in mammalian cell cultures with and without cytopathic effect(1-9). As yet, however, no *in vitro* system has been shown to be as sensitive as the suckling mouse for assay of all available dengue serotypes. Recent application of viral interference in tissue culture to the study or isolation of common cold(10), rubella(11) and several arthropod-borne viruses(12-15) stimulated a search for an analogous system for dengue viruses. This paper reports the successful use of an interference test to demonstrate the susceptibility of several primary and stable mammalian cell cultures to mouse adapted dengue virus types 1-4, and to 2 new dengue prototypes, TH-36 and TH-Sman, recovered from hemorrhagic fever patients in Thailand (16).

Materials and methods. Viruses. The following viruses were employed: dengue type 1, Hawaiian strain, 70th mouse passage; dengue type 2, Trinidad 1751 strain, 68th mouse passage; dengue type 3, strain H-87, 35th mouse passage; dengue type 4, strain H-241, 35th mouse passage; TH-36 (tentatively designated as dengue type 5 by Hammon)(16), 15th mouse passage; TH-Sman (tentatively designated as dengue type 6 by Hammon) (16), 15th mouse passage; chickungunya, Ross strain, 175th mouse passage; Coxsackie B1, strain unknown, multiple monkey kidney cell and 10 hamster kidney cell passages and polio type 1, strain unknown, with more than 50 monkey kidney cell passages. Dengue viruses were received from Dr. W. McD. Hammon, chickungunya from the Rockefeller Foundation Virus Laboratory and enteroviruses from the Virus Department, Walter Reed Army Institute of Research.

Cell cultures. Continuous cell cultures employed were as follows: grivet monkey kidney, BS-C-1(17), received in December 1962 from Microbiological Associates, Bethesda, Maryland via Walter Reed Army Institute of Research; rhesus monkey kidney, LLC MK2, series 2(18), received April 1963 from Dr. Robert Hull, Biological Research Division, Eli Lilly, Indianapolis, Ind.; porcine kidney cells, PS(19), received November 1962 from Dr. Y. Kanda Inoue, Virus Research Inst., Kyoto, Japan; and a Microbiological Associates strain of HeLa supplied December 1961 by Lt. Cdr. Irving Green, NAMRU-2, Taipei, Taiwan.

Trypsinized monolayers used were monkey