

EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS: THE  
EFFECT OF 6-MERCAPTOPYRIMIDINE\*

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Experimental allergic encephalomyelitis (EAE), an experimental model of demyelinating diseases of the central nervous system, has been intensively studied in recent years. Definition of the pathogenic mechanisms has been sought with a wide range of experimental approaches, but some of the basic questions are still unanswered. The relative role of delayed hypersensitivity and classical circulating antibody in the pathogenesis of the disease has been one of the most controversial of these questions. This problem is considered in several recent reviews of EAE (1-3).

One means of studying the character of this immunologic disease is to consider the ways by which it can be modified or prevented. Treatment with salicylates (4), ACTH and cortisone (5-7), and nitrogen mustard (8, 9) has been shown to affect the incidence and severity of EAE. None of these drugs was effective, however, if treatment was started after injection of the encephalitogenic preparation. Moreover, none of these experiments established that the disease was modified by the anti-immunologic activity of the drugs, and not by their antihematologic, anti-inflammatory, or non-specific toxic effects.

To consider whether or not EAE can be modified by a more specific suppression of immunologic processes, the effect of 6-mercaptopurine (6-MP) has been studied. The anti-immunologic properties of this drug, developed as an anti-leukemic, were first noted by Schwartz *et al.* (10). Their demonstration that 6-MP suppresses the primary antibody response to bovine serum albumin in rabbits stimulated the investigation of the effect of the drug on EAE and later studies in which it has been shown that the drug affects the secondary immune response (11), antibody production in highly sensitized rabbits (12), homograft rejection (13), inflammation (14), and delayed hypersensitivity (15).

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We have previously reported that 6-MP prevented EAE when rabbits were treated with the drug from the day of injection of the spinal cord-adjunct emulsion (16). This paper presents the results of further studies of the effects of 6-MP on EAE, with special emphasis on the prevention of the disease with delayed 6-MP administration, and a consideration of the possible mechanisms by which the drug may be affecting the disease.

### *Materials and Methods*

*Animals.*—Animals used in these experiments were albino hybrid rabbits of both sexes, weighing from 2 to 3 kg, obtained from a single local breeder, and Hartley strain albino guinea pigs of both sexes, weighing between 400 and 550 gm, obtained from a single breeder. These animals were kept in the laboratory for at least 2 weeks prior to experimentation to insure a disease-free stock acclimated to laboratory conditions. Except in the fasting experiment, they were offered laboratory chow and water *ad libitum*. Rabbits in the fasting study received only water. No antibiotic supplements were used.

*6-Mercaptopurine.*—6-Mercaptopurine (purinethol), kindly supplied by Burroughs, Wellcome and Co., Inc., Tuckahoe, New York, was dissolved in 1 N NaOH (0.15 gm/ml). Fresh preparations were made up every other day and kept under refrigeration. The solution had a pH of 10 and was mildly irritating when injected. In rabbits it was administered by intravenous injection in the marginal ear vein, and in guinea pigs it was administered intraperitoneally or intramuscularly into the deep muscles of the back.

The dosages of 6-MP used caused moderate toxic mortality in some experiments. Animals were considered to have died from the toxic effects of 6-MP if death occurred within the period of drug administration or during the 4 following days, the animals had shown no signs of paralysis, and histologic study of the central nervous system showed no lesions of EAE.

*Encephalitogenic Agent.*—Fresh whole rabbit spinal cord was freed from meninges and large blood vessels by forcing it through a stainless steel tissue press containing a no. 80 mesh copper wire screen. Rabbits were sensitized with an emulsion containing: 20 gm wet weight of spinal cord, 10 ml of complete Freund's adjuvant (Difco Laboratories, Detroit), 10 ml of bayol F (Esso Standard Oil Company, New York), 2 ml of arlcel A (kindly furnished by the Atlas Powder Company, Wilmington), and 500 mg. of killed, dried, ground *Mycobacterium butyricum* (Difco). Rabbits received 0.1 ml. of this emulsion intradermally in each hind foot pad.

The encephalitogenic emulsion for guinea pigs was prepared with smaller quantities of nervous tissue and *Mycobacterium*. In experiments GP-1 and GP-2 it contained: 500 mg of fresh rabbit spinal cord in 20 ml of saline, 17 ml of bayol F, 3 ml of arlcel A, and 100 mg of the *Mycobacterium*. In experiments GP-3 and GP-4 it contained: 1 gm of lyophilized rabbit spinal cord, 8.5 ml of bayol F, 1.5 ml of arlcel A, and 100 mg of *Mycobacterium butyricum*. Each guinea pig received 0.1 ml of the emulsion intradermally on the anterior aspect of the thorax.

These encephalitogenic preparations resulted in at least 75 per cent incidence of EAE in control rabbits and guinea pigs.

*Observation.*—Signs of EAE appear 11 to 18 days after the injection of the spinal cord-adjunct emulsion, and include weakness, weight loss, paralysis, loss of sphincter control, and death. Paralysis is the most consistent sign of the disease, and was the index used in all these studies. Animals were observed daily and were considered to have EAE only if definite signs of paralysis were observed in either the front or hind quarters. Central nervous system tissue of treated and control animals was examined to compare the clinical and histologic signs.

*Histologic study.*—Sections of the brain and spinal cord were examined in animals sacrificed during the experiments, in all animals that died without prior paralysis, and in about one-third of those that died after demonstrating paralytic signs of EAE. At least three sections of each of five areas of the central nervous system: lumbar spinal cord, cervical spinal cord, medulla, pons, and cerebrum, were examined in each case. The tissue was fixed in formalin and stained with hematoxylin and eosin. The pathologic material was evaluated without knowledge of the clinical course of the animal.

TABLE I  
*Effect of Early Treatment with 6-MP on EAE in Rabbits*

Experiment No.	6-MP dosage	Treatment period	6-MP mortality	Incidence of paralysis§			
				By day 18		By day 40	
				6-MP-treated	Controls	6-MP-treated	Controls
	<i>mg/kg/day</i>	<i>days*</i>					
R-1a	6	0 to 18	0/10	4/10	} 8/9	8/10	} 9/9
R-1b	9	0 to 18	0/9	3/9		9/9	
R-1c	12	0 to 18	1/8	0/8		6/7	
R-2	12	–2 to 18	2/9	0/9	6/9	6/7	8/9
R-3	12	0 to 18	3/13	0/11	8/10	6/7	10/10
Summary of R-1c R-2, and R-3	12		6/30 (20%)	0/28 (—)	22/28 (80%)	18/21‡ (86%)	27/28 (96%)

\* Day 0 = day of injection of spinal cord

‡ Three animals were sacrificed on day 18.

§ Numerator is cumulative incidence of paralysis (including those few deaths from EAE not preceded by observed paralysis, but confirmed histologically); the denominator is the number of animals in the experiment less the number of deaths from 6-MP toxicity.

## RESULTS

*Early Treatment with 6-MP.*—Rabbits were given daily intravenous injections of 6-MP in several dosages, beginning on the day of injection of the encephalitogenic preparation and continuing for 18 days. Early studies indicated that most rabbits tolerated 18 days of 6-MP treatment fairly well, but that signs of severe 6-MP toxicity appeared when the drug was continued for longer periods.

Table I summarizes the results of a series of experiments using three dosage levels of 6-MP. It is apparent that the lower dosages of 6-MP (6 and 9 mg/kg/day) reduced the incidence of paralysis by day 18, although some treated animals showed clinical signs of EAE. When 6-MP was given in a dosage of 12 mg/kg/day, however, it completely prevented paralysis during the period of drug administration. Data from experiments R-1c, R-2 and R-3 are graphed in Fig. 1 to demonstrate the latent period which separated the discontinuation of 6-MP treatment and the onset of paralysis. After 6 to 20 days these rabbits

developed paralytic encephalomyelitis which differed in no way from the disease observed in control animals.

Histologic study confirmed the clinical observations. 3 animals from experiment R-3 were sacrificed on day 18, and autopsies were performed on the 6 animals that died of 6-MP toxicity. All had normal central nervous system tissue and no histologic signs of EAE. Nervous tissue of 11 rabbits which became paralyzed after discontinuation of 6-MP was also examined. These sections showed the characteristic perivascular inflammatory lesions of EAE (1, 2).

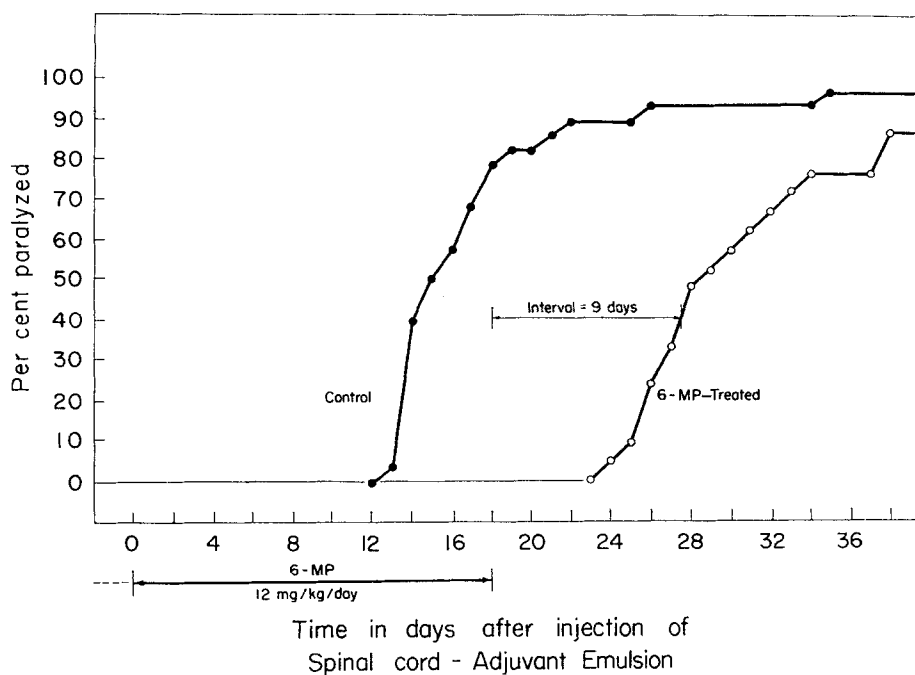


FIG. 1. The effect of 6-MP on EAE in rabbits.

*Delayed Treatment with 6-MP.*—To characterize more generally the effects of 6-MP, we investigated treatment schedules started after the injection of the spinal cord-adjuvant emulsion. Since control rabbits first show paralytic signs of EAE after day 12, treatment was initiated 9 and 12 days after administration of the encephalitogenic preparation, during what would seem to be the most active period of the immunologic reaction. The data of Table II indicate the effectiveness of 6-MP in suppressing EAE, despite the delay in treatment.

A better picture of the relationship between the period of 6-MP treatment and suppression of EAE is presented in Figs. 2 and 3 which graph the data of

experiments R-5 and R-6 (Table II). The dosage level was 18 mg/kg/day, administered from day 9 (Fig. 2) and day 12 (Fig. 3). When treatment was started on the 9th day, there were statistically significant differences in the incidence of paralysis in control and 6-MP-treated animals from day 14 to day 23 ( $p$  was less than 0.05 when the two groups were compared by the chi-square test with the Yates modification). When 6-MP treatment was delayed until the 12th day (Fig. 3), the incidence of paralysis in the treated and control

TABLE II  
*Effect of Delayed Treatment with 6-MP on EAE in Rabbits*

Experiment No.	6-MP dosage mg/kg/day	Treatment period days*	6-MP mortality	Incidence of paralysis†			
				By day 18		By day 40	
				6-MP-treated	Controls	6-MP-treated	Controls
R-4	12	9 to 25	5/10	2/10	8/10	3/5	10/10
R-5a	18	9 to 18	1/10	1/10	7/10	8/9	7/10
R-6a	18	9 to 18	3/10	2/8	8/9	7/7	9/9
Summary . . . . .		After day 9		5/28 (18%)	23/29 (79%)	18/21 (86%)	26/29 (90%)
R-5b	18	12 to 21	1/10	4/10	7/10	6/9	7/10
R-6b	18	12 to 21	1/9	5/9	8/9	6/8	9/9
Summary . . . . .		After day 12		9/19 (47%)	15/19 (79%)	12/17 (71%)	16/19 (84%)

\* Day 0 = day of injection of spinal cord

† Numerator is cumulative incidence of paralysis (including those few deaths from EAE not preceded by observed paralysis, but confirmed histologically); the denominator is the number of animals in the experiment less the number of deaths from 6-MP toxicity.

groups was about the same until day 14. Very few 6-MP-treated rabbits became paralyzed after day 15, however, while there was no change in the rate at which control animals became paralyzed. The difference between the control and 6-MP-treated groups was not statistically significant ( $p$  was 0.09 for day 18) because so many treated rabbits had become paralyzed by day 15, before drug treatment began to have any effect. The break in the curve was most striking, however, and a relative plateau after day 15, such as that of Fig. 3, has never been observed in control groups in which EAE antigen has been administered to over 500 rabbits.

Once paralytic signs of EAE have developed, spontaneous remissions are very rare. Although 6-MP injections were continued in the animals which

became paralyzed from days 12 to 15 (Fig. 3), remissions were not observed. Thus, 6-MP prevented EAE in many animals even when its administration was delayed for 12 days, but had no effect when paralysis was imminent or already present.

*The Latent Period after Discontinuation of 6-MP Treatment.*—In all of the experiments in which EAE was prevented, the animals remained well for

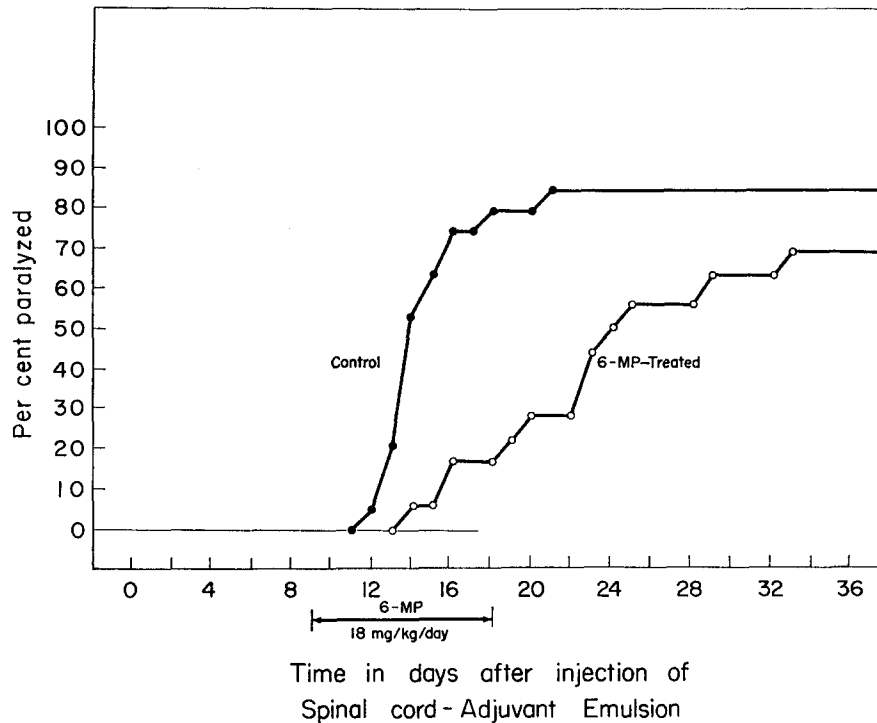


FIG. 2. The effect of 6-MP on EAE in rabbits when treatment started on day 9.

several days after discontinuation of 6-MP, but developed paralytic signs of the disease after a latent period. To compare the development of paralysis in different groups of animals, we determined the time interval before 40 per cent of the rabbits became paralyzed in each experiment. This arbitrary value, equal to one-half the total incidence of paralysis in most experiments, proved to be a highly reproducible measure for the several control groups as well as for separate treated groups with the same protocol.

As Figs. 1 and 4 indicate, 40 per cent of control rabbits were paralyzed by the 14th day after spinal cord-adjutant injection, while 40 per cent of the 6-MP-treated animals became paralyzed 9 days after the drug was discontinued,

whether it had been given for 9 days (Fig. 4) or 18 days (Fig. 1). Four other treatment schedules were also investigated (Table III) and each was followed by a latent period of either 9 or 10 days, regardless of the duration of 6-MP treatment. The group given 6-MP for 5 days is a good example. Paralysis was not delayed for 5 days, but developed at the same rate as in the control animals, so that 40 per cent of the animals were paralyzed by the 14th day (9 days after 6-MP was discontinued).

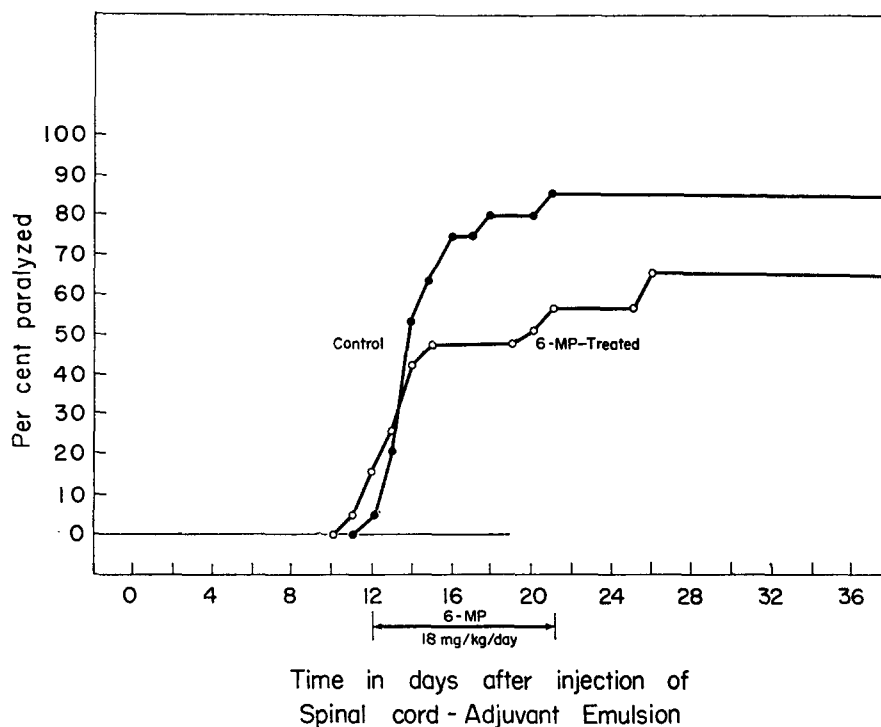


FIG. 3. The effect of 6-MP on EAE in rabbits when treatment started on day 12.

*The Effect of 6-MP on EAE in Guinea Pigs.*—Field's (17) report that 6-MP did not prevent EAE in the guinea pig suggested that the effect of 6-MP on EAE might only obtain in rabbits. Our own studies, however, summarized in Table IV, showed suppression of EAE by 6-MP in the guinea pig as well, but we found that dosage and route of administration were more critical in this species than in the rabbit.

When the drug was given to guinea pigs by the intraperitoneal route, a large dosage was required to obtain an effect. As shown in Table IV, the onset of paralysis was delayed considerably in guinea pigs given 150 mg/kg/day intra-

peritoneally for 12 days. The drug was more effective, however, when administered into the deep muscles of the back. Nine daily injections of 50 mg/kg/day by this route prevented the development of EAE, with little increase in 6-MP mortality.

As in the case of the rabbit, delayed treatment with 6-MP was effective in preventing the disease. Treatment started 5 days after the injection of the

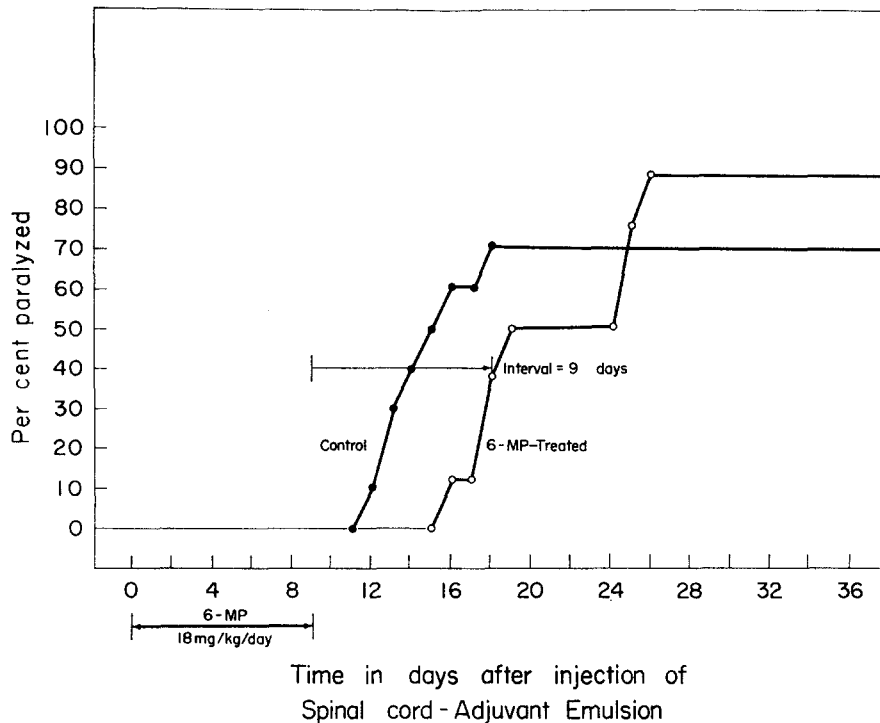


FIG. 4. The development of paralysis after discontinuation of 6-MP treatment, 18 mg/kg/day given for 9 days.

spinal cord-adjuvant emulsion prevented paralytic signs of EAE, but when 6-MP was not given until the 7th day, it had little effect on the disease (Table IV).

Hence, although larger and more toxic dosages of 6-MP are required in the guinea pig than in the rabbit, the drug effectively suppresses EAE in both species. This is in contrast with x-irradiation, which does not delay or suppress EAE in the guinea pig, but is effective in the rabbit (18, 19).

*The Effect of 6-MP on Leukocytes.*—To consider the possibility that the effect of 6-MP on EAE was due to leukopenia or neutropenia, total and differential



leukocyte counts were determined on animals in several experiments: R-2 and R-3 (Table I), and R-6a and b (Table II). 6-MP did not produce a profound leukopenia in any of these experiments. Fig. 5 illustrates the findings in the

TABLE III  
*Development of EAE after Discontinuation of 6-MP Treatment in Rabbits*

Number of rabbits	6-MP dosage	Last day of 6-MP treatment*	Day 40 per cent of animals paralyzed†	Interval between last day of treatment to day of 40 per cent incidence
	mg/kg/day			
48§	—	—	14§	—
8	12	3	13	10
9	12	5	14	9
8	12	7	17	10
8	18	9	18	9
9	12	13	23	10
21	12	18	27	9

\* 6-MP was started on the day of administration of the spinal cord-adjutant preparation in all but the 48 control animals (given the EAE antigen only).

† The day of 40 per cent incidence of paralysis in each group was approximated (to the nearest day) from a graph of the cumulative incidence of paralysis as a function of time, as illustrated in Figs. 1 and 4.

§ The control summary is composed of 5 groups of 9 or 10 rabbits each. 40 per cent paralysis was noted by day 13 in one group, day 14 in 3 groups, and day 15 in one group.

TABLE IV  
*Effect of Early and Delayed Treatment with 6-MP on EAE in Guinea Pigs*

Experiment No.	6-MP dosage	Treatment period	6-MP mortality	Incidence of paralysis			
				By day 14		By day 28	
				6-MP-treated	Controls	6-MP-treated	Controls
	mg/kg/day	days*					
GP-1	150, ip	0 to 12	12/25	3/13	9/10	9/13	10/10
GP-2	50, im	0 to 9	17/30	0/13	10/17	4/13	13/17
GP-3	50, im	5 to 14	3/10	0/8	7/8	4/7	7/8
GP-4	50, im	7 to 16	1/10	4/9	7/8	7/9	7/8

\* Day 0 = day of injection of spinal cord emulsion

animals of experiment R-3, treated with 12 mg/kg/day of 6-MP for 18 days, and is representative of the data from the other groups. The total number of leukocytes and granulocytes rose in the control rabbits while the lymphocyte level remained relatively constant. In the 6-MP-treated animals, the average total white count fell slightly (10 to 30 per cent, but never below 7000 cells/

mm<sup>3</sup>), as did the granulocytes (10 to 50 per cent, but never below 2000 cells/mm<sup>3</sup>), but the number of lymphocytes (3000 to 6000 cells/mm<sup>3</sup>) again remained relatively constant.

The 20 per cent toxic mortality might be considered evidence of leukopenia; however, only 2 of 10 rabbits dying of 6-MP toxicity during hematologic study had decreased granulocytes or lymphocytes when last tested before death.

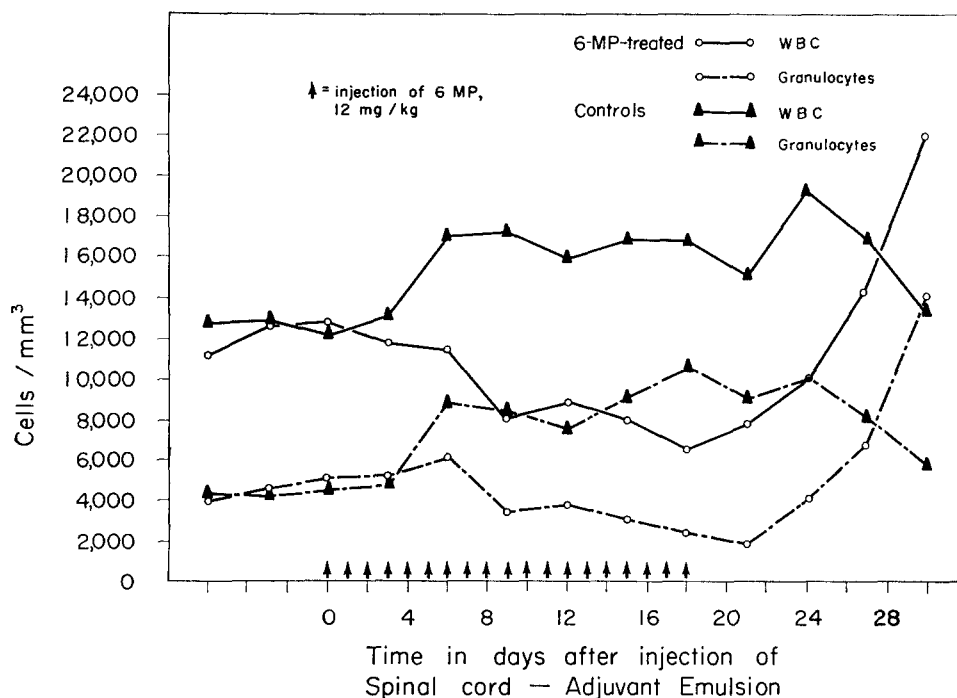


Fig. 5. The effect of 6-MP on leukocytes in rabbits stimulated with a spinal cord-adjuvant emulsion.

Autopsy of animals dying from 6-MP toxicity often revealed gastrointestinal obstruction and ulceration, and peritonitis: findings consistent with reports of a relatively high concentration of 6-MP in the rapidly proliferating epithelial tissue of the gastrointestinal tract (20) and a high incidence of gastrointestinal lesions after 6-MP treatment (21).

*Non-specific Toxicity and Debilitation.*—Because the most effective 6-MP dosage was quite high (12 mg/kg/day in rabbits) and resulted in 20 per cent mortality, one might consider the suppression of EAE a reflection of toxicity and debilitation rather than specific effects of 6-MP on immunologic processes. The majority of the animals did not show signs of debilitation, however, and

were healthy enough to manifest the disease after the drug was discontinued. Moreover, when lower dosages of 6-MP were used (6 and 9 mg/kg/day, Table I), there was a delay in development of EAE in the absence of any toxic signs.

The data in Tables V and VI emphasize the lack of correlation of signs of toxicity and suppression of EAE. Rabbits were pretreated with 6 mg/kg of 6-MP for 12 days, with completion of the course 2 days before administration

TABLE V  
*Effect of Pretreatment with 6-MP on EAE in Rabbits*

Treatment	6-MP Mortality	Incidence of Paralysis	
		By day 16	By day 40
6-MP, 6 mg/kg/day, Days -13 to -2*.....	2/10	5/8	8/8
Control rabbits, not given 6-MP.....	—	3/9	9/9

\* Day 0 = day of injection of spinal cord-adjuvant emulsion

TABLE VI  
*Comparative Effects of 6-MP Treatment and Fasting on Weight and Incidence of EAE*

Treatment*	Number of Animals	Mean weight on		Weight loss over the 15 days		No. of rabbits paralyzed on day 15	Effect of treatment on EAE
		Day 0	Day 15	kg	per cent		
		kg	kg				
None.....	19	2.60	2.29	0.31	12	12/19	—
6-MP, 12 mg/kg/day, Days 0 to 18 (8 rabbits), Days -2 to 18 (9 rabbits).....	17	2.63	2.27	0.36	14	0/17	Complete suppression
Fasting, days 0-15.....	10	2.40	1.61	0.79	33	5/10	None

\* All animals received 0.1 ml of spinal cord-adjuvant in each hind foot pad on day 0.

of the encephalitogenic emulsion (Table V). While this dosage did not cause any toxic mortality in rabbits which had previously received the encephalitogenic emulsion (Experiment R-1a, Table I), this schedule caused moderate weight loss and 20 per cent toxic mortality in these unsensitized rabbits. In spite of the evident toxic effects of 6-MP in the rabbits pretreated with 6-MP, the subsequent development of EAE was not delayed. Rather, these animals showed paralytic signs somewhat earlier than the control animals.

The weight loss in 6-MP-treated rabbits in experiments R-1c and R-2 was no greater than in control animals developing EAE (Table VI). To consider more directly the factors of weight loss and debility, a group of rabbits was

fasted for 15 days after administering of the encephalitogenic emulsion. Fasting produced much greater weight loss (Table VI) and more evident debility than 6-MP treatment, but did not prevent the development of paralytic signs of EAE.

#### DISCUSSION

Before concluding that 6-MP suppressed EAE by affecting a basic part of the immunologic response to the nervous tissue antigen, we have to consider a number of other possible mechanisms. We believe that the evidence indicates that 6-MP did not merely mask clinical signs of the disease, and that its effects were not the result of leukopenia, non-specific toxicity or debilitation, or anti-inflammatory activity.

The histologic studies, the eventual development of EAE in the 6-MP-treated animals, and the length of the latent period all suggest that 6-MP did not merely mask clinical signs of the disease. Histologic study of central nervous system tissue from animals sacrificed or dying during the period of drug administration revealed no lesions of EAE. After 6-MP was discontinued, the treated rabbits became paralyzed. In this way, each animal served as its own control, and showed that an adequate encephalitogenic stimulus had been injected. None of the treated animals became paralyzed until 6 days after 6-MP treatment was discontinued, however. Since 6-MP activity is virtually absent 24 hours after injection (20), the length of the latent period also argues against the masking effect.

6-MP's suppression of EAE cannot be ascribed to leukopenia, neutropenia, or non-specific toxicity and debilitation. Although 6-MP prevented the usual granulocytic response to the spinal cord-adjuvant emulsion, it did not produce either severe leukopenia or neutropenia. The 6-MP-treated rabbits lost no more weight than the control animals given only the encephalitogenic antigen, yet the disease was completely suppressed. In contrast, fasting caused marked weight loss and severe debilitation, but did not prevent the disease.

Finally, 6-MP might have prevented EAE by its anti-inflammatory effect. This seems unlikely, however, for at least 6 days of pretreatment were necessary for 6-MP to have even a moderate anti-inflammatory effect in rabbits, and marked suppression of inflammation developed only after 8 days of pretreatment (14). In contrast, 6-MP suppressed EAE 3 days after treatment was started. While 6-MP-treated animals did not become paralyzed until the drug had been stopped for 6 days, normal inflammatory cycles were observed in rabbits 2 days after discontinuation of 6-MP administration (14). If the prevention of EAE were a result of 6-MP's anti-inflammatory properties, one would have expected an earlier onset of paralysis after discontinuation of treatment.

How, then, does the drug affect EAE? It seems to us to be interfering with a basic part of the immunologic response to the nervous tissue-adjuvant emul-

sion and suppressing the development of immunologically competent cells and/or antibody formation. 6-MP has been shown to interfere with cellular metabolism in several ways (22). Especially suggestive is the demonstration that the drug limits nucleic acid biosynthesis by inhibiting the essential conversion of inosine ribonucleotide to adenine ribonucleotide (23). The synthesis of adenine-containing coenzymes is also inhibited by 6-MP (24, 25). By one or more of these several metabolic effects, 6-MP modifies immunologic activity.

Our findings emphasize that 6-MP's effectiveness must be considered in the light of the species, the route of injection, the dosage, the duration of treatment, and the relationship of the treatment period to antigenic stimulation. Moreover, one must specify the nature and intensity of the stimulation. The effect of each of these variables has been illustrated in these experiments.

The dosage of 6-MP used is of great importance, and a relatively small increase, as from 9 to 12 mg/kg/day, was associated with striking enhancement of effect. Negative reports on the effects of 6-MP (17, 26) may reflect inadequate dosage rather than ineffectiveness of the drug. Toxicity is a limiting factor, with reference to both dosage and duration, but it was our experience that the drug's anti-immunologic properties were more sensitive to dosage than were the toxic effects.

These studies showed very clearly that 6-MP's effectiveness depends on continued administration. Very short courses of treatment had little effect, and rabbits developed EAE at the same rate as controls if given 6-MP for less than 6 days. Pretreatment alone, with no 6-MP after injection of the spinal cord, was also ineffective. It is apparent that 6-MP must be given in a manner quite different from that used for cytotoxic drugs (*e.g.* nitrogen mustard) and x-irradiation. Only when the antigen is essentially eliminated, as following primary stimulation with a simple protein antigen (10), can 6-MP be discontinued without subsequent evidence of immunologic activity. If the antigen persists in the stimulated animals, as it does when the encephalitogenic antigen is given in Freund's adjuvant, a return of immunologic function follows the discontinuation of drug treatment (13).

One of the most debated aspects of EAE concerns the nature of the hypersensitivity involved in its pathogenesis. Both circulating antibody and delayed hypersensitivity have been suggested as essential, but at present neither has experimental support which is not subject to alternative interpretation. It was hoped that these studies with 6-MP might contribute to the understanding of this problem.

The initial immunologic studies with the drug established its effect on antibody production following primary stimulation (10), and subsequent experiments have shown that the drug suppresses antibody production following secondary stimulation (11) and in highly stimulated animals producing large quantities of antibody (12). Hoyer and Condie have demonstrated that 6-MP

prevents delayed hypersensitivity after BCG infection in both rabbits and guinea pigs (15), so there does not seem to be any fundamental difference between this phenomenon and antibody production in response to early 6-MP treatment.

Delayed hypersensitivity and antibody production differ in their response to delayed treatment with 6-MP, however. Antibody production in the rabbit is very sensitive to delayed treatment with 6-MP, both in the primary immune response (10) and in repeatedly stimulated animals (12). In contrast, delayed treatment does not affect tuberculin hypersensitivity in rabbits. Although 6-MP completely prevented delayed sensitivity if treatment was started on the same day as BCG infection, large doses of the drug had no effect when initial treatment was delayed for as little as 5 days (15). 6-MP treatment can be delayed for 9 or 12 days, however, and still suppress EAE. Although indirect, this is further evidence for the hypothesis that circulating antibody is essential in the pathogenesis of EAE in the rabbit.

The temporal relationships of 6-MP treatment and onset of disease in these studies suggest that two phases of activity separate administration of the encephalitogenic emulsion and the appearance of paralytic signs of EAE. Rabbits normally develop paralysis (40 per cent incidence) in 14 days, while 6-MP-treated animals become paralyzed (40 per cent incidence) 9 or 10 days after discontinuance of treatment. Although the activity of the other 4 or 5 days is essential in the pathogenesis of EAE, it is not affected by the 6-MP treatment. This period may be non-immunologic, perhaps a period of accumulation of antigen in the popliteal nodes after the emulsion has been injected in the hind foot pads. Alternatively, the 4 or 5 day difference may indicate that the early stages of the immunologic response to the EAE antigen are less sensitive to 6-MP than late "productive" stages.

The prevention of an experimental autoimmune disease, EAE, with 6-MP, may have clinical implications. Dameshek and Schwartz have used 6-MP in treating patients with some human "autoimmune" diseases (acquired hemolytic anemia, lupus erythematosus, and lupoid hepatitis) and they have reported good results (27). Page (14,28) subsequently treated three young women with plasma cell hepatitis, and in each case 6-MP treatment was followed by remission of the liver disease (14,28).

Drug-induced remissions in human autoimmune diseases are not easily understood if it is considered essential that 6-MP treatment accompany the initial antigenic stimulation. As Schwartz has suggested, the effectiveness of delayed treatment in preventing EAE and suppressing antibody production may be analogous to the drug's effect in the human diseases (29). An alternative explanation has been offered by Page, who attributes the remissions in cases of plasma cell hepatitis to the anti-inflammatory properties of 6-MP (14).

Many investigators have suggested that EAE has the same basic pathogene-

sis as a number of human neurologic diseases, including the acute encephalitides which follow vaccination and the exanthems of childhood (30, 31). These develop so quickly that considerable neurologic disturbance is invariably present when treatment is initiated and it seems unlikely, as with EAE, that 6-MP could reverse the changes which have already occurred.

In subacute and chronic neurologic diseases, however, the immunologic component of pathogenesis—if there is one—might be more amenable to 6-MP treatment. Some neuropathologists consider EAE a useful model for multiple sclerosis (30–34) and feel that allergy is very important in that disease. If continuing immunologic activity is necessary in the development of this demyelinating disease, 6-MP offers possible treatment. It seems reasonable to consider experimental use of 6-MP in patients with multiple sclerosis, since there is as yet no satisfactory treatment.

#### SUMMARY

1. 6-Mercaptopurine (6-MP) prevents experimental allergic encephalomyelitis (EAE) during the period of drug administration in both rabbits and guinea pigs. The disease is suppressed even when treatment is started as late as the 5th day after antigenic stimulation in guinea pigs and the 12th day in rabbits.

2. After discontinuation of 6-MP treatment, there is a latent period before the disease is noted. The length of this latent period is not modified by the duration of 6-MP treatment.

3. The effect of 6-MP on EAE is not the result of leukopenia, non-specific toxicity and debilitation, anti-inflammatory activity, or mere masking of clinical signs of the disease. It is, rather, the result of 6-MP's specific anti-immunologic activity.

4. The effects of 6-MP on antibody production, delayed hypersensitivity, and EAE are compared. This provides indirect evidence for the importance of circulating antibody in the pathogenesis of EAE.

5. The important considerations in the use of 6-MP are discussed and the possible usefulness of 6-MP in human neurologic diseases is considered.

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