Prevention of Experimental Allergic Encephalomyelitis with 6-Mercaptopurine.* (25460)

LEON W. HOYER,† RICHARD M. CONDIE‡ AND ROBERT A. GOOD
Pediatric Research Laboratories, Variety Club Heart Hospital, Univ. of Minnesota

Despite extensive investigation, the mechanism whereby administration of nervous tissue in adjuvants results in production of experimental allergic encephalomyelitis (EAE) remains to be conclusively demonstrated(1). Extensive indirect evidence suggests that EAE has, in a poorly understood manner, an immunologic basis(1,2). Although development of EAE has been prevented by a number of agents which interfere with the immune response, repeated failures to achieve passive transfer of the disease with serum or cells from involved animals to normal animals raises a question concerning other evidence indicating that EAE is an immunological disease. Studies by Sterzl(3) on use of 6-mercaptopurine to influence the immune response, and the more recent demonstration by Schwartz(4) that 6-MP will prevent production of circulating antibody in response to first parenteral injection of simple protein antigens, would seem to provide another approach to elucidation of the pathogenic mechanisms in development of EAE. This paper reports results in which treatment with 6-MP prevented development of EAE.

Material and methods. Rabbits employed were albino hybrids of both sexes, weighing 2-3 kg, obtained from a single breeder and kept in the laboratory for at least 2 weeks prior to experimentation to insure a disease-free stock acclimated to laboratory conditions. 6-MP§ was dissolved in 1N NaOH (0.15 g/ml) and the solution kept under refrigeration. Fresh solution was made up every other day. All injections of 6-MP were made into marginal ear vein. The encephalitogenic agent was whole rabbit spinal cord, freed of menінges and large blood vessels by being forced through stainless steel tissue press containing #80 mesh copper wire screen, and emulsified in a modified Freund's adjuvant: 20 g wet weight spinal cord; 10 ml complete Freund's adjuvant (Difco); 500 mg ground, killed, dried Mycobacterium butyricum; 10 ml Bayol F; and 2 ml Arlacel A. Rabbits received 0.1 ml of this emulsion intradermally in each hind footpad. Five groups of animals were used. Group 1a received 12 mg/kg/day, starting 2 days prior to injection of spinal cord-adjuvant emulsion and continuing through 18th day after injection. Group 1b (12 mg/kg/day), Group 2 (9 mg/kg/day), and Group 3 (6 mg/kg/day) received 6-MP daily, starting day of injection of spinal cord-adjuvant emulsion and continuing through 18th day after injection. Each experimental group contained 10 animals. A group of 20 animals served as controls and received no injections with 6-MP.

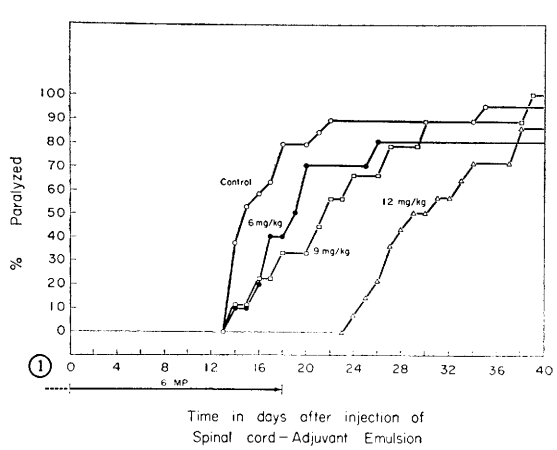
Results. Although EAE may be manifested by paralysis, tremors, convulsions, coma, and death, the most consistent clinical manifestation of this disease in rabbits is paralysis. Indeed, in our experience paralysis is a most satisfactory index for occurrence of EAE in this species. In observations of EAE in more than 500 rabbits, paralysis has never been present without confirmatory histologic lesions, including perivascular cuffing and evidence of demyelination or necrosis within the central nervous tissue. On the other hand, in almost all animals with demonstrable histologic lesions, paralysis of some degree has been observed. Alvord, Magee, Kies, and Goldstein(5) report that in the guinea pig, as well, paralysis is almost always associated with microscopic lesions of the central nervous system. Consequently, in this study, paralysis was used as primary index of presence or absence of disease. Animals were observed daily

* Aided by grants from U. S. Public Health Service, Am. Heart Assn., Minn. Heart Assn., and Minn. Division of Am. Cancer Soc.

† National Science Foundation Medical Student Summer Research Fellow.

‡ Research Fellow of Child Development Study Group, Univ. of Minnesota.

§ "Purinethol" 6-Mercaptopurine was generously provided by Burroughs Wellcome & Co.



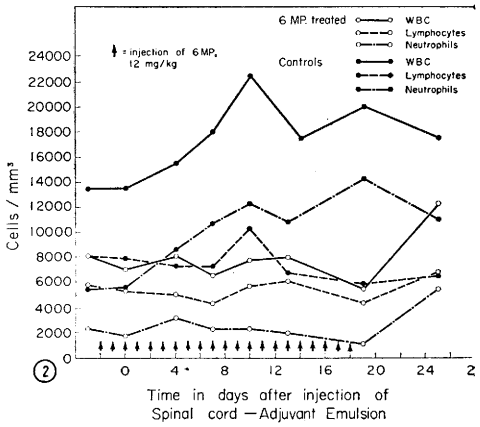


FIG. 1. Effect of 6-MP on experimental allergic encephalomyelitis.

FIG. 2. Effect of 6-MP on leukocytes in rabbits stimulated with a spinal cord-adjuvant emulsion.

and were considered to have the disease only if definite signs of paralysis were observed in either the front or hind quarters. In a number of animals dying of EAE, histological examination revealed the characteristic lesions (1).

Fig. 1 shows the time course of development of paralysis, Groups 1a and 1b being plotted together. Eight of the 60 animals in Groups 1-3 were not used to determine percentages of paralyzed animals given in Fig. 1. These animals include: one of the control group which died on fourth day after injection of spinal cord-adjuvant emulsion; one animal of Group 3 and 3 animals of Group 1 which died immediately following heart puncture; and 3 animals of Group 1 which died of apparent toxicity due to 6-MP. (All died on nineteenth day after injection of spinal cord-adjuvant emulsion.) These animals showed no evidence of paralysis prior to death. Although lower dosages of 6-MP (6 and 9 mg/kg/day) delayed appearance of the disease, they did not prevent appearance of paralysis even while they were being given. However, when 6-MP was given in a dosage of 12 mg/kg/day, it completely prevented development of the disease during drug administration.

Because previous studies had indicated that animals given 12 mg/kg/day of 6-MP remained in apparent good health for 18-20 days, and then began to sicken and die if injections of 6-MP were continued, administration of the drug was discontinued on eighteenth day in this experiment. After discontinuation of the drug, and following a latent period of 7-18 days, the rabbits developed paralytic encephalomyelitis which differed in no way from the disease observed in controls. It is notable, however, that the interval between discontinuation of 6-MP and appearance of paralysis was somewhat shorter than the interval between injection of spinal cord-adjuvant emulsion and appearance of paralysis in control animals. It is unlikely that 6-MP merely masked clinical manifestations of EAE in this group, for after drug treatment was discontinued at least 7 days elapsed before paralysis due to EAE was observed in these animals.

Effect of 6-MP on circulating leukocytes. Administration of 6-MP to animals in Group 1a produced slight depression in total number of circulating leukocytes (Fig. 2). Continued administration of 6-MP to animals not stimulated with an antigen, results in a severe leukopenia(6), but this was prevented in our experiments by the stimulation provided by injection of the spinal cord-adjuvant emulsion. The hematologic data for a group of control rabbits are also given in Fig. 2. It is apparent that the increase in total number of leukocytes associated with EAE is due to increase in numbers of neutrophils. In these control animals, as well as in 6-MP-treated animals, the number of lymphocytes in peri-

pheral blood remained nearly constant. 6-MP in these doses prevents the increase in circulating neutrophils and produces a mild neutropenia.

Discussion. A number of studies have shown that it is possible to inhibit or diminish the severity of EAE. These have included excision of lymph nodes draining the injection site, irradiation of these nodes prior to injection of the spinal cord-adjuvant emulsion, prior injections of aqueous suspensions of nervous tissue in newborns(7) and adult animals(8) and treatment with salicylates(9), ACTH and cortisone(10,11,12).

Our experiment indicates that a third type of chemical, a nucleic acid antimetabolite, will also prevent occurrence of EAE while it is being given. The effect of 6-MP on immune response is probably a result of its antimetabolic properties. Schwartz *et al.*(4,13) found that antibody production is inversely related to dose of 6-MP administered, and interpreted this to mean that the drug specifically interferes with a first-order chemical reaction rather than having a general cytotoxic effect similar to that of nitrogen mustard and X-irradiation. Whether DNA metabolism or RNA metabolism is affected is not clear at present. Interference with either induced proliferation or differentiation of cells may be the basis of this effect.

Only very large doses of 6-MP prevented occurrence of encephalomyelitis. However, the majority of rabbits so treated tolerated the drug quite well and, following its discontinuation, were healthy enough to express the disease in its characteristic form. Certainly the rabbits were not debilitated during treatment with 6-MP and the effects observed cannot be attributed to drug toxicity *per se*. It is of interest that, even with the intensive stimulus represented by injection of spinal cord-adjuvant emulsion administered, some delay in development of EAE was observed with non-toxic doses of 6-MP (6 mg/kg and 9 mg/kg).

While the immunological nature of EAE seems well established, the nature of the hypersensitivity involved is not yet clear. The effect of 6-MP on delayed hypersensitivity has not been reported, but Schwartz *et al.*(13) were able completely to suppress formation of circulating antibody by continued injections of 6-MP. Whether or not 6-MP will aid in identifying the nature of the hypersensitivity involved in pathogenesis of EAE, remains a question to be answered by further experimentation. It is clear, however, that EAE can be suppressed by 6-MP without a reduction in circulating level of lymphocytes, the cell type most usually implicated in the mechanism of delayed hypersensitivity.

Summary. Experimental allergic encephalomyelitis is prevented during administration of 12 mg/kg/day of 6-Mercaptopurine. The number of lymphocytes in peripheral blood is not reduced during this period in which 6-MP prevents EAE, although the number of neutrophils is reduced by half.

1. Condie, R. M., Good, R. A., *Progress in Neurobiology: IV. The Biology of Myelin,* 1959, S. R. Korey, Ed., Hoeber-Harper, New York, p321.

2. Waksman, B. H., *Int. Arch. Allergy App. Imm.,* 1959, Suppl. 14, 1.

3. Sterzl, J., Holub, M., *Csl. biol.,* 1957, v6, 75.

4. Schwartz, R., Stack, J., Dameshek, W., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 164.

5. Alvord, E. C., Jr., Magee, K. R., Kies, M. W., Goldstein, N. P., *J. Neuropath. and Exp. Neurol.,* 1959, v13, 442.

6. Philips, F. S., Sternberg, S. S., Hamilton, L., Clark, D. A., *Ann. N. Y. Acad. Sci.,* 1954, v60, 283.

7. Paterson, P. Y., *ibid.,* 1958, v73, 811.

8. Condie, R. M., Monson, R., Good, R. A., *Fed. Proc.,* 1959, v18, 563.

9. Good, R. A., Campbell, B., Good, T. A., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 341.

10. Moyer, A. W., Jervis, G. A., Black, J., Koprowski, H., Cox, H. R., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 387.

11. Kabat, E. A., Wolf, A., Bezer, A. E., *J. Immunol.,* 1952, v68, 265.

12. Ferraro, A., Roizin, L., *J. Neuropath. and Exp. Neurol.,* 1953, v12, 373.

13. Schwartz, R., Eisner, A., Dameshek, W., *J. Clin. Invest.,* 1959, v38, 1394.

Received September 4, 1959. P.S.E.B.M., 1960, v103.