10. At any rate the minimal duration of spermatogenesis is 48 days, or the duration of three cycles; this is the time taken by a type B spermatogonium to transform into spermatozoa (Fig. 1).

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Methotrexate: Suppression of Experimental Allergic Encephalomyelitis

Abstract. *Methotrexate inhibited the production of allergic encephalomyelitis in guinea pigs when administered between the time of sensitization and the onset of disease. If treatment was delayed until the day of onset of encephalomyelitis, the disease was still suppressed and the death rate was reduced. The protective effect of methotrexate was reversed by folinic acid.*

The antifolic acid agent methotrexate (amethopterin), has been shown to inhibit both antibody formation and the development of the delayed hypersensitive state to purified protein antigens in guinea pigs (*1*). Suppression of immune host-graft reactions have also been demonstrated with this agent (*2*). The purpose of this study was to determine what effect, if any, was produced by methotrexate on an experimental autoimmune disease. Experimental allergic encephalomyelitis (EAE) in the guinea pig was selected as the model because the disease can be produced regularly and because methotrexate has relatively little toxicity in this species.

Table 1. Effect of methotrexate (Mtx) on the incidence of EAE in guinea pigs.

Group I control	Group II Mtx begun day 1 to 5	Group III Mtx begun day 6 or 8
	EAE by day 19	
17/22* (77%)	1/32 (3%)†	4/18 (22%)
	EAE by day 39	
17/22 (77%)	11/32 (34%)	8/18 (44%)
	Death	
13/22 (59%)	8/32 (25%)	4/18 (22%)

* Ratio of the number of animals with EAE to the total number of animals given the emulsion containing spinal cord and adjuvant. † Developed EAE on day 19, 2 days after last injection of methotrexate.

Guinea pig spinal cord was ground in a "Tri-R Teflon" tissue homogenizer, suspended in a solution of 0.25 percent phenol in water, and emulsified in an equal volume of complete Freund's adjuvant containing 4 mg of heat-killed *Mycobacterium tuberculosis* per milliliter. Male Hartley guinea pigs were injected intradermally; each guinea pig received 0.4 mg of mycobacteria in a total volume of 0.2 ml of emulsion. The concentration of spinal cord in the emulsion varied from 5 to 12.5 percent, but in each trial the control and methotrexate-treated guinea pigs to be compared were injected with the same preparation. With this procedure, the incidence of disease in any control group was not less than 60 percent.

Methotrexate was injected intraperitoneally in a dose of 5 mg, usually daily, sometimes on alternate days. Drug administration was begun on day 0, 2, 4, 6, or 8 of the experiment; day 0 represents the day of sensitization. Drug treatment was terminated on either the 17th or the 23rd day.

The diagnosis of EAE was based on the appearance of grossly obvious, definite paralysis or paresis which was always associated with sphincter disturbances and weight loss, and was usually associated with a wet chin, tremor, or convulsions. No histologic studies were made.

The pattern of response was similar in each of the separate trials and the results are therefore combined in Table 1. Animals that received methotrexate treatment beginning on day 0, 2, or 4 are tabulated together (group II) as are those started on day 6 or 8 (group III). Days 19 and 39 were chosen as reference points because no control animals developed EAE later than day 19 while none of the treated animals developed it after day 39.

In group I, the untreated controls, 17 of 22 animals developed EAE by day 19. In group II, which received methotrexate beginning on day 0, 2, or 4, no animals developed the disease during treatment, and only one of 32 developed it by day 19. In group III, methotrexate administration was begun on day 6 or 8, and four of 18 guinea pigs developed the disease by day 19. Many of the treated animals developed it from 2 to 22 days after cessation of methotrexate injections. This temporal sequence is illustrated in Fig. 1.

Some of the animals lost weight temporarily or failed to gain normally without showing definite signs of EAE after the methotrexate injections were stopped. In those animals there may have been an attenuated form of the disease, and the true incidence of late disease may be higher than that illustrated. Histologic studies will be required to deal with this question. The mortality was reduced in both methotrexate-treated groups (Table 1).

Table 2. Reversal of methotrexate (Mtx) effect by folinic acid (FA) in guinea pigs.

Controls	Mtx day 5-18	Mtx plus FA day 5-18
	EAE by day 14	
6/9*	0/9	7/9
	EAE by day 39	
6/9	6/9	7/9

* Ratio of the number of animals with EAE to the total number of animals given the emulsion containing spinal cord and adjuvant.

Methotrexate exhibited little if any gross toxicity in this experiment. One animal died on the last day of an 18-day course of 5 mg daily with no evidence of EAE, and this was therefore considered a toxic death. Two other animals with no evidence of disease failed to gain weight while receiving methotrexate. The remainder of the treated animals that were protected gained weight and looked outwardly normal while receiving the drug.

Because folinic acid (citrovorum factor) prevents the other known effects of methotrexate, an effort was made to determine whether it would alter the protective effect of methotrexate. In one trial, a control group was compared with a methotrexate-treated group, while a third group was injected daily with the same dose of methotrexate plus 20 mg of folinic acid subcutaneously. The results are shown in Table 2. The addition of folinic acid completely reversed the protective effect of methotrexate.

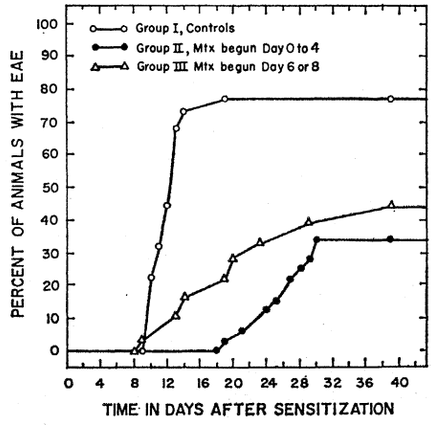

Fig. 1. Effect of methotrexate (Mtx) on the development of EAE.

To determine whether drug treatment could influence the course of EAE after its onset, guinea pigs were sensitized and, as signs appeared, animals were either treated with methotrexate or served as controls. The animals were numbered and were assigned to either the treated or the control group at the time of sensitization and the two groups were considered to be closely matched. The mean weight loss for the 24 hours preceding the appearance of signs of EAE was 38.4 g for the control group and 41.6 g for the treated group. Methotrexate was given for a 2- to 3-week period from the day of onset of disease. For the first 3 to 6 days 10 mg was injected daily, and thereafter, 5 mg was injected daily. The mortality rate in the control group over the 2-week period after onset of the disease was 85 percent (23 of 27 animals) whereas the rate in the methotrexate-treated group was 33 percent (10 of 30 animals). All animals dying in the control group did so within 9 days of onset. During the period of drug administration none of the treated animals died later than 5 days after the onset. The death rate in the two groups was similar at the third day after onset of disease. From the fourth day however, a clear difference in mortality emerged. The surviving animals showed weight gain and improvement or reversal of paralysis. Twenty of the treated animals were studied for more than 4 weeks after treatment with methotrexate was stopped. There was temporary weight loss and worsening of paralysis in six of these animals. Three others became worse and died with EAE from 8 to 16 days after the last dose of a 2-week course of methotrexate. These three deaths occurred from 21 to 29 days after the onset of signs of the disease.

Hoyer *et al.* have reported similar suppression of the production of EAE in rabbits and guinea pigs with the antimetabolite 6-mercaptopurine (*3*). However, no effect of 6-mercaptopurine was seen when administration was begun after the onset of disease. The toxicity of 6-mercaptopurine appeared to be considerable, although it was felt not to be important as a nonspecific factor in the inhibition of EAE. The mechanism of action of these drugs in such inhibition is as yet uncertain. Although 6-mercaptopurine and methotrexate both suppress antibody production, the role of circulating antibody in the pathogenesis of this disease is disputable. Suppression of the inductive or proliferative cellular changes associated with the production of circulating antibody or with the development of the delayed hypersensitive state may be important. The possibilities of an anti-inflammatory action or of still other effects have not been ruled out.

Experimental allergic encephalomyelitis is a useful experimental model for human demyelinating diseases such as postinfectious encephalomyelitis and multiple sclerosis. In any consideration of the use of methotrexate in humans with diseases that might have an autoimmune basis, the great variation in susceptibility of different species to the toxic effects of methotrexate is important. In the human, doses of 0.5 mg/kg of methotrexate daily for 5 days often produce severe toxicity; in contrast, the guinea pig tolerates more than 10 mg/kg daily for at least 2 to 3 weeks with little evidence of toxic effects.

MICHAEL W. BRANDRISS
National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

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Montmorillonite-Vermiculite Interstratification in Clays from Eocene Chalk Soils

Abstract. *Soils of the Eocene chalk often show interstratification in their clay fractions, though discrete 14- and 18-Å spacings are the rule rather than the exception. It is also frequent that a 16-Å intermediate shows in place of these diffraction maxima. This intermediate is indicative of complex interstratification. It is a unique feature of this body of soils and a strong indication that existing data on random and regular interstratification are both correct and diagnostic. The rare but interesting diffuse basal spacings which are observed at 22 Å are another unusual feature of the clays of this group of soils.*

Interstratification of vermiculite and montmorillonite occurs in the Prairie soils of northeastern Mississippi, and irregularities are common in the x-ray diffraction patterns. A 16-Å diffraction maximum is found in many of the samples. This sharp, regular, and intense diffraction maximum is one of the best examples of layer-silicate interstratification I have yet found, and the collapse of the materials caused by potassium-saturation and gentle heat treatment is another point of interest. The 14- to 18-Å pair is a more common characteristic of the soils being described, and the unusual cases described here were selected to demonstrate a specific point.

The Houston clay (sampled 7 miles west of Columbus, Mississippi) illustrates the unusual characteristics of some of the soils. Saturation with magnesium and glycerol solvation at room temperature were a part of the sample preparation procedure. The presence of montmorillonite, mica, and kaolinite is evident from the data presented in Table 1. However, the presence of the 16-Å diffraction maximum in coarse clay fractions at all depths and its presence in fine clay taken from the lowest depth sampled are worthy of note. The 16-Å component is probably a weathering product, and in lower layers even the fine clay has a most unusual interstratification. The sharpness of all diffraction maxima suggests that some interstratification may be common in these soils. The presence of accessory minerals was verified by heat treatments and other techniques. Perhaps the most unusual feature of the clays is the dif-

Table 1. X-ray diffraction maxima in various fractions of Houston clay.

Fraction (μ)	Relative intensity at various D-spacings			
	17.6 Å	16.3 Å	10.0 Å	7.1 Å
	Depth 0 to 6 inches			
<0.2	29		2	6
2–0.2		32	1	28
CC*			23	
	Depth 6 to 12 inches			
2–0.2		26	7	20
	Depth 18 to 24 inches			
2–0.2		27	7	30
CC*			20	
	Depth 24 to 30 inches			
<0.2	20			4
	Depth 30 to 36 inches			
<0.2		34	4	10
2–0.2		19	4	10
CC*			14	

*Coarse clay, potassium-saturated samples heated to 500°C.