tion was productive of a mild dehydration, the results stated herein, together with the reports of others(10,11) indicate that the restricted food consumption associated with intoxication by aminomethylPGA cannot account for the observed protein and water losses.

The mechanism by which aminomethylPGA elevated plasma creatinine and depressed plasma calcium and phosphorus concentrations is obscure. Starvation did not duplicate these effects. Probably part of the lost calcium was that combined with the plasma protein extruded into the intestine.

It is difficult to evaluate the significance of the observed biochemical changes. Both the starvation and the dehydration induced by administration of aminomethylPGA seem compatible with life. The failure of forced hydration to prevent death in intoxicated mice further minimizes the significance of the dehydration. The loss of plasma protein may be of greater importance. It may be calculated from the observed changes in body weight, plasma volume and plasma protein concentration that approximately 40% of the initial plasma protein was lost during the period of intoxication with this drug.

It is of interest to compare the effects of the present folic acid antagonist with those of the nitrogen mustards. The actions of both groups of compounds are characterized by anorexia, weight loss, bone marrow damage, diarrhea associated with intestinal lesions, and delayed death. Previous investigators(12,13), studying the effects of the mustards in dogs, found that fatally poisoned animals lost significant amounts of body water and protein, principally from the intestinal tract. However, as concluded in the present study with aminomethylPGA, these changes were not considered sufficient to account for the lethal actions of nitrogen mustards.

Summary. Administration of 4-amino-N^{10}-methyl-pteroylglutamic acid to rats caused reduction of food consumption, extracellular fluid volume, plasma volume and plasma concentrations of protein, calcium, and phosphorus. Plasma creatinine concentration was elevated. Starvation alone did not duplicate the effects of this compound. Increased urinary excretion of nitrogen and water did not occur. The loss of body water and plasma protein appears to be the consequence of a previously described intestinal lesion produced by anti-folic acid agents. The relation between these biochemical changes and the mechanism of lethal action remains obscure.

The author is indebted to Dr. Oscar Bodansky for the flame photometric determinations of sodium and potassium and to Dr. Frederick S. Philips for advice and criticism. Supplies of 4-amino-N^{10}-methyl-pteroylglutamic acid were furnished by investigators of the Lederle Laboratories and the Calco Chemical Division, American Cyanamid Co.

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Received May 11, 1951. P.S.E.B.M., 1951, v77.

A Spontaneous Epizootic of Mouse Encephalomyelitis.* (18744)

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Two main groups of mouse encephalomyelitis viruses were described by Theiler(1,2). The TO type virus produces flaccid paralysis,

* Aided by a grant from The National Foundation for Infantile Paralysis.

1. Theiler, M., *J. Exp. Med.*, 1937, v65, 705.

2. Theiler, M., and Gard, S., *J. Exp. Med.*, 1940, v72, 49.

has a long incubation period (7-30 days), and requires a relatively high concentration of virus to infect (dilutions of 10^{-3} to 10^{-5}). The GD-FA types produce encephalomyelitis, usually without paralysis, have a short incubation period (2-9 days), and infect in relatively high dilutions (10^{-6} to 10^{-8}). According to Olitsky(3) the viruses are antigenically related but not identical. Spontaneous paralysis due to the TO type has been reported many times(4), but the total incidence is only in the neighborhood of once in every 10,000 mice. Occurrence of the encephalitic-type of disease has been reported a number of times following intracerebral injections of various materials. Theiler(2) isolated encephalitic virus from mice which became ill following inoculation with yellow fever virus. Melnick and Riordan(4) reported seven instances in which the disease followed intracerebral inoculation of Lansing-type polio virus or of noninfectious material. In one instance the disease occurred spontaneously without previous inoculation. This appears to be the only reported case of spontaneous occurrence. To our knowledge no epizootic due to either type virus has ever been reported.

Circumstances under which the epizootic occurred. The epizootic to be described occurred during the course of experiments on the passive transfer of immunity to Lansing virus from immune mothers to their own, or to foster, young(5). Female mice were immunized intraperitoneally or subcutaneously with Lansing virus or with normal mouse brain-cord suspensions. In the particular experiment concerned, the mother mice were vaccinated during pregnancy. At birth, half the young were left with their own mothers, and half transferred to a mother of the opposite group, that is from immune to control mothers, or vice versa. The young were weaned at 28 days. The object of this experiment was to determine the duration of the passive immunity, and challenge injections were to be made at 2, 4, 6, and 8 weeks after weaning. It was during the period of waiting before challenge that the epizootic deaths occurred in most groups.

At detection of pregnancy the mothers were placed in glass jars with sawdust bedding. The food, fox checkers, was placed in the bottom of the jars. Water was supplied by gravity feed bottles with glass tubing. At weaning, the mothers were removed and the young left in the jars. Additional sawdust bedding was added as necessary, but in most cases the jars were not cleaned out during the experiment.

The Epizootic. Occasional deaths had occurred among the mothers in the experimental breeding cages, during November, December, and January. During January a few deaths occurred in some groups of uninoculated young, but no nervous symptoms were noted. Early in February the young began to die off rapidly, and nervous symptoms were noted in about 50% of the mice which died. About 150 of a total of 240 uninoculated young mice died during the acute stage of the epizootic. The peak was reached February 14th when 49 mice died. Scattered deaths occurred during late February and March.

There was no relation of the outbreak to the exposure of the mothers to Lansing virus. The control young, whose mothers had no contact with this virus, had a death rate of 57% in the epizootic. The "immune" young, born of and fed by mothers immunized by active Lansing virus, had a death rate of 60%. The young transferred from control to immunized mothers had a mortality rate of 67%, and those from immunized to control mothers, one of 54%. There was apparently no relationship of the epizootic to the age of the young mice. The different age groups had mortality rates as follows: 60-66 days, 80%; 50-59 days, 57%; 40-49 days, 30%; 30-39 days, 81%. Only one group of unweaned mice was concerned: of 6 mice, 19 days of age, 3 died with typical symptoms. The outbreak was confined to the mice in the experimental room. No deaths occurred in the normal breeding room. In the experimental room all jars were

3. Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1945, v58, 77.

4. Melnick, J. L., and Riordan, J. T., *J. Immunol.*, 1947, v57, 331.

5. Thompson, R., and Meyers, F. P., *Am. J. Hyg.*, 1950, v52, 213.

affected, but the majority of deaths at the height of the epizootic occurred in one part of the room.

Isolation of virus. At the height of the epizootic on February 14th, 6 mice with typical nervous symptoms were killed. A 20% emulsion of the pooled brains and cords was inoculated intracerebrally into 10 normal 28-day-old mice. All of these died within 2 days with typical nervous symptoms. Two other consecutive passages were made with similar results. Cultures on blood plates and in broth, of the brain-cord emulsions used for passage, were consistently negative. No evidence of the growth of bacteria or of pleuropneumonia organisms was seen. A 5% broth emulsion of the second-passage virus after passage through a Seitz filter produced typical symptoms and death in 5 of 5 mice.

Symptoms were similar in the spontaneously-infected mice and in the experimentally-infected. Death without symptoms was somewhat more frequent among the spontaneously-infected. Paralysis was never seen in the spontaneous cases while, as noted below, it was occasionally seen in the experimentally-inoculated mice. Often the earliest symptom was a pulling of the head to the left or right, with a tendency to circling in the same direction. The circling increased with the progress of the disease and with stimulation by noise. As the disease progressed, the circling became a pivoting with the base of the tail as the center of the circle. Frequently this progressed to a rolling in the same direction. Sometimes the rolling was spontaneous, but most frequently was produced by noise stimulation. Some mice showed merely excitability and generalized convulsions on noise stimulation. Some showed marked generalized tremors, and in others great respiratory difficulty was the only symptom. Occasionally the front paws were clenched up under the lower jaw. Weakness or flaccid paralysis of the hind legs occurred occasionally in some of the experimental mice.

Preliminary titration studies indicated that the virus isolated had a 50% end-point dilution titer of 10^{-6} to 10^{-7}. The short incubation period, the titer, and the symptoms all indicated a virus of the encephalitic type. Careful comparative studies were made of the new virus and of the GD VII virus which had been passed for some months in the laboratory. In view of the possibility that this GD VII virus (designated GD VII A) had been contaminated while in our laboratory, we obtained also another GD VII passage from Dr. Theiler, and this virus (designated GD VII B) was used in the comparisons. The three viruses were similar in all respects. Injected intracerebrally into 28-day-old mice they all produced the symptoms described above.† The incubation period was 2 to 9 days in all cases. The epizootic virus had a 50% end-point dilution titer of 10^{-6} to 10^{-7}, as compared with the 10^{-5} to 10^{-6} range of the two stock viruses. Both the epizootic virus and the GD VII A virus produced typical disease on the intraperitoneal or intranasal inoculation of undiluted 10% emulsion in 28-day-old mice. The GD VII B strain was not tested in this respect. All 3 viruses on intracerebral injection into 30-day-old hamsters produced encephalitic or paralytic disease. No symptoms were produced on intracerebral inoculation of young guinea pigs by either the epizootic or the GD VII A virus.

To determine the antigenic relationship of the GD and new viruses, 110 mice from 28 to 34 days of age were divided into 3 groups with

TABLE I. Cross-Immunity Between GD VII Virus and the Virus Isolated from the Epizootic.

Virus used to immunize	Virus used for challenge	No. of mice challenged	No. of mice surviving	% of mice surviving
GD VII B	GD VII B	19	11	58
"	Epizootic	19	9	47
Epizootic	GD VII B	19	13	68
"	Epizootic	19	6	31
0	GD VII B	17	1	6
0	Epizootic	17	1	6

† Extensive pathologic studies could not be done, but Dr. Karl Neuberger of our department of pathology examined sections of several of the spontaneously-affected mice. Microscopic changes were surprisingly scanty. Lymphocytic infiltration was present around several small vessels, especially in the medulla. There was a tendency to fragmentation of the nuclei of these infiltrates and a patchy disintegration of the nerve cells with glial proliferation.

TABLE II. Cross-Neutralization Between GD VII Virus and the Virus Isolated from the Epizootic.

Virus used to produce serum	Dilution of serum	Virus used for inoculation	No. of mice inoculated	No. of mice surviving	% of mice surviving	Normal serum controls* No. of mice inoculated	Normal serum controls* No. of mice surviving	Normal serum controls* % of mice surviving
GD VII B	500	GD VII B	14	12	86	10	0	0
	1000	"	14	6	43	17	0	0
	100	Epizootic	10	10	100	10	0	0
	500	"	10	9	90	10	2	20
	1000	"	11	1	9	—	—	—
	1500	"	11	1	9	—	—	—
Epizootic	500	Epizootic	12	8	67	8	2	25
	1000	"	12	4	33	8	3	37
	10	GD VII B	7	7	100	7	3	43
	500	"	10	7	70	10	0	0
	1000	"	17	10	59	17	0	0
	1500	"	12	1	8	—	—	—

* Inoculated at the same time as the corresponding experimental group with pool virus from same vial. The normal serum dilution used was the same as the immune serum dilution.

litter mates divided evenly between the groups. Group 1 was immunized against the new virus; Group 2 against the GD VII virus; and Group 3 was left as control. The immunizations consisted of intraperitoneal injections of the virus suspensions at 3-day intervals over a 5-week period, beginning with a 0.1 cc injection of a 1:10,000 dilution, and building up to a 0.3 cc injection of a 1:10 dilution. Immune and control mice were challenged by an intracerebral injection of 0.03 cc of a 10^{-4} dilution of each of the viruses. The results are shown in Table I.

Each virus produced a considerable degree of immunity against the other. There was no indication of any significantly greater degree of immunity against the homologous than against the heterologous strain. The epizootic virus would appear to be antigenically identical or very similar to the GD VII strain. Since the cross-resistance could conceivably be due to virus interference, rather than to antigenic relationship, it seemed necessary to perform cross-neutralization tests. Using the same methods and dosages as in the active immunity experiment, one group of 32 mice was immunized to GD VII B virus, and another group of 32 mice to the epizootic virus. Both groups were bled by decapitation on the 10th day after the last injection. The serums from the animals in each group were pooled, distributed in vials in 1 cc amounts, and preserved at —70°C until used. Serum from normal animals of corresponding age was similarly prepared and preserved. For neutralization tests serial dilutions of the serums were mixed with equal quantities of a $10^{-3.5}$ dilution of the virus to be tested, and 0.03 cc of the mixture inoculated intracerebrally into etherized 28-day-old mice. The viruses were from previously prepared and titrated pools kept at —70°C. The results of the neutralization experiments are shown in Table II. These results confirm the findings of the active cross-immunity experiment, inasmuch as serum protected against the other virus at least equally as well as against the virus used to produce it.

Discussion. We have no explanation as to why this unusual epizootic occurred at the time it did. The epizootic was apparently due to a virus identical or almost identical to the GD VII virus. Whether the virus originated from the GD VII strain being used in the laboratory, or whether it arose from our mouse colony or from wild mice which were present in the laboratory at times, cannot be determined. Spontaneous paralysis had occurred in the mouse colony on several occasions, and a virus of the TO type had been isolated on 3 separate occasions several years ago. No encephalitic-type virus had ever been isolated previously from this colony. The rather unsanitary conditions in which the experimental mice were kept may have been a factor. We can only speculate as to whether the series of injections of brain suspensions into the mother mice played any role. In retrospect, it would have been desirable to make

more than one isolation of virus from the affected mice, but the great similarity of the disease in the spontaneously-affected and the experimentally-infected mice would seem to be a good indication that the virus isolated was actually responsible for the epizootic.

Summary. A spontaneous epizootic of encephalomyelitis which killed 62% of 240 uninoculated mice is described. A virus was isolated which resembles GD VII virus in its properties, and which is antigenically identical or very similar to the GD VII virus.

Received May 18, 1951. P.S.E.B.M., 1951, v77.

Detection of Phosphatase Activity in Polarized Light Following Glycerophosphate Incubation.* (18745)

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The differences in the exact localization of phosphatase activity such as reported in the literature are due to several factors. Among these is the fact that in the Gomori technic of histochemical detection(1), the white precipitate formed is transformed into a black mass by the cobalt nitrate-ammonium sulfide treatment. Unfortunately such a treatment produces also some artificial blackening part of which has already been recognized, such as that of the ground substance of the cartilage. On the other hand, in the black areas of phosphatase activity, it is possible that some local changes in the amount and position of the precipitate might take place during the transformation of lead or calcium phosphate into sulfide.

In order to control these two factors of error, one might try and examine the initial precipitate, a thing which is made easy by using polarized light and an environment which will not reduce the anisotropism of the mineral salts.

Material and methods. Rat and hamster tissues were fixed in 80% alcohol or cold acetone. The tissues were embedded routinely in paraffin or low viscosity celloidin, according to a technic described previously(2). Sections of already mineralized material such as bones and teeth were treated with a buffer decalcifying solution of formic acid and sodium citrate at pH 4.9 according to Greep, Fischer, and Morse(3). By treating 10 mμ sections instead of blocks, the time in that solution was reduced from a matter of days to a maximum of 15 minutes. Incubation was carried out at 37°C in Gomori's solutions of sodium alpha-glycerophosphate at pH 5.0 and pH 9.4 for acid and alkaline phosphatase respectively, and also other substrates such as glucose-1-phosphate, adenylic acid, ribonucleic acid, desoxyribonucleic acid and sodium desoxyribonucleate.

Following 3 hours of incubation, the sections were brought to room temperature and dehydrated in graded alcohols and finally mounted with a coverslip in 5% celloidin in alcohol-ether. The controls were similarly treated and all preparations had to be thoroughly dry before examination. In examining these preparations regular polarizing equipment has been used but in comparison, it has been possible to do just as well with a simple addition to a monocular microscope of 2 pieces of Polaroid material of type JG30FAP commonly used in antiglare goggles. These pieces are cut to appropriate sizes to fit the filter slot at the base of the condenser (polarizer) and the inside of the eyepiece (analyser) where the last one rests on the metal insert midway between the top and the bottom lenses. With this equipment, a red extinction of the field is obtained when the

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