Propagation of a Strain of Egg-Adapted Distemper Virus in Suckling Mice.* (20525)

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Several workers have adapted the canine distemper virus to growth on the chorioallantoic membrane (CAM) of the embryonating egg(1-4). Its continued passage in the egg has resulted in a gradual loss of pathogenicity for naturally susceptible species. Simultaneous retention of the antigenicity and immunogenicity of the virus have led to its use as a vaccine(3,5).

Serial passage of an egg-adapted strain of distemper virus in suckling mice was accomplished in this laboratory recently. Two lines of virus were propagated by intracerebral inoculation through 18 and 10 passages, respectively. Nervous signs of moderate degree which were apparent in a small proportion of mice of the first passage of each line became quite pronounced and prevalent with subsequent passages.

Materials and methods. The FXNO strain of egg-adapted distemper virus was used in this study. This strain, originally isolated from infected fox spleens(4), was carried through 80 egg passages in this laboratory. By the 45th passage, the virus induced small, widely scattered, opaque, papuloid areas of the CAM. Fiftieth egg passage tissues elicited only a mild infection in ferrets. After recovery these animals were immune to challenge with virulent distemper virus(6). A Rockefeller line of Swiss albino mice originating from Carworth Farms stock served as the host. Mice varying in age from 1 to 4 days were inoculated intracerebrally in the right hemisphere with 0.01 ml quantities of inoculum. Infected chorioallantoic membranes, ground and centrifuged for 10 minutes at 2500 rpm in an International centrifuge furnished the supernatant fluids used as inoculum for the initial passage. Twenty percent suspensions of infected brains in tryptone broth were centrifuged similarly and used for all subsequent passages. For titrations, 10-fold serial dilutions were made in tryptone broth. Mice with definite nervous signs were killed with ether and stored in a deep freeze at —20°C. Hyperimmune sera collected from ferrets after vaccination with the FXNO strain and subsequent challenge with virulent virus (Delavan strain) were used in virus neutralization experiments. Sera collected from ferrets which had not had previous experience with the distemper virus served for control purposes. All sera were heated at 60°C for 30 minutes just prior to use. The Delavan strain of virulent distemper virus used for challenge exposure of ferrets was obtained by Dr. John R. Gorham from a natural outbreak of distemper on a mink ranch near Delavan, Wis. Ten or 11 days after inoculation of ferrets with this strain, the skin of the abdomen and the chin became reddened. By the 13th day, a purulent exudate of the eyes was present and the eyelids became pasted together. The appetite was gradually lost; the animals became sluggish and died in 24 to 30 days. It was not uncommon for those surviving longer than 24 days to show nervous signs.

Mouse passages. Line 1, now in its 18th mouse passage, was initiated from the 65th egg passage of the FXNO strain. Line 2 originated from the 75th egg passage and is now in its 10th passage in suckling mice. The observations of the passages of both lines are summarized in Table I. Hyperkinesis was the most striking sign in the early passages of both lines. This manifestation, rapid running and jumping at the sides of the cages, persisted for 2 to 3 days at which time the mice were sacrificed for further passage of the virus. By the 8th passage ataxia, as well as hyperkinesis, was observed on the 4th day

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TABLE I. Intracerebral Passage of 2 Lines of FXNO Distemper Virus in Suckling Mice.

Passage No.	Age of mice in days		No. affected/ No. inoculated		Time in days after inoculation until appearance of nervous signs	
	Line 1	Line 2	Line 1	Line 2	Line 1	Line 2
1	1	3	2/8	1/11	8	15
2	3	4	6/6	5/9	6	13
3	2	4	2/11	4/8	7	7
4	3	3	6/7	5/6	5	5
5	3	2	9/10	6/6	4	6
6	4	3	9/9	8/8	4	5
10	4	4	9/9	5/7	3	4
18	3	—	8/8	—	3	—

after inoculation and was succeeded rapidly by muscular twitching, disequilibrium, paresis, and clonic spasms. With the 10th passage, nervous signs appeared in 3 days with Line 1 and in 4 days with Line 2. Deaths occurred within 5 to 8 days of inoculation. The LD_{50} titer/g of infected brain material gradually increased with passage. The approximate LD_{50} titers for the 5th, 10th, and 16th passages of Line 1 were 10^3, $10^{4.5}$, and 10^6, respectively.

Infectivity of mouse-passaged virus for embryonating eggs. Twenty percent suspensions of brain material from the 5th, 6th, and 11th passages, respectively, of Line 1, when ground in tryptone broth containing penicillin and streptomycin (1000 units and 1000 μg per ml, respectively), and inoculated onto the CAM of 7-day eggs, produced lesions typical of the original FXNO strain. In each case, the area of CAM affected was slight so that a repassage was necessary to produce prominent lesions.

Immunizing effect on ferrets. Seven 4-month-old ferrets not previously exposed to the distemper virus were inoculated intraperitoneally with one ml quantities of 20% suspensions in tryptone broth of the following: brains of normal mice into 2 ferrets, brains of mice infected with the 6th mouse passaged FXNO strain, Line 1, into 3 ferrets, and chorioallantoic membranes infected with the original FXNO strain into 2 ferrets. Clinical signs of infection did not develop in any of the ferrets during the subsequent 3 weeks. At this time the 7 ferrets and 2 additional ones received an injection of distemper virus of the Delavan strain. The ferrets which had received the infected chorioallantoic membranes or the infected mouse brains did not show clinical signs of disease during the 3-month observation period following challenge with the virulent Delavan virus. The ferrets previously injected with normal mouse brain as well as the untreated controls exhibited the typical clinical signs of distemper in this species. Ferret protection tests indicated that after 6 intracerebral passages in mice, Line 1 had retained the immunogenicity of the original egg-adapted virus for ferrets against clinical distemper. Since the virus titer of the fluid expressed from chorioallantoic membranes infected with the FXNO strain is approximately 10^3 to 10^4 ID_{50} per ml. it is not likely that a sufficient quantity of the original virus may have been carried through 6 passages in mice to bring about the protection indicated.

Neutralization tests. Attempts to evaluate the neutralizing activity of the ferret sera by intracerebral inoculation of the serum-virus mixtures in suckling mice have yielded equivocal results. However, a modification of the technic of Belcher(6) employing the CAM of 7-day embryonating eggs as the test tissue has given definite results. A tryptone broth suspension of virus from the 11th mouse passage, after 2 subsequent passages in eggs, was used as inoculum. This suspension contained approximately 1000 ID_{50} per ml. Immune serum dilutions of 1-4 to 1-64 were admixed with equal aliquots of inoculum and 0.2 ml quantities injected into 4 eggs per dilution. A mixture of virus and non-immune ferret serum was employed as a control for the experiment. The results are shown in Table II.

The hyperimmune serum pool completely inhibited the formation of lesions of the CAM

TABLE II. *In ovo* Neutralization of 100 Embryo ID_{50} of Mouse-Brain-Propagated, Distemper Virus by Distemper Immune Serum.

Final serum dilutions	Normal serum	Immune serum
1-8	4/4*	0/4
1-16	"	"
1-32	"	"
1-64	—	1/4
1-128	—	"

* No. egg membranes showing lesions/No. inoc.

in dilutions as high as 1-32 while normal serum had no observable neutralizing effect.

Summary. Two separate lines of the FXNO strain of egg-adapted canine distemper virus were passed intracerebrally in suckling mice 18 and 10 times, respectively. Both lines induced signs of protracted hyperkinesis. After the 7th passage central nervous system involvement became quite prominent and severe. Ataxia, muscular twitching, disequilibrium, paresis, and clonic spasms were common manifestations. The time of the first nervous disturbance decreased progressively from 8 days to 3 days in Line 1 and from 15 days to 4 days in Line 2. *In ovo* neutralization tests and ferret protection tests establish the identity of the mouse passaged agent with the original egg-adapted FXNO strain.

Comment. While this manuscript was in preparation, a Lederle strain of avianized distemper virus (Lederle Distemper Vaccine Mink Serial No. 6368-17A) was passaged 6 times in suckling mice with results in agreement with those reported for the FXNO strain. This would suggest the lack of strain specificity in the mouse adaptability of the avianized distemper virus.

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Infrared Spectrophotometry as a Means for Identification of Mosquitoes.* (20526)

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In addition to the conventional taxonomic methods, various types of differentiating procedures have been employed in attempts to distinguish between closely related species of mosquitoes. The *Culex pipiens* complex in this country, for example, has been studied through cross-breeding experiments, temperature responses, susceptibility to parasitic infections as well as immunologically(1). Such investigations have been made in the search for distinguishing characteristics whereby the exact relationship between morphologically similar species might be determined. These biological tools, however, are very time-consuming and might well be supplemented by other methods. Until recently, no attempt was made to employ biochemical procedures as a means of comparing mosquito species. The first step in this direction was made by Micks and Ellis(2) in which the free amino acids of 7 species of mosquitoes were analyzed by paper chromatography. Certain quantitative differences were found between the

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