Encephalomyelitis and Pneumonitis in Hamsters Infected with Newcastle Disease Virus by Different Routes.* (26718)

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Intracerebral injection of Newcastle disease virus (NDV) has been shown to cause encephalomyelitis in hamsters(1) and monkeys(2). Similarly, mice(3,4) and cats(5, 6) develop encephalomyelitis, pneumonitis or both following intracerebral or intranasal administration of this virus. Among mammals, there have been no reports of successful infection with this virus administered by routes other than these. The purpose of this communication is to describe the pattern of disease and pathological findings of encephalomyelitis and pneumonitis in hamsters caused by NDV injected by peripheral routes.

Materials and methods. The virulent chicken-adapted CG strain of NDV was obtained from F. B. Bang. The pool of infected allantoic fluid harvested 48 hours after inoculation of 11-day-old embryonated eggs and used in these experiments titered $10^{8.2}$ plaque forming units (PFU) per ml in chick embryo fibroblasts.

The virus, diluted in phosphate buffered saline, was administered by either the intranasal (0.05 ml); intracerebral (0.05 ml), intramuscular (0.15 ml), subcutaneous (0.10 ml) or intraperitoneal (0.5 ml) routes to 21-day-old hamsters obtained from Lakeview Farm, Newfield, N. J. Virus was administered intranasally by dropping diluted inocula into the external nares of anesthetized animals and hyperextending the head to allow adequate contact of the nasopharyngeal mucosa with infective material. Intracerebral injections were made through the mid point of the skull. Subcutaneous, intraperitoneal, and intramuscular inoculations were made into the interscapular region, through the lower right abdominal wall and into the right thigh respectively. In each experiment, sterile diluent fluid was administered to a control group of hamsters by the same route as the virus.

The hamsters were placed in individual cages and examined daily during a period of 3 weeks for signs of illness or death. Partial autopsies were performed on nearly all animals and complete autopsies were made on the 179 animals examined histologically. Tissues were fixed in 10% formol-saline. Brains were embedded in celloidin and sectioned coronally or sagittally at 20 μ and stained with cresyl violet or hematoxylin and eosin. Other organs were embedded in paraffin, sectioned at 8-10 μ, and stained with hematoxylin and eosin.

In some experiments, brains and lungs were removed aseptically, ground in Ten Broeck grinders, and diluted to 10% (W/V) with buffered saline (0.01 M phosphate, pH 7.3). Virus isolations from tissue homogenates were made by subinoculations of 180 mm $\times$ 16 mm tube cultures of chick embryo fibroblast or 10-day-old embryonated eggs. The chick fibroblast cultures were inoculated 2 days after seeding, and maintained with mixture 199 and 10% calf serum for a week. Tissue culture fluids collected from cultures showing cytopathic effect were also tested for their capacity to agglutinate chick red blood cells. Ten-day-old embryonated eggs were injected into the chorioallantoic sac and observed daily for 5 days. Allantoic fluid collected from dead embryos was also tested for presence of hemagglutinins.

Results. The results of several titrations of NDV shown in Table I are expressed in terms of average number of PFU required to produce death in 50% of hamsters receiving virus by each route. As noted, hamsters were extremely susceptible when injected intracerebrally and least susceptible when in-

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TABLE I. Number of Plaque Forming Units Required to Produce Encephalomyelitis by Different Routes of Inoculation.

Route of inoc.	No. of animals	PFU / LD_{50}
Intracer.	199	.30
Intranasal	182	5.70
Intraper.	358	3.9×10^3
Intramusc.	208	4.4×10^6
Subcut.	212	Indeterminate

jected by the subcutaneous and intramuscular routes.

The mean time of death of animals which died of NDV infection in each experiment is shown in Fig. 1. For each route of inoculation there was a correlation between amount of virus injected and mean time of death. In view of the observation that few animals died after the 12th day following administration of virus, it may be concluded that if an animal survived this period the probability of survival beyond the 3 weeks of observation was excellent.

Effects of physiologic stress. The effect of physiological stress on susceptibility of hamsters to NDV was tested in 2 ways. In one series of experiments, NDV was titered in hamsters kept in controlled temperature rooms maintained at 13°-15°, 21°-23°, or 28°-30°C. In another series of experiments, NDV was titered in hamsters made to swim for 1 hour, at different intervals after infection, in individual beakers containing water at 36°-38°C. In none of these experiments was there any difference observed in the signs of disease, pathology of lesions, or in amount of virus required to produce illness.

Pattern of disease. Hamsters infected with lethal doses of NDV, regardless of route, developed signs of encephalomyelitis, pneumonia, or frequently both. The onset of illness was characterized by hunching of the trunk, ruffled fur and inactivity. Some animals when disturbed exhibited hyperexcitability. Left alone they were quiet. These initial signs were followed by development of segmental or generalized jerking movements of the head and neck, jaw, trunk, or fore and hind quarters (myoclonus). The rate of these predominantly flexor spasms varied from 40-160 per minute. Paralysis of one or more limbs was often observed; terminally, the animals were usually prostrate. With progression of the illness, respirations became labored and in those animals with generalized myoclonus the respiratory rate became synchronous with the myoclonic spasms. Death usually occurred one to 3 days after onset of the first signs of illness. In some instances, animals were found dead in their cages without previous signs of illness.

TABLE II. Results of Histological Studies of Hamsters Administered NDV.

Route of infection	Animals examined	Encephalitis	Pneumonitis
Intracer.	50	32	44
Intranasal	53	25	43
Intraper.	46	22	42
Subcut.	30	11	25

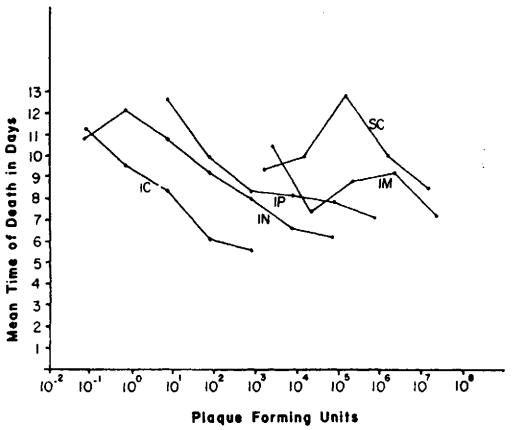

FIG. 1. Relationship between virus dose and mean time of death.

The predominant manifestations of encephalomyelitis in these animals were myoclonus and paralysis, both of which were observed in approximately 70% of animals that died. In general, there was a correlation between number of animals which developed myoclonus and paralysis and dose of virus administered.

Histopathology. Semi-serial sections of brains and single sections of lungs of hamsters dying after infection with NDV were examined and compared with those from control animals. The number of brains and lungs showing histologic evidence of en-

cephalomyelitis or pneumonitis, respectively, is shown in Table II.

Perivascular cuffing was the most prominent histologic lesion of the brain irrespective of route of infection. This finding was most evident in the midline nuclei of thalamus, hypothalamus, tegmentum of the mid brain and brain stem, and basal cerebellar nuclei. In addition, brains of animals with more advanced perivascular cuffing showed considerable vascular dilatation. This was sometimes associated with red cell diapedesis into the neural tissue. Glial nodules and focal inflammatory changes were found in these same regions in brains with more severe encephalomyelitis. Specific neuronal lesions were rare. Except for an occasional animal with focal meningitis and contiguous perivascular spread into the brain, the cerebral and cerebellar cortices were essentially normal.

In animals which received NDV by the intranasal route, cuffing of blood vessels, glial nodules and focal inflammatory changes were found in the olfactory bulb and tract, the preoptic areas and fornices. The amygdaloid nuclei, paraolfactory and septal areas, and the cornu ammonis showed similar lesions.

In the lung sections of those animals with pneumonitis, there was evidence of severe passive congestion. The alveoli were commonly filled with edema fluid or edema fluid and blood. Interstitial infiltrations of mononuclear cells and erythrocytes and patchy atelectasis were observed. Hemorrhagic consolidation of entire lobes was a frequent finding regardless of route of virus inoculation. Epithelial degeneration was most prominent in the hilar bronchi, but in more advanced stages of pneumonitis, this process extended into the smaller bronchi.

Virus recovery. Attempts were made to recover virus from brains and lungs of sick animals. Ten-fold dilutions of brain or lung homogenates were inoculated into cultures of chick embryo fibroblasts and embryonated eggs. Virus was recovered from brains but not from lungs of 12 animals, injected 6 each by the intracerebral and intraperitoneal routes respectively. The average titer of virus recovered from brains in embryonated eggs was less than 10^3 50% egg infectious doses.

In other experiments no virus was recovered in embryonated eggs from the blood of hamsters inoculated with varying doses of NDV by the intraperitoneal route and collected immediately after inoculation and daily for 4 days.

Uninfected Animals. Of 214 animals which received sterile diluent inoculated by different routes, 27 died during the 3 week period of observation. Of these animals, 6 died from diarrheal disease and rectal prolapse. Nine showed histological evidence of pneumonitis. In the remaining 11, cause of death was not established. None of the control animals given sterile diluents developed encephalomyelitis.

Discussion. The significant finding of this study was the production of encephalomyelitis in hamsters following inoculation of the chicken-adapted CG strain of NDV by peripheral routes. The manifestations and pathology of the disease were similar in all respects to that produced by intracerebral injection of this virus. Encephalomyelitis, pneumonitis or both developed 5 to 13 days after inoculation. Length of the incubation period was related to dose and route of virus administration. The results of the present observations indicate that the hamster is more susceptible to disease produced by this virus than any mammal yet studied.

Demonstration of viremia as an essential precursor to many naturally acquired and experimentally induced viral encephalomyelitides raises important questions about exact sites and conditions favoring virus penetration into the CNS. Judging from the prominent perivascular cuffing and vascular congestion in the CNS of hamsters with NDV encephalomyelitis, it is suggested that the primary action of NDV in the hamster is on the blood vessels rather than nerve cells.

The experiments in which inoculated hamsters were maintained in controlled temperature rooms or made to swim for varying lengths of time were designed to determine whether physiological stress could alter the amount of virus required to kill 50% of animals, or increase the probability of infec-

tious particles making contact with susceptible cells in the CNS. The failure of these experiments would seem to indicate that either the physiological stresses were inadequate, or at the time at which they were applied the virus was absorbed by other tissues and was no longer available to the CNS. It is of interest, in this connection, that no virus was recovered in the prodromal period from blood or lungs; very little virus was recovered from brains. From the finding that very little virus is required to initiate infection in animals by intracerebral inoculation, it may be concluded that there is an abundance of susceptible cells in the CNS; but their capability to produce virus is of a relatively low order as compared to chick cells in ovo or tissue culture. The amounts of NDV recovered from brains of infected hamsters were of the same order of magnitude as those amounts recovered from brains of other mammals(4).

From the observation that several hamsters inoculated with sterile diluents developed pneumonitis, it is possible, although evidence is lacking, that the inoculum may have provoked the multiplication of a latent infectious agent other than NDV. The pneumonitis in the inoculated animals, therefore, may be due to either: a) NDV infection despite poor recovery of virus from the lung tissues. b) accumulation of cytotoxic products of cells infected with NDV as suggested by others(7), or c) multiplication in the lung of a latent agent which interfered with multiplication of NDV.

Summary. Hamsters injected intracerebrally or by peripheral routes with NDV developed encephalomyelitis and pneumonitis. The amount of virus required to produce infection varied for each route, being least by the intracerebral route and highest by the subcutaneous route. Virus was recovered from the brain but not from the lungs or blood in the prodromal stages. The manifestations and pathology of the resultant encephalomyelitis were similar irrespective of route of inoculation.

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Postsystolic Myocardial Augmentation: 1. Effect Upon an Induced Shock State.* (26719)

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Different types of mechanical assistance have been considered in management of central circulatory insufficiency. Four major maneuvers have been considered: (1) total or partial cardiopulmonary bypass; (2) postsystolic myocardial augmentation; (3) veno-arterial shunt; and (4) selective right or left ventricular bypass. In postsystolic myocardial augmentation the augmenting ventricle (Fig. 1) is a blind-ended diverticulum which aspirates blood during the true ventricular systole and returns the blood to the arterial tree during true ventricular diastole, when the aortic valve is closed. Thus, the ventricle contracts against reduced resistance. Such counter-cycling can reduce and

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