THE BEHAVIOR OF POX VIRUSES IN THE RESPIRATORY TRACT

I. The Response of Mice to the Nasal Instillation of Vaccinia Virus

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PLATE 12

Our earlier studies on fowl coryza and mouse catarrh (1, 2) had dealt with infective agents which were essentially specific for the mucous membrane of the respiratory tract. It seemed of interest to extend this work to diseases in which the causal agent provoked an initial coryza but was not necessarily restricted to the respiratory tract. The pox viruses were chosen as being illustrative of this situation and also because they afforded an opportunity for an intimate comparison of the elementary bodies and the coccobacilliform bodies. Aside from observations on the rabbit, little attention appears to have been paid to the upper respiratory tract, in experimental studies of the pox viruses, in spite of its obvious importance as a portal of entry under natural conditions.

The production of a coryza in rabbits by vaccinia virus is well established. Gordon (3) found that the nasal instillation of vaccinia virus in doses containing as little as 0.01 mg. of calf lymph gave rise to a nasal catarrh after 6 to 8 days. There were no general symptoms. Rabbits tested 10 days after recovery were protected against vaccinia virus administered cutaneously. Nicolau and Kopciowska (4) noted that a coryza was a characteristic feature of a spontaneous epizootic attributed to vaccinia. Rosahn, Hu, and Pearce (5) observed that a coryza with a nasal discharge was a prominent manifestation in rabbits experimentally infected with a vaccinia-like virus from a natural outbreak.

The literature on vaccinial pneumonia in rabbits has been reviewed by Armstrong and Lillie (6). They described a highly fatal and characteristic pneumonia

in the rabbit produced by a selected strain of vaccinia virus following intranasal or intratracheal injection. The virus was carried through 8 successive lung transfers with no apparent loss in infectivity.

In the present paper observations are presented on the susceptibility of the white mouse to vaccinia virus introduced by the nasal route.

The initial strain of virus used in this work was a suspension of elementary bodies originally obtained through the courtesy of Dr. Thomas Rivers. The virus was propagated in the chorio-allantoic membrane of embryonated 10 day hen eggs incubated at 37°C. for 2 to 3 days. The method of inoculation was essentially that of Burnet (7). Membranes which showed a characteristic vaccinial reaction and numerous elementary bodies were removed, finely ground, and each suspended in 2 to 5 cc. of saline solution. These suspensions were used both for egg passages and mouse injection. The history of the mice employed has been given elsewhere (2). The mice were usually infected in groups of 5 which were kept together in one cage and held in a quarantine unit. The virus was introduced into etherized mice either by dropping a suspension on the nose or by dipping the nose directly into the suspension.

The introduction of vaccinia virus into the nasal passages of mice was regularly followed by a coryza, and later a pneumonia which was often fatal.

Symptoms

The onset of the vaccinial reaction was marked by obvious signs of discomfort which generally began on the 3rd day after injection but were occasionally delayed until the 4th day. Often the first indication was a tendency to remain huddled in a corner of the cage. By the 4th day symptoms were usually unmistakable. The mice were thin with a lean hunched appearance and ruffed coats; they were generally inactive and showed a markedly accelerated respiration. A cutaneous reaction was never observed. Nasal irritation was indicated by snuffling and often an intermittent chattering. There was no nasal discharge. The disease usually reached a crisis between the 5th and 7th days, terminating in death or rapid recovery. Occasionally recovery was retarded, symptoms persisting as long as 2 weeks after injection.

Pathology

Postmortem examination on the 3rd to the 7th day showed inflammation of the nasal mucosa but no indication of pock formation. A copious amount of turbid semifluid exudate was often present in the nasal passages. The exudate showed numerous tissue and mononuclear cells; polynuclears were generally present but never predominated as in the exudate of infectious catarrh. Elementary bodies

were detectable by the Morosow stain and were generally present in considerable numbers. An otitis media was sometimes observed but was not a characteristic feature, as it was in mouse catarrh. Bacteria were rarely conspicuous in the nasal exudate, save in mice which had been dead for some time when examined, and coccobacilliform bodies were never seen.

A pneumonia was generally present in infected mice, macroscopic changes being detectable in the lung by the 3rd or 4th day. Involved lobes showed grey or pink to red translucent areas of consolidation. At first these areas were small and patchy, later through coalescence they occupied a considerable portion of the lobe. The pleural surface was often moist, and occasionally free fluid was present in the chest cavity. In the lungs of mice that died it was not unusual to find areas of consolidation in all 5 lobes.

Histologically the vaccinial reaction in the lung was quite unlike any of the native pneumonias that we have seen in mice. The reaction involved both the bronchi and the alveoli. The bronchi frequently showed necrosis which varied in extent from case to case. In some instances there was a central plug of tissue debris. The alveoli were often filled with a coagulated serous deposit, occasionally with red cells. The alveolar walls were usually congested and areas of necrosis were often present. The alveolar reaction was further characterized by the deposition of fibrin as fibrils or dense strands. There was usually a marked increase in mononuclear cells in involved areas. Polynuclear leucocytes were generally present but were rarely numerous. Acidophilic cell inclusions resembling the Guarnieri bodies were not conspicuous. They were never found in the epithelial cells of the bronchi but were sometimes observed, as late as the 5th day, in the cytoplasm of large cells in areas of alveolar involvement. These inclusions were generally multiple, and sharply outlined. They often appeared to be embedded in a clear unstained zone.

The Mortality Rate

The nasal instillation of vaccinia virus was attended by a high though variable mortality, the actual rate being influenced by the mode of injection.

In the earlier experiments the suspension was dropped from a pipette directly in the nasal openings of etherized mice. Later the method used by Shope (8) in his work on influenza in mice was employed. The nose of an etherized mouse was dipped into a thin layer of the suspension which was drawn into the nasal passages with inhalation. Depending on the size of the animal, from 0.1 to 0.2 cc. of fluid was taken up in this way.

As indicated in Table I the percentage mortality was more than doubled in mice infected by dipping, the respective rates being 34 and 72 per cent. This high mortality was also maintained in a second

series of mice infected by dipping. Inoculated in this way a larger volume of suspension was drawn into the nasal passages and was probably drawn deeper. Regardless of the method, however, the mortality rate fluctuated from group to group, ranging from 0 in one group to 100 per cent in 4 groups. These irregularities in mortality were probably referable to variations in dosage. Most of the deaths occurred between the 5th and 7th days after infection. An advanced pneumonia was invariably present and was presumably the cause of death. There was no indication that the mortality was significantly affected by secondary bacteria.

TABLE I

The Mortality Rate in Mice Infected by Injection and by Dipping

Infected by injection			Infected by dipping		
Suspension	Number of mice	Number of deaths	Suspension	Number of mice	Number of deaths
Egg membrane	5	2	Egg membrane	5	4
Exudate	5	0	Exudate	5	1
"	5	0	"	5	0
"	5	2	"	5	5
"	5	3	"	5	4
Egg membrane	5	1	"	5	5
"	5	2	"	5	3
"	5	1	"	5	5
Exudate	5	2	"	5	4
"	5	4	"	5	5
Total............	50	17	Total............	50	36

Transmission by Passage

The respiratory disease invoked in mice by vaccinia virus was readily transmitted by passage. Exudate removed from the nasal passages of sick mice at autopsy or from dead mice was regularly infective for normal animals. No significant variations in the disease in respect to symptoms or mortality were noted with continued passage. Two experiments with 10 successive passages were carried out, one being still in progress for the maintenance of infective exudate. The results of the earlier passage are shown in the sixth column of Table I. The suspensions used were approximately a 1:10

dilution of nasal exudate in saline. The morbidity rate of the 50 mice employed in this test was 100 per cent and the mortality rate 72 per cent.

Acquired Immunity

It was not uncommon for mice with severe symptoms, seemingly about to die, to rally suddenly and within several days regain their normal sleek appearance. From time to time mice which had thus recovered were reinjected with an infective suspension of the virus. The interval between the two injections was variable but never less than 2 weeks. Generally the reinfected mice were kept under observation for 10 to 14 days and then autopsied, but in a few instances they were killed within a week of the second injection. 25 mice were tested, with a single non-specific fatality. In no case was there any indication of a reaction to the virus, either during life or at autopsy. Recovery clearly imposed a solid immunity to reinfection by the nasal route. The results with a few mice which were tested after an interval of a year indicated that the immunity does decline. Thus, of 4 reinfected mice, 1 showed a typical vaccinial reaction and died, 1 was normal during life and at autopsy, and 2 had a slight pneumonia when killed.

Communicability by Direct Contact

In each of 5 contact experiments 5 normal mice were placed in the same cage with an equal number of infected mice. The actual period of contact was variable depending on the survival time of the infected animals. None of the 25 exposed mice died and none showed symptoms. A few were sacrificed early with negative findings at autopsy. Most of the exposed mice were reinjected with an infective suspension of vaccinia virus. The susceptibility of the mice in 3 of the groups was normal, the morbidity rate being 100 per cent and the mortality 80 per cent. 2 groups of mice showed definite evidence of protection. There was only one death (10 per cent) and no indication of disease save in this one mouse. In these 2 groups, transmission of the virus was favored by a longer survival of the infected animals.

The outcome of the contact experiments indicated that vaccinial catarrh was not communicable by cohabitation. The amount of virus transmitted from infected to normal mice was regularly below the threshold required to establish an active infection. The actual dissemination of virus during cohabitation was intimated by the acquired resistance of 2 groups of mice in which exposure was prolonged.

The Limiting Infective Dilution of Virus

An infected egg membrane weighing 200 mg. was finely ground and a 10 per cent suspension prepared in saline. Graded dilutions were then made in steps of 10, and 5 mice infected with each dilution. The recorded dilutions which ranged from 2.5×10^{-2} to 2.5×10^{-5} were approximate, as the mice were infected by dipping. The actual variation, however, was slight. 2 embryonated eggs were inoculated with dilutions from 2.5×10^{-2} through 2.5×10^{-6}. They were

TABLE II

The Limiting Infective Dilution of Vaccinia Virus in Mice and in
Embryonated Eggs

Mouse test				Egg test		
Dilution	Number of mice	Number showing symptoms	Number of deaths	Dilution	Number of eggs	Reaction
2.5×10^{-2}	5	5	5	2.5×10^{-3}	2	+
2.5×10^{-3}	5	5	2	2.5×10^{-4}	2	+
2.5×10^{-4}	5	0	1*	2.5×10^{-5}	2	+
2.5×10^{-5}	5	0	0	2.5×10^{-6}	2	+

* Accidental death, not specific.

TABLE III

Protection in Mice Injected with a Subinfective Dilution of Virus

Group	Number of mice	Number showing symptoms	Number of deaths
Mice injected with dilution 2.5×10^{-4}.................	5	0	0
Survivors injected with undiluted suspension..........	5	0	0
Normal mice injected with " "	5	5	4

opened on the 3rd day after incubation at 37°C. and the membranes were examined for elementary bodies. A protection test was made 4 weeks after injection on mice which showed no reaction to a 2.5×10^{-4} dilution of virus.

The results of these experiments which are recorded in Tables II and III show that the amount of vaccinia virus required to establish infection in the mouse was at least 1000 times greater than that required to infect an embryonated egg and at least 10 times greater

than the immunizing dosage. The end-point was not reached in the eggs, but a dilution of 2.5 × 10⁻⁶ was infective. In mice the limiting infective dilution was 2.5 × 10⁻³ whereas the immunizing dilution was at least 2.5 × 10⁻⁴.

Distribution of the Virus

1. In the Nasal Passages.—The nasal scrapings or exudate from infected mice regularly showed elementary bodies, with the Morosow stain, during the acute stage of the disease. One experiment was made to determine how soon the elementary bodies could be detected after infection.

TABLE IV

The Detection of Virus in the Nasal Passages during the Incubation Period

Time of autopsy	Number of mice	Symptoms	Rhinitis	Elementary bodies	Injection of nasal washings			Protection test		
					Number of mice	Number with symptoms	Number of deaths	Number of mice	Number with symptoms	Number of deaths
hrs.										
24	2	—	—	—	5	—	—	5 survivors	0	1*
48	2	—	—	+	5	5	5	5 normal	5	4
72	2	+	+	+	5	5	5			

* Accidental.

Mice were infected by dipping and killed in groups of 2 at daily intervals through the 3rd day. The nasal scrapings were examined microscopically with the Morosow stain and tested for infectivity, using a 1:10 saline suspension. Survivors of the latter group were tested for protection.

The results of this experiment, as summarized in Table IV, indicate that elementary bodies were present in the nasal passages throughout the incubation period. There was no apparent increase, however, until the 2nd day, and no evidence of a rhinitis until the 3rd day. Additional observations indicate that active multiplication may be delayed until the 3rd day when symptoms are first apparent. Nasal washings removed on the 1st day contained sufficient virus to afford protection in normal mice and on the 2nd day to establish infection.

2. In the Lung.—The numerous granules in the lung tissue films interfered with the examination for elementary bodies, and their presence was never determined with certainty. Infectivity tests, however, indicated that an infective amount of virus was present, at least during the acute stage. Suspensions made of lung removed from 3 sick mice on the 6th, 6th, and 7th day of the disease produced a characteristic vaccinial reaction in each of 15 normal animals. The mortality rate was 55 per cent. Elementary bodies were again

TABLE V

The Detection of Vaccinia Virus in the Blood by Culture in Embryonated Eggs

Blood sample		Reaction		
Number	Day drawn	Lung	Nasal passage*	Egg
1	2nd	—	—	+
2	2nd	—	—	+
3	2nd	—	—	+
4	3rd	—	+	+
5	3rd	—	+	+
6	3rd	—	+	+
7	4th	—	+	+
8	4th	—	+	+
9	5th	+	+	+
10	5th	+	+	+
11	6th	+	+	+
12	6th	+	+	—
13	6th	+	+	—
14	6th	+	+	—
15	7th	+	+	—

* Includes presence of elementary bodies.

demonstrable in the nasal exudate at autopsy. A suspension made of lung removed on the 14th day from a mouse with persistent symptoms was not infective for 5 normal mice but did protect them against active nasal exudate.

3. In the Blood.—

Mice infected by dipping were bled from the heart under deep ether anesthesia. To avoid possible contact with the lung the chest cavity was exposed prior to bleeding. The aspirated blood, which varied from 0.2 to 0.5 cc. in volume, was made up to 1.0 cc. with saline and approximately 0.1 cc. of the suspension was implanted on the chorio-allantoic membrane of an embryonated egg. The inocu-

lated eggs were incubated at 37°C. for 3 days and opened. Membranes which showed a visible reaction were examined for elementary bodies.

As shown in Table V, 11 of the 15 samples of blood contained sufficient virus to produce egg membrane lesions. The reaction was confined to discrete foci of varying size, in which elementary bodies were generally demonstrable. The number of foci varied from 5 to 50 per membrane, the latter indicating a virus content in the blood of at least 500 elementary bodies per cubic centimeter. None of the eggs showed a diffuse necrotic reaction or death of the embryo indicative of a high virus concentration. Virus was detectable in the blood as early as the 2nd day, prior to the appearance either of a rhinitis or a pneumonia. 4 of the 5 samples removed on the 6th or 7th day contained no virus.

4. *In the Skin.*—Development of the virus in the skin following nasal instillation was never observed. The strain employed does not produce the characteristic pox of vaccinia even when introduced directly into the skin. It does, however, produce a superficial necrosis with considerable reaction in the subcutaneous tissue, followed by scab formation but no scar. Since the virus was generally demonstrable in the blood, it was thought that injury to the skin prior to nasal instillation might induce cutaneous localization of the virus. 6 mice were shaved over the abdomen and infected by dipping. 4 died on the 4th to the 6th day with no skin lesions. 2 mice which recovered also showed no evidence of a cutaneous reaction.

DISCUSSION

Vaccinia virus implanted on the untraumatized nasal mucosa of mice multiplies actively in the local epithelial cells. After a lag of several days, following the introduction of the virus, elementary bodies are demonstrable microscopically and exudation is apparent. The nasal surfaces are evidently highly permeable to the virus as it is cultivable from the blood prior to its microscopic appearance in the nasal tract. From its point of entrance the virus may be carried to the lung by two routes: directly, by way of the blood, and indirectly, along the bronchi. There is no apparent cutaneous development of the virus although it is probably carried to the skin in the circulating blood.

The behavior of vaccinia virus administered to mice by nasal instillation resembles that in rabbits similarly infected. In both hosts there is a catarrhal reaction with a coryza and pneumonia. These two manifestations are almost invariably associated in mice. In rabbits, however, the reported observations indicate that they are generally not associated, one occurring in the absence of the other. If these observations are indicative of strain differences either in the host or the virus, similar irregularities may be expected with other vaccinia strains in mice.

The factor of dosage is important in establishing vaccinia virus in the respiratory tract of mice. With the present strain the amount required to infect a mouse by the nasal route was at least 1000 times that required to infect an embryonated egg. The importance of dosage was also indicated by the failure to establish a vaccinial catarrh by contact infection. In the rabbit, however, vaccinia or vaccinia-like viruses may be naturally transmitted by the nasal passages and result in outbreaks of epidemic proportions. It appears probable that the mouse is endowed with a greater natural immunity to vaccinia than is the rabbit and is susceptible only when a critical concentration of virus is introduced.

The general manifestations of vaccinial catarrh in the mouse resemble those of the catarrh produced by the coccobacilliform bodies. Both show similar symptoms and both are characterized by a coryza and pneumonia. In specific details, however, the two reactions are quite unlike. Infectious catarrh is of slow onset and chronic. The disease progresses slowly over an extended period and is invariably fatal. The inflammatory reaction in all involved loci is characterized by a predominance of polynuclear leucocytes. The disease is readily transmissible by direct contact, and the specific agent is demonstrable in the nasal passages throughout the life of the host. Vaccinial catarrh is of rapid onset. The course of the disease is short and is abruptly terminated by death or rapid recovery. Recovered mice are immune to reinfection for a considerable period of time. The inflammatory reaction is characterized by a predominance of mononuclear cells. Vaccinial catarrh is not communicable by cohabitation, but sufficient virus may be disseminated thereby to produce immunity in exposed individuals.

SUMMARY

A catarrhal reaction manifested by a coryza and a pneumonia of characteristic pathology was regularly produced in mice by the nasal instillation of vaccinia virus. Inoculation into embryonated eggs indicated that the virus entered the circulation as early as the 2nd day after injection.

The vaccinial catarrh was readily transmissible by the passage of nasal exudate but not by contact. Dosage was important in establishing the virus in the nasal passages, the limiting dilution being approximately 10^{-3} of an egg membrane suspension (at least 1000 times the amount required to infect an embryonated egg).

The morbidity rate was variable but in general high, reaching 70 per cent in 2 groups of 50 mice. An immunity which was effective against reinfection for several months but ultimately declined was attendant on recovery. The amount of virus required to produce this immunity was significantly less than the infective dosage.

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EXPLANATION OF PLATE 12

FIG. 1. Elementary bodies in nasal exudate. Morosow stain. × 1000, enlarged to 3000.

FIG. 2. Vaccinial foci in egg membrane inoculated with blood. Approximately natural size.

FIG. 3. Late vaccinial reaction in lung. Phloxin methylene blue stain. × 125.

FIG. 4. Alveolar cell with multiple cytoplasmic inclusions. Phloxin methylene blue stain. × 1200.

FIG. 5. Alveolar reaction showing strands of fibrin. Phloxin methylene blue stain. × 800.
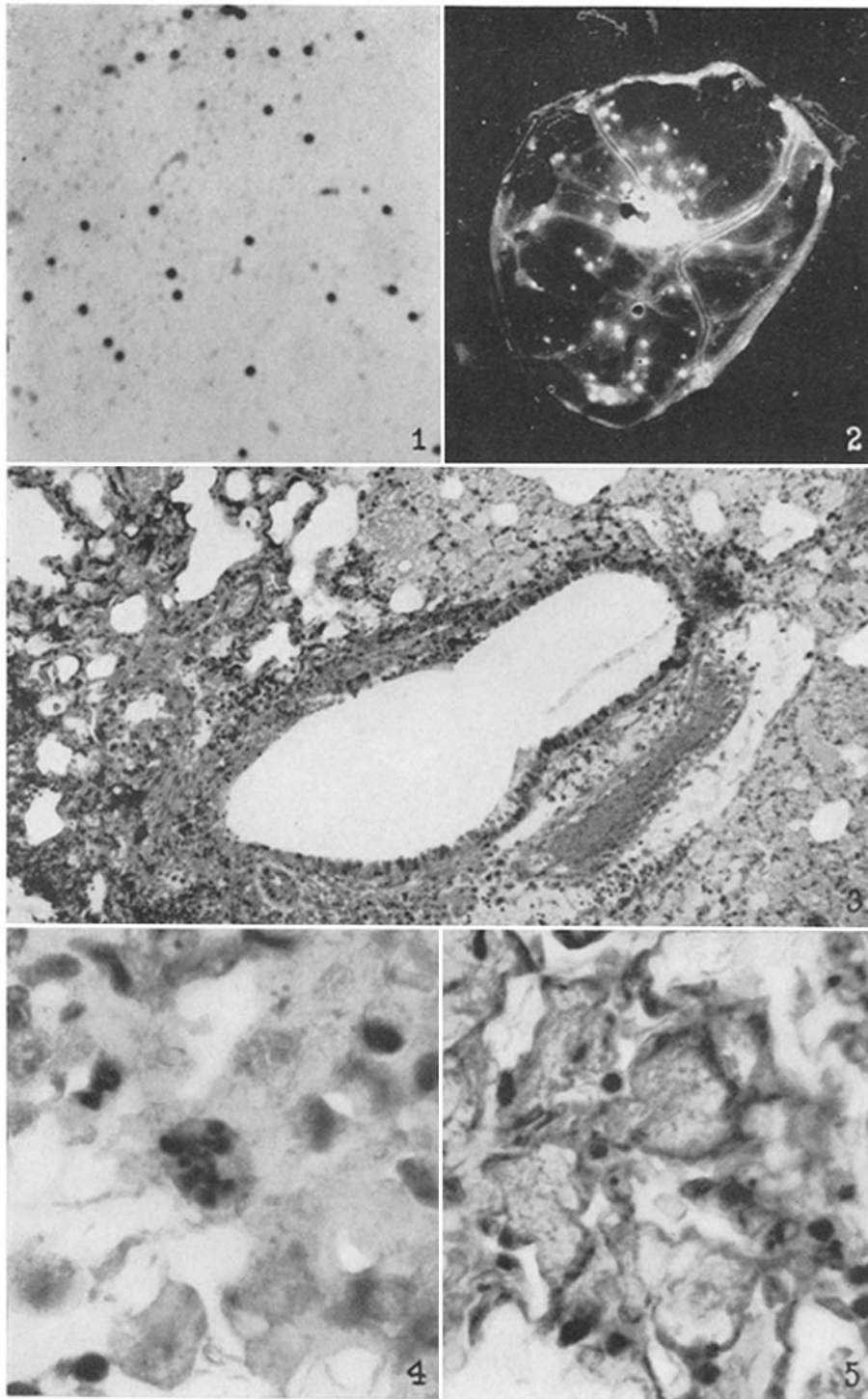
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