Susceptibility of some rodent species to monkeypox virus, and course of the infection *

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The authors studied the susceptibility of five species of rodent to monkeypox virus inoculated by various routes and the course of the infection. Reactions varied from complete resistance to lethal generalized infection with rash. Rabbits and white mice appeared to be the most susceptible species and young animals were more susceptible than adults. Monkeypox virus was found to infect young animals by natural routes, i.e., per os *and intranasally. Transmission by contact occurred among 10-day-old rabbits. Since antibodies to monkeypox virus may persist for over a year in the sera of convalescent animals, serological examination of animals is recommended for studying the ecology of this virus.*

When it was discovered that monkeypox virus can induce a pox-like infection in man (2, 7) numerous problems remained to be solved, since the ecology of the agent had not been adequately studied. It has still not been determined for certain what is the source of human infection and whether monkeys are the only carrier of this virus.

During the outbreak of monkeypox in Rotterdam Zoo (3) it was observed that not only primates but also the giant ant-eater can be infected with this virus. It therefore seemed expedient to study the susceptibility of various animal species to monkeypox virus and to determine the pecularities of the course of infection.

MATERIALS AND METHODS

Viruses

The reference strain of monkeypox virus Copenhagen was used in the form of a chorioallantoic membrane (CAM) culture from the 7th–15th passages on chick embryos. The infective dose varied from 10^1 to 10^2 pock-forming units (PFU), depending on the species of animal and the aim of the experiment.

Animals

Adult rabbits (chinchilla strain) weighing 2.5–3.0 kg and 10-day-old rabbits; guinea-pigs weighing 250–300 g; 3-week-old hamsters; adult and newborn white rats; and white mice aged 1–2, 8, 12, and 15 days were used in the experiments.

Methods of inoculation

Routine methods were used to inoculate animals by the intracerebral, intravenous, intracardial, intraperitoneal, intranasal, and intradermal routes, and on scarified skin. For foot-pad inoculation of guinea-pigs and white mice, 0.1 ml and 0.01 ml of virus suspension, respectively, were used. A special needle was used to infect mice *per os*. Young rabbits were infected with a probe or by adding the virus to the milk on which they fed. Adult rabbits were infected by means of a pipette.

Virus isolation from the blood and viscera

At the peak of the disease or 1, 2, 3, 4, and 5 weeks after inoculation, some of the animals from each group were killed and their blood and viscera (lungs, liver, kidneys, spleen, lymph nodes, brain, and testicles) were collected under aseptic conditions. To isolate the virus from these organs, the latter were ground in a mortar and 20% suspensions (by weight) in McIlvain buffer were prepared. Centrifugation was not used, but before inoculation the suspensions were shaken vigorously. The suspensions were tested by inoculation on CAM of 12-day-old chick embryos. These were opened after 72 h of incubation at 35°C.

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If there were no lesions on CAM or if their morphology was doubtful, another passage was performed with a CAM suspension as the inoculum. If the inoculum produced lesions typical of monkeypox virus, the results were considered positive. The blood for virus isolation was diluted 1 : 3 in distilled water. The viscera and blood were tested either immediately after collection or after storage at 4°C for 1–3 days. Sera for testing were prepared by the standard method.

Serological tests

The haemagglutination inhibition test was performed with 2 and/or 4 haemagglutination (HA) units of vaccinia virus and a 1% suspension of rooster red cells. Virus neutralization and precipitation tests were carried out as previously described (1, 8). Sera were diluted 1 : 5 and then heated at 65°C for 20 min before testing.

RESULTS

The results of the experiments performed on each species of animal are given below.

Rabbits

The susceptibility of rabbits to monkeypox virus was seen to depend on the route of inoculation and the age of the animal. Intravenous inoculation of adult animals with the virus in a dose of 10^7 PFU caused a severe generalized process with fever from the 3rd to the 7th day, conjunctivitis, rhinitis, extensive rash on the skin and mucous membranes, and loss of weight. The rash was observed 5–6 days after inoculation and had the appearance of papules, which developed later into pustules (Fig. 1). In some cases the lesions became haemorrhagic. As a rule, the formation of crusts began on about the 8th day. After a fortnight the crusts fell off and the scabbing stage ended within 3 weeks. Eleven out of twelve rabbits survived the infection and one died of cachexia a month later. During the first days of the disease the virus could be isolated from the blood. Later, it was isolated from some lymph nodes and from the kidneys (7th day of the disease). The virus was also detected in the tissue of the testicles in one of three convalescent animals 22 days after inoculation (neither crusts nor other signs of the disease were observed). The rabbits inoculated by the intravenous route developed antibodies 7 days after infection. By the 14th day the antihaemagglutinin titre rose to 128–256 and persisted at the level of 40–80 for more than 12 months. Virus neutralizing antibodies in a titre of 640 and precipitating antibodies were also detected in the sera taken more than a year later.

Rabbits infected on scarified skin with a virus dose of 10^5 and 10^6 PFU per 0.1 ml developed a localized papulopustular eruption at the inoculation site. In some cases the infection was followed by generalization of the process (fever, rash on the skin and mucous membranes). Intradermal inoculation of an equal dose of the virus induced dense infiltrates with necrosis and haemorrhages in the centre. No clinical reaction was observed in adult rabbits infected *per os*, even with a high concentration of the virus (1.4×10^9 PFU per 2 ml). An increase of virus neutralizing antibodies (up to 20) was observed 25 days after oral inoculation in one of two animals.

Young rabbits appeared to be much more susceptible to monkeypox virus: the 10-day-old rabbits infected *per os* with a dose of 10^6–10^7 PFU per ml of virus developed an acute generalized process with rash. Adynamia, loss of appetite, and diarrhoea appeared 4–6 days after oral inoculation. Eruptions on the inner side of the ears and around the lips and nose were observed in most of the animals, with subsequent suppurative conjunctivitis and rhinitis and rash spreading over the body (Fig. 2). The disease was accompanied by considerable loss of weight and as a rule ended in death 4–14 days after inoculation. At the acute stage of the disease the virus could be isolated easily from the viscera, and it was isolated from the blood (a few pocks on CAM), lungs, liver, and spleen (confluent pocks on CAM inoculated with a 20% suspension of those organs) of a young rabbit killed at the acute stage of the disease (4th day after inoculation). On the 7th day, the virus could be isolated from the lungs (in a titre of $>10^5$ PFU per ml) and kidneys (in a titre of 2.6×10^3 PFU per ml), but an attempt to isolate it from the blood was unsuccessful.

The 10-day-old rabbits were also found to be highly susceptible to intranasal inoculation. In the course of the disease they lost appetite and weight, the disease terminating in death within 4–5 days. No rash appeared. When uninfected animals and animals infected either *per os* or intranasally were kept together (the whole litter was kept in one cage together with the mother), the infection was transmitted by contact: 4 uninfected young rabbits sickened and 3 of them died 14 days after the experiment started and 6–8 days after the first signs of infection became evident in inoculated animals.

Table 1. Susceptibility of some rodent species to monkeypox virus

Species	Inoculation route	Age or weight	Symptoms	Mortality		Antibody formation
				No. [a]	%	
Rabbit	intravenous	adult, 2.5–3.0 kg	general disease with fever and rash	1/12	8	+
	per os		none	0/2	0	± [b]
	intradermal		dense infiltration with necrosis	0/10	0	+
	on scarified skin		local eruption followed by generalization	0/10	0	+
	intranasal	10 d	loss of weight, adynamia	5/6	83	+
	per os		acute general disease with rash	17/20	85	+
White mouse	intranasal	8-15 d	loss of weight, adynamia	17/17	100	n.d.
	per os	8 d	loss of weight, adynamia	4/10	40	n.d.
		12 d	loss of weight, adynamia	7/29	24	+
	intraperitoneal	8 d	loss of weight, adynamia	10/10	100	n.d.
	intradermal	8 d	local infiltration	5/10	50	n.d.
	foot pad	8 d	foot oedema, general disease	18/18	100	n.d.
		12 d	foot oedema, general disease	3/5	60	n.d.
White rat	intranasal	adult	none	0/6	0	n.d.
	intravenous		none	0/6	0	n.d.
	on scarified skin		none	0/6	0	n.d.
	intranasal	1-3 d	adynamia	24/24	100	n.d.
Guinea-pig	intracardial	250-300 g	none	0/5	0	+
	intranasal		none	0/5	0	+
	per os		none	0/5	0	—
	foot pad		foot oedema	0/5	0	+
Hamster	on scarified skin	3 weeks	none	0/6		+
	intranasal		none	0/6		+
	per os		none	0/3		+
	intracardial		none	0/75		+

[a] Numerator = number of dead animals; denominator = total number of animals inoculated.
[b] Antibody formation in one out of two animals.

A high concentration of virus (10^2 PFU per ml) was detected in the viscera of the dead animals. The mother rabbit had no symptoms of the disease, but haemagglutination-inhibiting antibodies (in a titre of 80 with 4 HA units) and neutralizing and precipitating antibodies were found in her serum 28 days after the young rabbits were inoculated with virus. Different types of antibodies to vaccinia virus were also found in the serum of a surviving young rabbit (virus neutralizing, precipitating, and haemagglutination-inhibiting antibodies in a titre of 160 with 4 HA units).

Guinea-pigs

The susceptibility of guinea-pigs was determined by *per os*, intranasal, intracardial, and foot-pad inoculation of the virus. No clinical reaction was observed in animals infected by these methods, except for foot oedema after foot-pad inoculation.

Seven days after inoculation virus could be detected only in the lungs of those guinea-pigs that had been inoculated by the intracardial route. After a fortnight the virus was not traceable in any of the animals infected by the above-mentioned methods. By that time haemagglutination-inhibiting antibodies in a titre of 16–64 were observed in the sera of guinea-pigs inoculated by the intranasal and intracardial routes and into the foot-pad.

White rats

No pathological symptoms developed in adult white rats infected by the intravenous or intranasal routes, or on scarified skin. The virus was not isolated from the blood and viscera of rats infected by the intranasal and intravenous routes, and it did not multiply in the skin of rats infected on scarified skin.

Different results were obtained by infecting newborn rats (1–3 days old). These rats were sensitive to monkeypox virus inoculated by the intranasal route, since they developed disease terminating in death on the 5th or 6th day. The virus was isolated from their lungs and liver in a titre of 10^2–10^3 PFU per ml.

White mice

White mice were shown to be highly susceptible to monkeypox virus inoculated by various routes. When 8-day-old white mice were inoculated by the intraperitoneal or intranasal route in doses of 1.2×10^6 PFU and even into the foot-pad (with a dose of 6×10^2 PFU) they developed disease the main symptoms of which were flabbiness, loss of appetite, and oedema of the foot (after foot-pad inoculation). All these mice died. Intradermal inoculation of the virus into 10 mice resulted in infiltrates with the death of 5 mice (50%). Mice inoculated by the oral route became flabby and lost appetite, and 40% of them died. Comparison of the susceptibility of 8-day-old mice to oral and to intranasal inoculation showed that the LD^{50} was lower when the virus was inoculated by the intranasal route. Higher sensitivity of white mice to intranasal infection was demonstrated by the inoculation of older animals. The 12-day-old mice infected *per os* sickened and died in only 14% of cases. On the other hand, 100% mortality was observed in 15-day-old mice after intranasal inoculation with the same dose of virus. The virus could be isolated (after oral inoculation) from the blood after one week and from the viscera (lungs, liver, spleen, and kidneys) after 3 weeks. The testing of sera from convalescent mice 42 days after infection *per os* revealed the presence of haemagglutination-inhibiting antibodies in a titre of > 256. A considerable amount of virus was detected in the lungs and other organs at the acute stage of the disease as a result of intranasal inoculation.

Hamsters

The animals were infected *per os*, by the intranasal and intracardial routes, and on scarified skin with a dose of $1.5–5.9 \times 10^7$ PFU. No symptoms of the disease were observed in these hamsters. Nevertheless, in animals infected by the intracardial route, the virus was detectable in the lungs, liver, and spleen during the first week after inoculation and in the kidneys during the first 3 weeks. Furthermore, despite the absence of clinical signs of the disease, marked pathological alterations were observed in the viscera of hamsters inoculated by the intracardial route. Those findings will be the subject of a separate report.

DISCUSSION

The pathogenicity of monkeypox virus for white mice inoculated by the intranasal and intracerebral routes and for rabbits inoculated by the intradermal, cutaneous, subcutaneous, intracerebral, intravenous, and intratesticular routes, as well as the resistance of guinea-pigs to the virus inoculated by the intravenous, intraperitoneal, and subcutaneous routes, were reported by von Magnus et al. (5), Prier & Sauer (9), Gispen et al. (4), and Marennikova

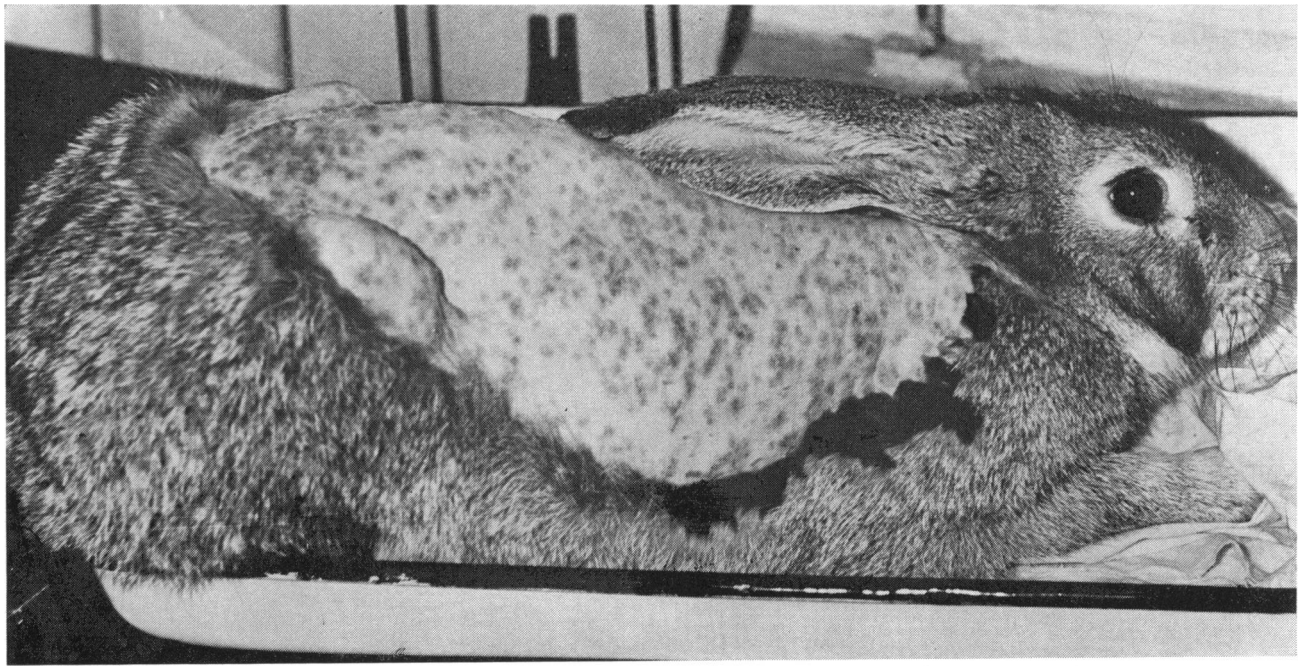
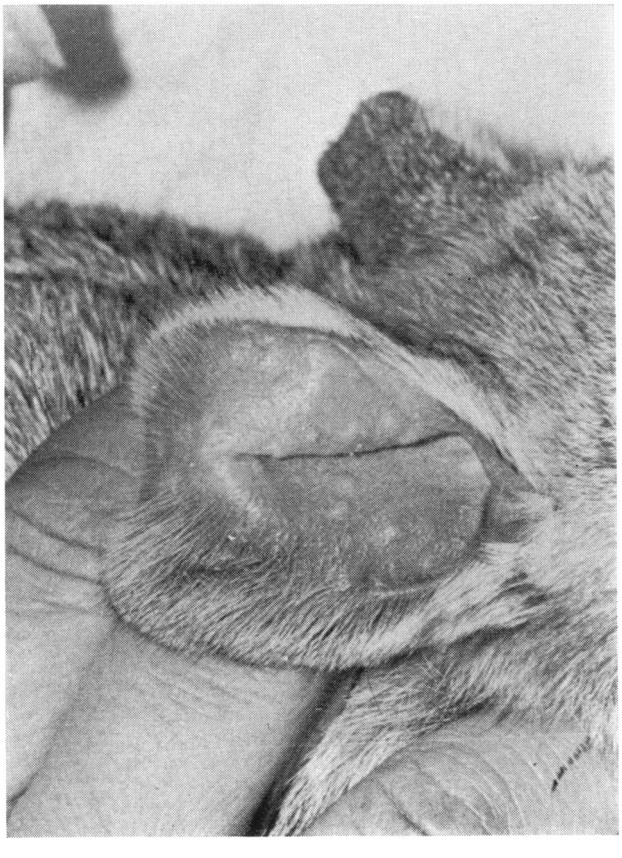


Fig. 1. Adult rabbit 7 days after intravenous inoculation with monkeypox virus.

Fig. 2. Generalized monkeypox infection in a 10-day-old rabbit: (*a*) general view; (*b*) inner surface of ear.



et al. (6). The susceptibility of hamsters had not been studied before.

The observations mentioned above were confirmed in our present study. These authors merely reported the pathogenicity of the virus, without details of the course of infection. Our task was to investigate the character of the infection process, the distribution of virus in the blood-stream and internal organs, and the immunological response after virus inoculation by various routes. Special attention was paid to the intranasal, *per os*, and contact routes, which are the possible ways of virus spread in nature, since they had never been studied previously, except for the intranasal route in mice.

The studies, performed with 5 species of rodent, showed that monkeypox virus can induce different kinds of reaction, ranging from complete resistance to a generalized lethal infection, depending on the species of animal, its age, and the route of inoculation. In our experiments, the disease developed in susceptible species (white mice and young rabbits) with infection methods that could be the ways in which the disease is transmitted in natural conditions (*per os*, intranasal infection). Generalized infection of rabbits with extensive eruptions on mucous membrane and skin permitted the release of the virus into the environment and the transmission of infection by contact.

The susceptibility of animals to monkeypox virus varied substantially depending on age. This was best demonstrated in white rats. Adult rats appeared to be resistant to large doses of virus inoculated by different routes, while newborn animals (1–3 days old) became sick on the 5th or 6th day after intranasal inoculation. The same effect was observed in rabbits. While adult rabbits developed no overt infection after *per os* inoculation, young rabbits inoculated by the same route responded with generalized infection with skin eruption and a high mortality rate. The possibility that infection was transmitted by contact (from infected to uninfected young rabbits) was established. This fact is particularly interesting as it indicates the possibility of horizontal transmission of monkeypox among animal species (e.g., rodents).

All the isolates of variola-like virus and one of monkeypox virus (strain 9411) were obtained from the kidneys of wild monkeys or apparently healthy laboratory monkeys. This fact served as a basis for studies of virus behaviour in animals with various types of infectious process.

Šeluhina et al. (10) observed that virus was continuously excreted by persons convalescing from smallpox. Despite the generalized type of infection, the virus could not be detected in rabbit kidney after the 7th day, although it was detected in testicle tissue for 22 days. On the other hand, the virus was found in hamster kidney for 1–3 weeks (though attempts to isolate it from other organs were negative and clinical features were absent). The long persistence of pox antibodies in the sera of animals convalescent after monkeypox infection, which we observed in our studies, coincides with the observation of R. Gispen et al. (unpublished observations, 1973), who found virus neutralizing and fluorescent antibodies in the sera of two convalescent orang-utans for more than 5 years. On the basis of our findings, serological examination of susceptible animals can be recommended for ecological studies of monkeypox virus.

The results presented here show that it was expedient to study the ecology of monkeypox virus and that it is necessary to investigate animal species other than monkeys in areas where monkeypox infection of man has been reported.

RÉSUMÉ

SENSIBILITÉ DE QUELQUES ESPÈCES DE RONGEURS AU VIRUS DU MONKEYPOX ET ÉVOLUTION DE L'INFECTION

On a éprouvé la sensibilité au virus du monkeypox de cinq espèces de rongeurs de laboratoire en le leur inoculant par différentes voies. Les réactions allaient de la résistance presque complète à l'infection léthale généralisée selon l'espèce de l'animal, son âge et la voie d'inoculation. Les jeunes lapins et les souris blanches de 8 à 12 jours étaient les plus sensibles: ils présentaient une infection généralisée après inoculation intranasale et *per os*. L'infection pouvait être transmise par contact parmi les rats de 10 jours. Il a été démontré que le virus du monkeypox était capable d'infecter des animaux par les voies naturelles de transmission virale. En raison de la persistance des anticorps spécifiques du monkeypox dans le sérum des animaux convalescents, il est recommandé de recourir aux examens sérologiques pour étudier la distribution du virus du monkeypox dans la nature.

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