A Study of New and Old World Monkeys to Determine the Likelihood of a Simian Reservoir of Smallpox

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The author presents data indicating that three species of New World monkeys (Cebus apella, Ateles paniscus, Lagothrix lagothricha) are not susceptible to Brazilian variola minor. Although the number of animals tested was not large, no experimental evidence was obtained to suggest that these species could form a non-human reservoir for smallpox in the Western Hemisphere. The results also indicate that Cercopithecus aethiops are not very susceptible to infection with either variola major or minor. Haemagglutination-inhibition tests of sera from monkeys from South America, Africa and the Philippines failed to reveal significant levels of poxvirus antibody. Previous studies have shown that Macaca irus, although susceptible to experimental infection with variola major, are not able to maintain the infection for more than a few generations of disease. The author concludes that there is as yet no clear experimental, serological or epidemiological evidence to support the hypothesis that smallpox can exist in wild simian populations.

The World Health Organization is sponsoring smallpox vaccination campaigns in all areas of the world where endemic smallpox exists. Since there is no known simian or other animal reservoir for smallpox (Arita & Henderson, 1968), the assumption in these eradication campaigns is that the perpetuation of smallpox is dependent solely upon continued human infection and that the virus will disappear from the earth if human infection is prevented. However, many Asian and African monkeys develop mild exanthems when experimentally infected with variola (Hahon, 1961) and smallpox-like illness has been reported to kill monkeys (Anderson, 1861; Bleyer, 1922).

We have studied the virulence of variola major and variola minor for selected species of Old and New World monkeys to determine whether these species could form a natural reservoir for smallpox. The first studies (Noble & Rich, 1969) showed that Macaca irus could transmit variola major (Harvey strain) to healthy control cage-mates by both contact and aerosol routes, but the infections died out after 2 and 6 generations of illness, respectively. Studies on the infectivity of variola major, variola minor, and monkeypox for three New World monkey species and poxvirus serological tests of 535 wild or captive monkeys from Africa, the Philippines, and South America are included in the present study.

METHODS

Cebus apella, Ateles paniscus (spider) and Lagothrix lagothricha (woolly) monkeys were imported from Peru and Colombia. Macaca irus monkeys were imported from Cebu Island, Philippines, to compare their response with that of the New World monkeys. On the first day of study pre-inoculation sera were obtained; then the monkeys were sedated with phencyclidine hydrochloride (Sernylan;4 Parke, Davis & Co., Detroit, Mich.), and inoculated intra-nasally with 1.5 ml of virus suspension. The intra-nasal route of inoculation was used in these studies to approximate to the natural route of infection. This

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method of inoculation had produced illness and lesions in *M. irus* subjected to transmission studies with variola major (Noble & Rich, 1969).

The animals were examined daily for the presence of lesions. Sera were obtained 2 weeks after inoculation and on termination of the studies, 3 weeks after inoculation. They were tested by the haemagglutination-inhibition (HI) test with a microadaptation of the method described by Kempe & St. Vincent (1964) and a partially purified vaccinia haemagglutinin.

The sixth egg passage of variola major (Harvey strain), titrating $2.5 \times 10^8$ pock-forming units (PFU) per ml, was used in study No. 1. This variola strain, isolated from a smallpox patient in India by Professor A. W. Downie, was also used in smallpox transmission studies in *M. irus* (Noble & Rich, 1969).

In study No. 2 the third passage of a Brazilian strain of variola minor titrating $10^7$ PFU/ml was used. The second egg-passage of a variola strain isolated from a smallpox patient in Pakistan was used in study No. 3. This Pakistani isolate grew at 38.5°C and was classified as a variola major. The Utrecht strain (65-32) of monkeypox was used in study No. 4. This virus was kindly provided by Dr R. Gispen, National Institute of Public Health, Utrecht, Netherlands, and titred $10^8$ PFU/ml. Variola major strains grew at both 35°C and 38.5°C in the ceiling temperature test (Nizamuddin & Dumbell, 1961). The Brazilian strain grew at 35°C but did not grow at 38.5°C. The infectivity of variola major and variola minor for *Cercopithecus aethiops* (African green monkeys) was also investigated in studies No. 1 and No. 5. The origin of these animals was unknown.

Animals in study No. 5 were inoculated intramuscularly and intraperitoneally with a second egg-passage of Brazilian variola minor titrating $10^7$ PFU/ml.

During these investigations and in collaboration with the World Health Organization we have also conducted tests on monkeys from Africa, South-East Asia, and South America for vaccinia HI antibody.

### RESULTS

Intranasal inoculation of variola major produced antibody responses in all of the animals tested in studies No. 1 and No. 3 (Table 1). One *M. irus* developed a facial lesion in study No. 3; the remaining animals inoculated with variola major in studies

| Study No. 1: Variola major (Harvey) |
|-----------------|-----------------|-----------------|
| Monkey No. 108  | Ateles paniscus  | <4              |
|                 | Ateles paniscus  | 8               | >256             |
| Study No. 2: Variola minor (Brazil) |
| Monkey No. 91   | Ateles paniscus  | <8              | <8               |
|                 | Ateles paniscus  | <8              | <8               |
| Study No. 3: Variola major (Pakistan) |
| Monkey No. 122  | Ateles paniscus  | <10             | 16               |
|                 | Ateles paniscus  | <10             | 64               |
| Study No. 4: Monkeypox (Utrecht 65-32) |
| Monkey No. 18  | Ateles paniscus  | 10              | 80               |
|                 | Ceboe aethiops  | <8              | 16               |
|                 | Ceboe paniscus  | 10              | 80               |

*Results expressed as reciprocal HI antibody titres.*

1. Table 1: Vaccinia Haemagglutination-Inhibiting Antibody Response Following Intranasal Inoculation of Variola Major and Minor and Monkeypox Into New and Old World Monkeys

2. Table 3: Comparative Results of Intranasal Inoculation of Variola Major and Minor and Monkeypox Into New and Old World Monkeys

3. Table 4: Comparative Results of Intranasal Inoculation of Variola Major and Minor and Monkeypox Into New and Old World Monkeys
No. 1 and No. 3 did not develop lesions and had no other manifestations of illness. Although *M. irus* are susceptible to variola major, there are occasional animals that develop very few lesions (Noble, unpublished data). In study No. 2 *M. irus* converted serologically after intranasal inoculation with Brazilian variola minor (Table 1) but did not develop lesions. The *Cebus apella*, *A. paniscus* and *L. lagothricha* did not develop lesions or vaccinia HI antibody after intranasal inoculation with Brazilian variola minor. Intranasal inoculation with monkeypox virus did not produce lesions on the animals tested (Table 1, study No. 4) but did cause rises in vaccinia HI antibody.

In study No. 5 (Table 2) Brazilian variola minor was inoculated intramuscularly and intraperitoneally to determine whether parenteral administration of a high-titred Brazilian variola minor strain recently isolated from a human case of smallpox could produce lesions in *M. irus* or New World monkeys. One of the *M. irus* (No. 134) developed 311 lesions. The other *M. irus* (No. 135), an *A. paniscus*, a *Cebus apella*, and a *Cercopithecus aethiops* did not develop lesions. Another *Cercopithecus aethiops* died without lesions 8 days after inoculation. The cause of death could not be established; however, autopsy revealed extensive haemorrhage about the site of a femoral venepuncture. The two *M. irus* and the surviving *Cercopithecus aethiops* developed vaccinia HI antibody (Table 2). The *Cebus apella* and *A. paniscus* did not convert serologically.

Some 95% of 535 monkey sera from various parts of the world were negative for poxvirus HI antibody (Table 3); 26 had HI titres of 1:10 or 1:20. Twenty-five of the positive sera were received from the Institut Mérieux, Lyon, France, in small volumes which permitted only 1 HI test. None of these sera titred above 1:20 and these titres may represent nonspecific reactions. A pet *Cebus capucinus* monkey in Riberalta, Bolivia, had a low HI antibody titre.

**TABLE 2**

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>Species</th>
<th>Serum sample&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Pre-inoculation</th>
<th>Post-inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>119</td>
<td><em>Cebus apella</em></td>
<td>&lt;10</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>123</td>
<td><em>Ateles paniscus</em></td>
<td>&lt;10</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>134</td>
<td><em>Macaca irus</em></td>
<td>&lt;8</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>135</td>
<td><em>Macaca irus</em></td>
<td>32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td><em>Cercopithecus aethiops</em></td>
<td>&lt;8</td>
<td>64</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Results expressed as reciprocal HI antibody titres.

<sup>b</sup> This animal was born in the United States of America and may have acquired accidental vaccinia infection.

We used black-tufted *Cebus apella* monkeys in these studies because this species is prevalent in tropical and subtropical forests of South America and usually has little contact with man. *Ateles paniscus* and *Lagothrix lagothricha* were studied because they often live close to villages in South America and have contact with both man and forest monkeys. Thus, they could transmit smallpox from the human population to species having little or no human contact.

In our studies, a laboratory strain of variola major and a second egg-passage of variola major recently isolated from a Pakistani patient produced antibody rise but no lesions in the New World species. None of the species inoculated intranasally with Brazilian variola minor developed lesions and only the *M. irus* had a rise in vaccinia HI antibody. The New World monkeys did not convert serologically following intranasal inoculation with this alastrim strain. Nor did a *Cebus apella* or *A. paniscus* convert serologically after intramuscular or intraperitoneal inoculation of the same virus. Great difficulty was experienced in conditioning and maintaining New World monkeys in captivity, making it impracticable to study a large number of these animals. However, our results, summarized in

**DISCUSSION**

Anderson (1861) and Bleyer (1922) reported that wild monkeys in Central and South America died in trees with a vesiculating smallpox-like exanthem. These reports prompted field and laboratory studies to determine the likelihood of simian smallpox, but no epidemiological evidence of nonhuman reservoirs of smallpox has been found (Arita & Henderson, 1968). Experimental studies have demonstrated limited transmission of variola major by aerosol and contact routes in *M. irus* (Noble & Rich, 1969); however, the infections were mild, did not increase in virulence on successive monkey passages, and then died out.

In our studies, a laboratory strain of variola major and a second egg-passage of variola major recently isolated from a Pakistani patient produced antibody rise but no lesions in the New World species. None of the species inoculated intranasally with Brazilian variola minor developed lesions and only the *M. irus* had a rise in vaccinia HI antibody. The New World monkeys did not convert serologically following intranasal inoculation with this alastrim strain. Nor did a *Cebus apella* or *A. paniscus* convert serologically after intramuscular or intraperitoneal inoculation of the same virus. Great difficulty was experienced in conditioning and maintaining New World monkeys in captivity, making it impracticable to study a large number of these animals. However, our results, summarized in
Table 3, suggests that these New World monkey species are not susceptible to variola minor infection and have little susceptibility to variola major.

When studying the pathogenesis of variola minor, Horgan & Haseeb (1939) inoculated several strains of virus intradermally into an African monkey, Cercopithecus sebaeus. The St. Louis strain of variola minor produced lesions and immunity, demonstrated by subsequent challenge vaccination. However, the Khartoum strain of variola minor failed to produce lesions when inoculated intradermally into this species, and the animals developed a primary reaction to subsequent smallpox vaccination. New World monkeys inoculated with Brazilian variola minor in studies No. 2 and No. 5 were not challenged with subsequent vaccination; however, they failed to develop vaccinia HI antibody.

Monkeys that developed antibody but no lesions after variola major or variola minor inoculation probably had an occult infection (variola sine eruptione). Virus isolation and contact transmission studies were not performed on these animals.
### Table 4
**Summary of Findings in Monkeys**

<table>
<thead>
<tr>
<th></th>
<th>Intranasal Inoculation</th>
<th>Intraperitoneal and Intramuscular Inoculation with Variola Minor</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Variola major</td>
<td>Variola minor</td>
</tr>
<tr>
<td>New World monkeys</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Macaca irus</em></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Ceropithecus aethlops</em></td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> 1 lesion observed in 1 of 2 monkeys.

<sup>b</sup> Of 2 monkeys inoculated, one presented generalized lesions, the other did not produce lesions, perhaps owing to previously acquired vaccinia infection.

<sup>c</sup> NA = not applicable.

However, transmission of variola major in *M. irus* occurred only when the infected animal had more than 4 lesions (Noble & Rich, 1969). In the absence of clinical illness and lesions, it is unlikely that the New World monkeys could have infected control cage-mates with variola major.

Monkeypox virus was inoculated into various monkey species to determine whether a type of fatal illness would be produced as Anderson (1861) and Bleyer (1922) had reported. The intranasal inoculation of *2 Cebus apella*, 1 *A. paniscus* and 2 *M. irus* monkeys did not produce cutaneous lesions, facial oedema, or death, suggesting that these species are not highly susceptible to this virus. However, the animals did develop vaccinia HI antibody, and the same virus inoculated intramuscularly and intraperitoneally into another *Cebus apella* monkey produced cutaneous lesions and stimulated development of HI antibody. Limitations of the experiment, owing to the few animals available, prevent further conclusions being drawn.

Serological testing of monkey sera obtained from South America, Africa, and the Philippines has not revealed significant levels of HI antibody suggesting previous infection with poxviruses in nature. Although sera from 20 *Erythrocebus patas* monkeys supplied by the Institut Mérieux had low levels of apparent antibody, sera from 133 other animals of the same species from the same area were negative. (During the screening of monkey sera for poxvirus antibody, laboratories in the Netherlands and Japan also observed nonspecific responses with the HI test (personal communications).) Authorities<sup>1</sup> working with primates and with the US/AID Smallpox Eradication/Measles Control Program of West Africa state that smallpox-like illness has never been observed in monkeys from Chad, nor in monkeys studied at the West African Council for Medical Research, Lagos, Nigeria, or at the Institut Pasteur, Dakar, Senegal. The *Cebus capucinus* monkey with antibody in Riberalta, Bolivia, was a pet and may have acquired vaccinia infection from man. No smallpox has occurred in that area of Bolivia since 1935.

Many of these sera could not be tested for vaccinia neutralizing antibody, and, since vaccinia HI antibody titre declines during the 12 months following infection, some of the monkeys that had no detectable HI antibody at the time of testing may have had a poxvirus infection in infancy. However, most of the animals studied at the National Communicable Diseases Center, Atlanta, Ga., were shipped directly from their places of origin; with several exceptions, they were young animals in the first or second year of life. The sera from Chad and Senegal were obtained from animals shortly after capture. If variola or monkeypox infection were present in the wild monkey populations from which these animals

<sup>1</sup> Dr Gerald Bearcroft, West African Council for Medical Research, Lagos, Nigeria; Dr Paul Brés, Acting Director, Institut Pasteur, Dakar, Senegal; Dr Bernard Lourie, formerly Medical Epidemiologist, US/AID Smallpox Eradication/Measles Control Program, Fort Lamy, Chad.
were captured, HI antibody would probably have been detectable in more of the sera.

In conclusion it may be said that, at the present time, there is no clear experimental, serological, or epidemiological evidence to support the hypothesis that smallpox can exist in wild simian populations.

ACKNOWLEDGEMENTS

Grateful appreciation is expressed to Mr James A. Rich and Mr James F. Freemont, who assisted in the animal handling and serological testing reported in this study. Monkey sera from the Institut Mérieux, Lyon, France, and from Chad were sent to the National Communicable Disease Center for testing by the World Health Organization.

RÉSUMÉ

ÉTUDE DE SINGES DU NOUVEAU ET DE L'ANCIEN MONDE EN VUE D'APPRÊCER LA VRAIESMBLANCE D'UN RÉSERVOIR SIMIEN DE LA VARIOLE

On a procédé à une étude expérimentale de la virulence de diverses souches de virus variolique pour un certain nombre d'espèces simiennes du Nouveau et de l'Ancien Monde et recherché la présence d'anticorps anti-poxvirus dans les sérum s de singes, sauvages ou en captivité, d'Afrique, des Philippines et d'Amérique du Sud.

Des singes de trois espèces du Nouveau Monde (Cebus apella, Ateles paniscus et Lagothrix lagotricha) n'ont présenté aucune lésion et n'ont pas élaboré d'anticorps IH après inoculation intranasale du virus de la variole mineure (souche brésilienne, la seule circulant dans l'hémisphère occidental). Bien que ne portant que sur un très petit nombre d'animaux, ces expériences ne plaident pas en faveur de l'existence éventuelle d'un réservoir non humain de la variole dans cet hémisphère. Chez Macaca irus, originaire des Philippines, les virus varioliques et le virus du monkeypox suscitent la production d'anticorps mais généralement sans provoquer de lésions cutanées. D'autres tentatives d'inoculation expérimentale indiquent que le singe vert d'Afrique (Cerco-pithecus aethiops) n'est pas très réceptif aux virus de la variole majeure ou mineure.

Sur 535 sérum s de singes examinés, 509 (95\%) se sont révélés, à l'épreuve d'inhibition de l'hémagglutination, dépourvus d'anticorps anti-poxvirus. Il est rappelé que si des travaux antérieurs ont montré la possibilité d'infecter expérimentalement M. irus par le virus de la variole majeure, cette infection ne se transmet de singe à singe que pendant un petit nombre de passages.

D'après l'auteur, aucune donnée expérimentale, sérologique ou épidémiologique précise n'étau e actuellement l'hypothèse selon laquelle la variole peut exister au sein de populations simiennes sauvages.

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