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# An outbreak of cowpox in captive cheetahs: virological and epidemiological studies

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## SUMMARY

This paper describes virological and epidemiological features of an infection which killed two of three affected cheetahs at Whipsnade Park in 1977. Two animals had profuse skin lesions and the third had an acute haemorrhagic pneumonia. The outbreak was shown to be caused by cowpox virus. Cowpox virus is believed to circulate in small wild animals, but the source of infection was not traced despite virological and serological tests on 93 captive and 102 wild animals.

Sub-clinical infections did not occur in susceptible contact cheetahs. Immune globulin did not influence the outcome and smallpox vaccine does not take in cheetahs. Management of any future outbreak will rely on prompt diagnosis and segregation of infected animals.

## INTRODUCTION

Until the 1930s the terms cowpox, smallpox vaccine, and vaccinia viruses were used synonymously. In 1938 Davies, Janes & Downie isolated from human cowpox a virus, which although closely related serologically and biologically to vaccinia virus, could be separated from it (Downie, 1939*a*, *b*) and all other accepted *Orthopoxvirus* species (Baxby, 1975).

In recent years cowpox virus and unclassified orthopoxviruses closely related to it have caused serious infections in valuable captive animals, notably okapis (Zwart, Gispen & Peters, 1971), lions, cheetahs, pumas, jaguars, panthers (Marennikova *et al.* 1977), and elephants (Baxby & Ghaboosi, 1977). We report here virological, serological and epidemiological aspects of an outbreak of cowpox in cheetahs (*Acinonyx jubatus*) at the Zoological Society of London's Whipsnade Park in February 1977.

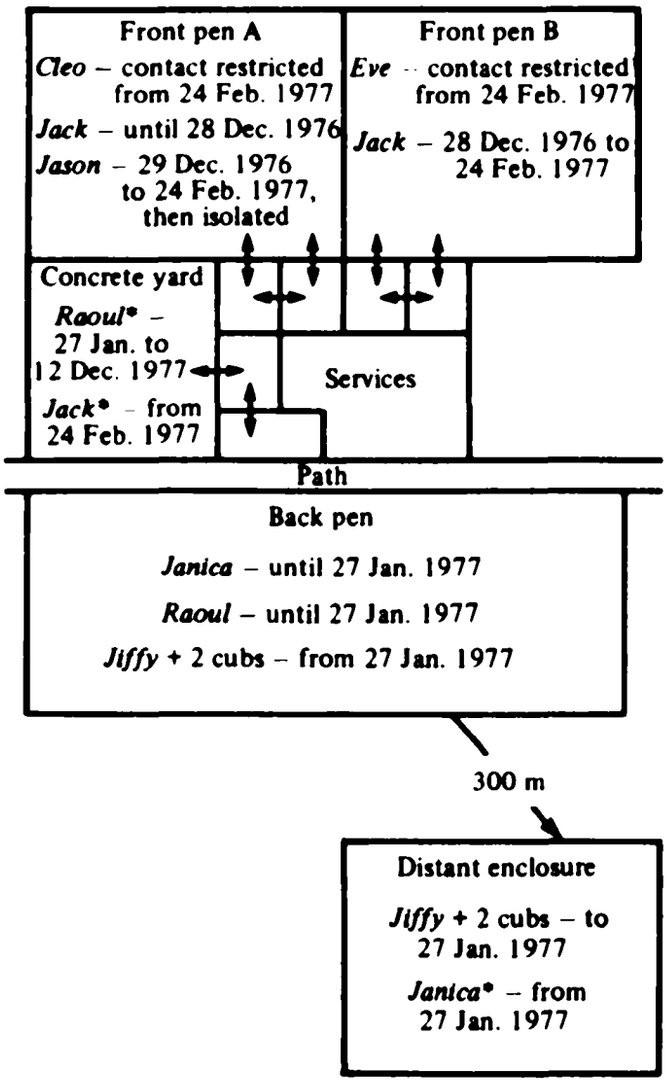


Fig. 1. Plan (not to scale) of cheetah accommodation showing location of the animals before and during the outbreak. *Animals showing clinical signs of illness. Arrows show possible movement of cheetahs, restrictable by sliding doors.

## THE OUTBREAK

### *Accommodation and movements prior to illness*

The group of cheetahs comprised seven adults and two cubs. All except one, which had been properly quarantined, had been born in the Park. Their accommodation and movements before and during the outbreak are summarized in Fig. 1. The main enclosure consisted of two grassed pens and one concrete yard round a central house, with interconnecting dens in which the animals could be separated by sliding doors. Animals in different pens could make some contact with each other through the outside chain-link fencing and through indoor partitions. Another enclosure (the 'back pen') was separated from the main complex by a path 1·5 m wide. A third enclosure 300 m away was also used.

Two of the animals to be affected, Raoul and Janica, had been housed together in the back pen until 27 Jan. 1977. Raoul was then moved to the concrete yard and Janica to the distant enclosure. The third animal to be affected, Jack, had been with Cleo in front pen A, but joined Eve in front pen B on 28 Dec. 1976. Direct contact between front pen B and the concrete yard was not possible; the nearest point of approach was across a 1 m wide central passage in the service area.

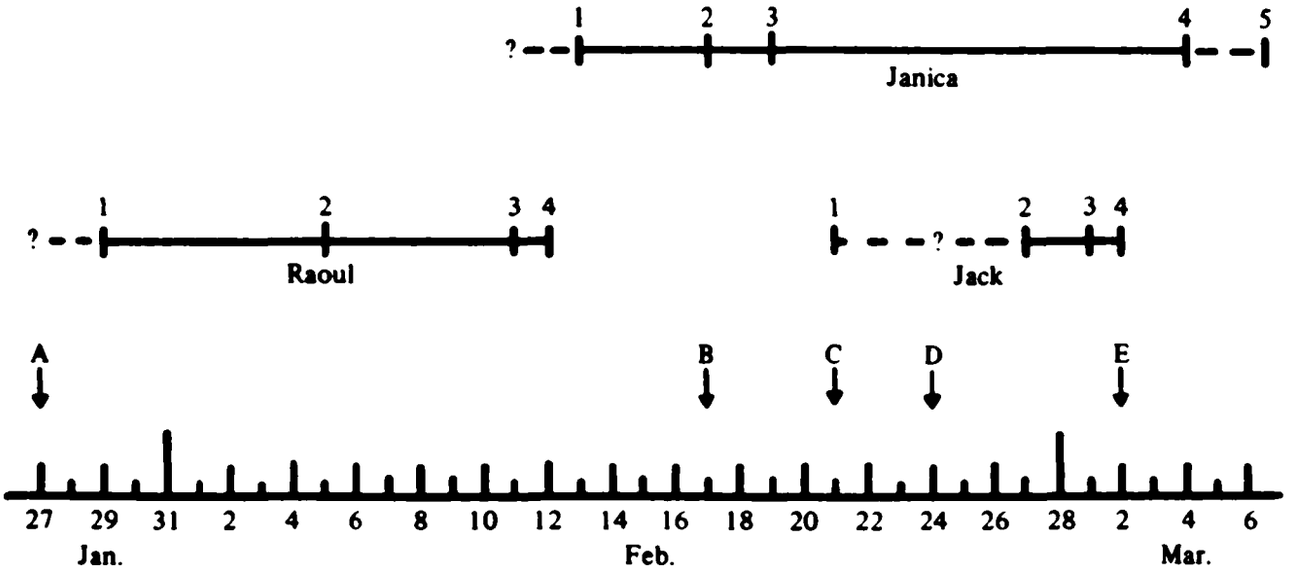


Fig. 2. Summary of the sequence of events during the outbreak. A. Raoul and Janica separated (see Fig. 1). B. *Orthopoxvirus* infection in Raoul confirmed. C. Anti-vaccinia $\gamma$-globulin given to all except Janica. D. Segregation of surviving cheetahs. Jason vaccinated. E. Cleo, Eve vaccinated.

*Raoul*. (1) Obviously ill. (2) First examination; biopsy and serum sample. (3) Second examination; serum sample. (4) Death, post-mortem samples.

*Janica*. (1) Obviously ill. (2) First examination; biopsy and serum sample. (3) Second examination; serum sample, $\gamma$-globulin given. (4) Obvious improvement. (5) (15 March), normal.

*Jack*. (1) Serum sample and mouth lesion tested for antibody and virus – both negative. Animal well. $\gamma$-globulin. (2) Obviously ill. (3) Second examination; pharyngeal swab, serum sample, $\gamma$-globulin given. (4) Death, post-mortem samples.

### *The affected animals*

Raoul and Janica had large numbers of ulcerated skin lesions which were not visible (except on the face) unless the hair was parted. Raoul also had oral lesions. His condition deteriorated despite treatment with broad-spectrum antibiotics and he died on 12 Feb. 1977; nine days after the lesions were first noticed and about 15 days after he had first shown signs of illness (Fig. 2).

Janica recovered from the infection. She was given 1000 mg human antivaccinia $\gamma$-globulin on 19 Feb. 1977, three days after skin lesions were noticed and about six days after first showing signs of illness (Fig. 2). She showed obvious improvement by 4 March and was completely well by 15 March.

Jack had only one skin lesion, behind the ear pinna, but showed severe respiratory signs and died only three days after first showing signs of illness (Fig. 2) Haemorrhagic pneumonia was the main post-mortem finding. The sequence of events in the three animals is summarized in Fig. 2. This shows the dates on which each animal became obviously ill, but less obvious signs were presumably present 1–2 days earlier.

### *Virological investigations*

Poxvirus infection was provisionally diagnosed when sections of Raoul's skin taken at biopsy were found to contain cells with large eosinophilic, cytoplasmic inclusions (Plate 1 A). These are characteristic of infection with some poxviruses,

Table 1. *Serological findings*

| Cheetah | Date in 1977 | Antibody titre by HI* | Antibody titre by Neut.† Raoul | Antibody titre by Neut.† Cowpox |
|---|---|---|---|---|
| Raoul | 3 Feb. | 256 | 400 | 375 |
| | 11 Feb. | 1280 | 1200 | 1500 |
| Janica | 17 Feb. | 256 | 240 | 280 |
| | 19 Feb. | 512 | 580 | N.T. |
| Jack | 21 Feb.‡ | < 4 | 10 | 10 |
| All others | 19–21 Feb. | < 4 | 10 | 10 |
| Jason | 14 March§ | 8 | N.T. | 20† |
| All others | March–April‖ | < 4 | N.T. | < 10† |

* Figure is reciprocal of serum dilution inhibiting 4 units of cowpox virus haemagglutinin.

† Figure is reciprocal of serum dilution permitting 50 % survival of virus isolated from Raoul and of cowpox virus, when held for 2 h at 37 °C. Neutralization tests on post-vaccination specimens were done with Lister Institute vaccine.

‡ Seven days before illness was apparent.

§ Three weeks after vaccination.

‖ Three to six weeks after vaccination.

notably cowpox (Downie 1939*a*), ectromelia (Barnard & Elford, 1931) and avian pox (Goodpasture, 1928). By that time, however, Raoul had died, and Janica was infected (although the latter information was unknown at the time).

Virus was isolated post-mortem from skin and lung specimens from Raoul inoculated into RK 13 and secondary monkey kidney cells. Inclusions were produced within 24 h (Plate 1 B) and haemabsorption of chicken erythrocytes strongly suggested infection with an *Orthopoxvirus* (Plate 1 C). This was confirmed by the detection of high titres of *Orthopoxvirus* neutralizing and haemagglutinin-inhibiting antibody when serum specimens taken from Raoul on the sixth and 13th days of illness were tested by standard methods (Boulter, 1957; McCarthy & Helbert, 1960) (Table 1). The virus isolated produced large haemorrhagic pocks on the chick chorioallantoic membrane (CAM). This, together with the production of the conspicuous inclusions, is diagnostic for cowpox (Baxby, 1975). Thin sections of biopsy material and infected CAM showed typical inclusions embedded with mature poxviruses (Plate 2 A, B).

An identical virus was isolated from the skin biopsy from Janica, who also developed high levels of antibody (Table 1). The virus was also isolated from the skin lesion, pharyngeal swab and internal organs of Jack.

No antibody was detected in serum samples from the other cheetahs (Table 1). This suggests that sub-clinical infections had not occurred.

*Management of the outbreak*

By the time cowpox was diagnosed in Raoul, Janica was showing signs of illness, and strict precautions were taken to prevent infection spreading to other animals. All the cheetahs were given 1000 mg human anti-vaccinia $\gamma$-globulin on 21 Feb. 1977. This did not prevent Jack becoming ill, nor did a second dose on 1 March prevent his death.

Redistribution of the animals was not possible before 24 Feb. On that date Jason was removed to separate quarantine facilities and Jack (not yet ill) was moved to the concrete yard, which had been thoroughly disinfected after Raoul's death. Cleo and Eve were kept separately in the two front pens, but were restricted so that they had no contact with each other or with other cheetahs. Jiffy and her cubs remained in the back pen.

The surviving cheetahs were given smallpox vaccine (Lister Institute). Jason had a minimal reaction and serological studies showed that he had a probably insignificant response. The other animals did not respond (Table 1).

*Epidemiological aspects*

Raoul, the first animal affected, became obviously ill on 29 Jan. 1977. Seven days later he had high levels of antibody and well-developed lesions. This suggests that infection occurred perhaps 2–3 weeks earlier, at which time he was in the back pen with Janica.

Janica was obviously ill on 13 Feb. 1977 and had high antibody titres and well-developed lesions by 17 Feb., which again suggests infection 2–3 weeks earlier. Raoul and Janica were separated on 27 Jan., two days before Raoul showed obvious signs of infection, and it is probable that Raoul infected Janica just before the separation. The alternative, that Janica was infected by a second unknown source when 300 m away in the distant enclosure, is possible but perhaps less likely.

Jack was given $\gamma$-globulin on 21 Feb. At that time a mouth lesion was noticed and sampled, but this, and a serum sample obtained on that day, showed no evidence that he was infected. By the time Jack became obviously ill (27 Feb.), Raoul had been dead for 15 days and Janica was being cared for 300 m away in the distant enclosure (Figs. 1, 2). It is possible that Jack was infected by virus shed by Raoul which spread across the 1 m wide passage which separated them. Jack was a particularly tame animal and may have been infected by keepers attending Raoul, although care was taken to prevent this. Three days before his illness was noted Jack was moved to the concrete yard which had been occupied by the sick Raoul. However, the yard had been thoroughly disinfected, and if Jack had been infected there by residual virus, it would have indicated an unusually short incubation period.

In the Moscow Zoo outbreak all the susceptible contact animals became infected (Marennikova *et al.* 1977). In the present outbreak Cleo and Jason were in contact with the sick Raoul through the chain-link fence and were not infected, and Eve shared a pen with Jack during the initial stages of his illness and was not infected.

*Attempts to trace the source*

Bovine cowpox is very rare. The current view is that the virus is not enzootic in cattle and that the virus reservoir is some so far unrecognized wild animal, possibly a wild rodent (Baxby, 1977). The cheetahs had been fed good local beef, but no cowpox was detected in local cattle and this source of infection seems unlikely.

To investigate the possibility that the source was a wild animal, serum samples and/or tissue specimens were taken from animals and birds trapped in the vicinity of the cheetah complex. In all 138 samples were collected from 90 mammals and

12 birds. An avian poxvirus was isolated from a sparrow, but no evidence of cowpox infection was found in any specimen. No evidence of cowpox infection was found by antibody tests on sera collected for various reasons in the year before the outbreak from 68 captive animals (including three cheetahs, four lions and five tigers). Similarly a further 25 samples obtained from captive animals after the outbreak were negative. Unfortunately the source of the outbreak remains a mystery.

## DISCUSSION

As far as we know no similar outbreak has occurred previously in Britain, but another outbreak killed two out of three cheetahs in another English zoo in November 1978 (Denham, Baxby & Thomsett, unpublished), and we have also seen cowpox in a domestic cat (Thomsett, Baxby & Denham, 1978). The outbreak reported here bears obvious resemblance to the outbreak in Moscow Zoo in 1973–4 (Marennikova *et al.* 1977). In both outbreaks the mortality was high, and in both an acute severe respiratory infection and a more prolonged dermal type were recognized. However, in the Moscow outbreak all susceptible animals became infected whereas at Whipsnade some cheetahs escaped, despite close contact. Minor differences have been found in the laboratory properties of the viruses isolated from the Moscow and Whipsnade outbreaks (Baxby *et al.* 1979) and evidently they also differ in infectivity for cheetahs.

The source of the Moscow outbreak was infected white rats used as food, and which became infected from wild rodents (Marennikova & Shelukhina, 1976). The source of the present outbreak was not found and is presumed to be a small wild animal. The only species tested in reasonable numbers were the brown rat (28 specimens) and the woodmouse (38 specimens). A very small proportion (2 of 28) of woodmice, and 7 of 20 short-tailed voles were shown to have *Orthopoxvirus* antibody in surveys carried out elsewhere but the infecting virus was not identified (Kaplan *et al.* 1980). We trapped six short-tailed voles but found no evidence of infection in them.

Management of future outbreaks may present some problems. Animals in zoo parks are impossible to segregate from small wild rodents. As far as we can tell $\gamma$-globulin has little or no value, but is probably worth using if available. Cheetahs appear to be insusceptible to Lister Institute smallpox vaccine, and are too scarce and valuable to permit trials of other *Orthopoxvirus* strains as possible vaccines. Consequently, at present management of future cases must depend on prompt diagnosis and segregation of affected animals. Segregation may not be possible because zoos may not have separate pens vacant at the crucial time. Cowpox virus can be isolated and identified quickly and easily, and the important point is to consider a clinical diagnosis of cowpox sooner rather than later.

This outbreak underlines an unfortunate trend already set by the Moscow outbreak and by infections caused in elephants in Germany by a closely related virus (Baxby & Ghabossi, 1977). This is that valuable and rare animals, endangered species in their natural environment, become susceptible to lethal poxvirus infections when in captivity. In zoo parks such animals are in contact with a variety of small wild animals. We trapped 12 species of mammal and the number could be increased. It is not known which species carry and transmit the infection, and more work is needed on the ecology of cowpox and related viruses.

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## EXPLANATION OF PLATES

### PLATE 1

(A) Section of skin lesion from Raoul. Cytoplasmic inclusions arrowed (H. & E. × 1600). (B) Secondary rhesus monkey kidney cells 24 h after inoculation with material from Raoul, showing many small cytoplasmic inclusions (some arrowed) (H. & E. × 1000). (C) As B, but after 48 h and showing haemabsorption of chick erythrocytes. This obscures the inclusions, but three are arrowed (H. & E. × 1600).

### PLATE 2

(A) Thin section of skin biopsy from Raoul showing large cytoplasmic inclusion (I) with mature poxviruses embedded in it. Immature viruses (V) in various stages of assembly can also be seen. This specimen was not fixed until virus had been isolated from it, hence tissue preservation is poor. (B) Thin section of CAM inoculated with virus isolated from Raoul, showing similar appearances to plate 2A (N = nucleus). Sections stained with uranyl acetate + lead citrate; magnification × 25000.

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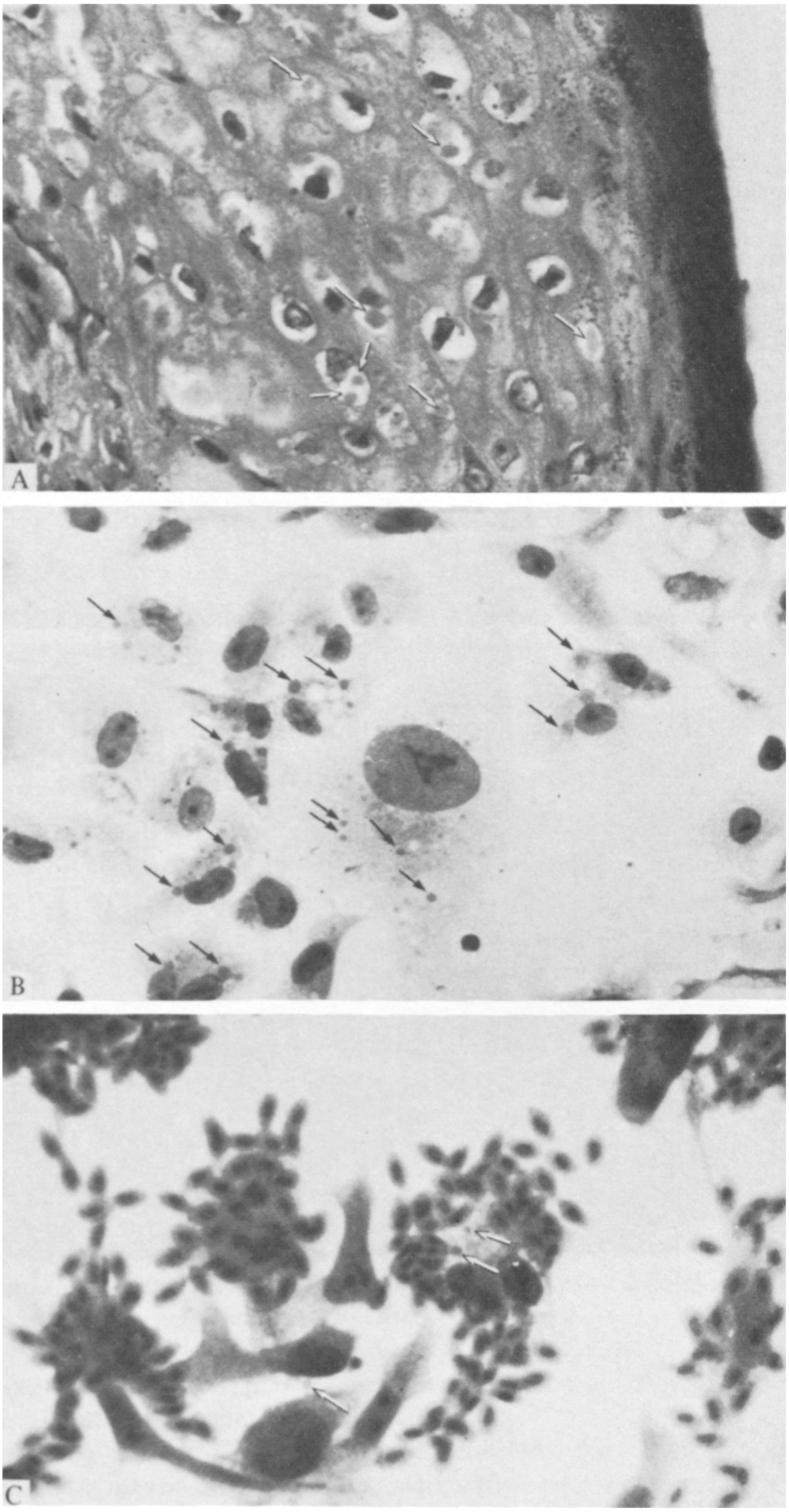


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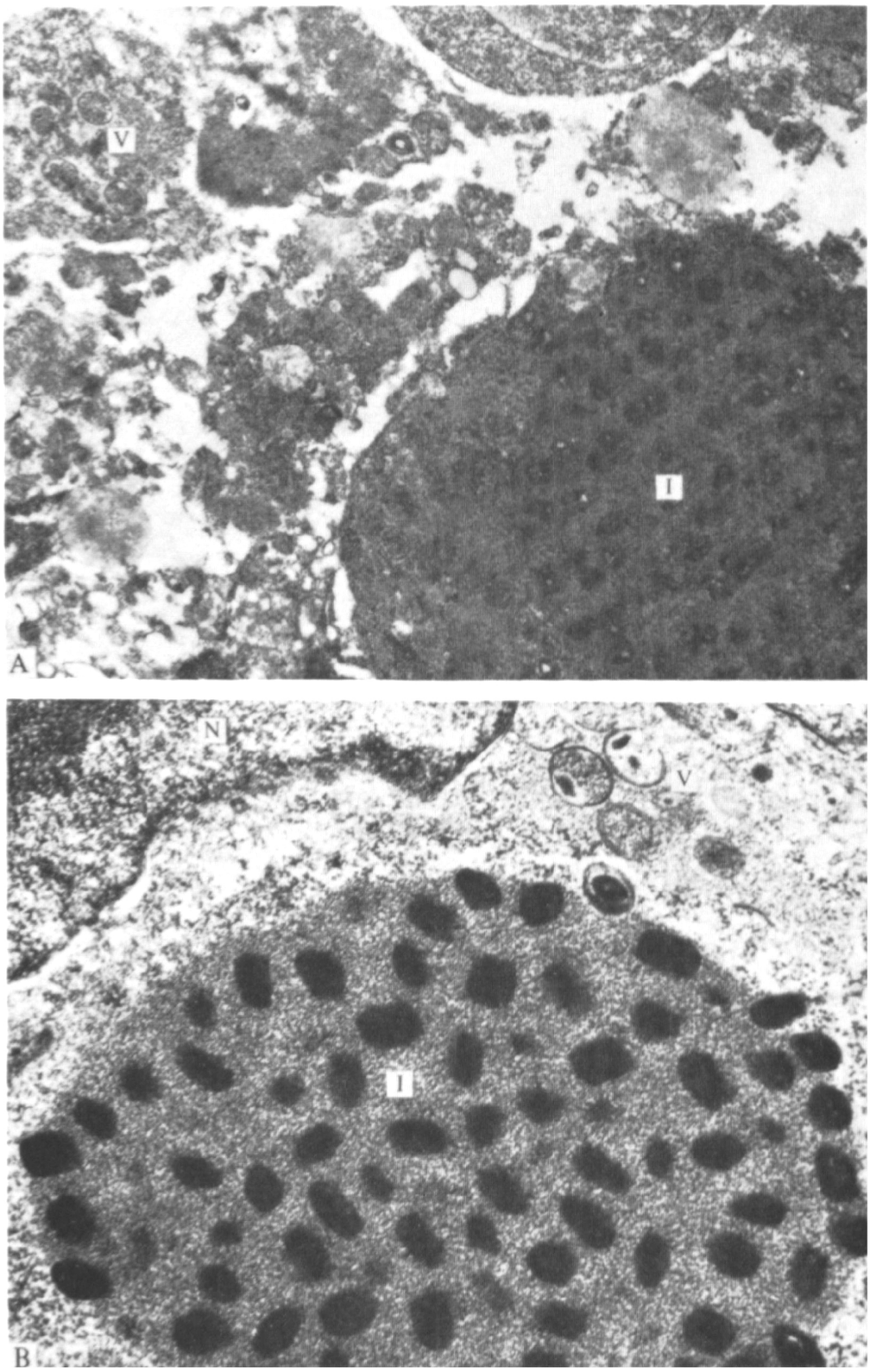


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