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Isolation and properties of the causal agent of a new variola-like disease (monkeypox) in man

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The causal agent of a case of disease in man occurring in the Democratic Republic of the Congo with a similar clinical picture to smallpox was isolated and studied. The agent was identified as monkeypox virus. A comparative study of the isolated strain (Congo-8) and of viruses isolated from similar cases of illness in Liberia (Liberia-1 and Liberia-2 strains) and Sierra Leone (V-70 1 266 strain) showed that they were identical. A number of local species of monkeys and apes were examined serologically in the Congo region to determine the probability of human infection with monkeypox virus. It was confirmed that the animals had had contact with an agent of the poxvirus group. In 2 of the 7 sera examined, antibodies of the variola–vaccinia group of poxviruses were discovered (virus-neutralizing antibodies, precipitins, and antihaemagglutinins). In a chimpanzee, antihaemagglutinins were found in a titre of 1 : 1 280, and in the same animal a variola-like virus was isolated from the kidneys. In the course of the investigation, it was shown conclusively that monkeypox virus and the strains under investigation could be distinguished from o dinary variola and vaccinia viruses on the basis of their behaviour in pig embryo kidney continuous cell line culture.

Until fairly recently it was believed that only two poxviruses other than variola and alastrim viruses could cause generalized infection in man accompanied by skin lesions. These were the true cowpox agent and the vaccinia virus. As a rule, both these viruses cause lesions at the point of infection, and the infection becomes generalized only in occasional cases. In 1958 a new representative of the poxvirus group, the monkeypox virus, was discovered (Magnus et al., 1959). Since then a number of outbreaks of monkeypox among trapped animals kept in nature reserves and zoological gardens have been recorded and studied (Prier et al., 1960; McConnell et al., 1962; Peters, 1966; Gispen, Verlinde & Zwart, 1967).

It has been established that the infection caused by the virus can be transmitted to some other animals (anteaters) and to various species of monkey and ape, including the anthropoid apes. The severity of the disease in monkeys varies and depends to a considerable extent on the species. The disease follows its most severe course in anthropoid apes. Nevertheless, no illness in man caused by monkeypox virus has been noted in any of these outbreaks. The data quoted in this paper, however, indicate that monkeypox virus can cause a disease similar to ordinary smallpox in man. The virus was isolated from material obtained from a 9-month-old unvaccinated child (A. I.) from the village of Bokenda in Basankusu Province, Democratic Republic of the Congo,[4] who was suffering from a disease suspected to be smallpox. The village concerned is situated in a remote locality in the depths of a tropical rain forest. No cases of smallpox had occurred during the 2 previous years either in the village or in the surrounding area. No cases of infection with the variola-like disease spreading from the child A. I. were recorded (Ladnyj, Ziegler & Kima, 1971). Practically the whole population of Bokenda village had been vaccinated against smallpox a year before this case

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occurred during a mass smallpox vaccination campaign throughout the country.

In addition to reporting the results of the isolation and identification of the Congo-8 strain of virus from the child A. I. this paper gives the results of a serological examination of a group of monkeys and apes from the focus, and also the results of a study of Liberia-1, Liberia-2, and V-70 1 266 viruses isolated in the Center for Disease Control, Atlanta, Ga., USA, from patients suffering from similar variola-like illnesses in Liberia and Sierra Leone.

MATERIAL AND METHODS

Material from the patients in the Democratic Republic of the Congo was obtained through the World Health Organization by air in a special packing, and consisted of the contents of skin lesions (scabs and smears on slides). The Liberia-1, Liberia-2, and V-70 1 266 strains were obtained through WHO on 11 December 1970, 18 December 1970, and 30 April 1971, respectively, in the form of suspensions of chorioallantoic membrane (CAM), infected CAM, and scabs from a patient (Liberia-1). These strains had been passaged once or twice in the laboratory and were used in the experiments in the form of first- or second-passage suspensions of CAM (disregarding passages undergone before they were received in the laboratory).

Strains Congo-8, Liberia-1, Liberia-2, and V-70 1 266 were compared with viruses of variola (strains MT-60 and MK-60-Harvey), cowpox (Brighton strain), monkeypox (Copenhagen strain), and vaccinia (strains Tanzania-3 and France). The viruses were used in the form of a suspension of infected CAM after one or two further passages in chick embryos.

The sera and organs of monkeys caught in the area of Bokenda village were received in the laboratory in a frozen state in a container of liquid nitrogen.

The usual methods were used to isolate the virus from materials taken from patients and from the organs of monkeys and to investigate them by means of the agar gel microprecipitation test (World Health Organization, 1969).

In studying the genetic features of the viruses (the type of pock on CAM and the plaques in cell cultures, haemagglutinating activity, pathogenicity for rabbits following infection by intradermal inoculation or scarification and other properties), standard methods were used (Marennikova & Šafikova, 1969; Marennikova, Gurvič & Šeluhina, 1970). The animals used in the experiments were white chinchilla rabbits weighing 2.5 kg, and randomly bred white mice weighing 10–11 g.

The type of cytopathic effect was determined in the following cell cultures: two primary (chick embryo fibroblast and monkey kidney cells) and four continuous (VERO, A-1, PEK, HEP-2). The morphology of the plaques was studied both with and without agar overlay (Porterfield & Allison, 1960; Gendon & Černos, 1964).

In the comparative studies equal doses of the viruses were used. The doses given to rabbits intradermally and by scarification were 10^6 pock-forming units per 0.1 ml. The doses used to study pathogenicity in chick embryos ranged from 10 to 10^7 pock-forming units per 0.1 ml, the results being read after 72 hours. In studying the ceiling temperature for the development of specific lesions, 100 and 1 000 pock-forming units were used. Except in special tests, the morphology of pocks was determined on CAM after 72 hours, when there were 10–100 pocks on the membrane. The type of cytopathic effect was studied by infection with doses of virus ranging from 10 to 10^7 TCD_{50}, the results being read from 24 hours to 7 days after infection.

Before examination the monkey sera were thawed and heated at 56°C for 30 minutes. The haemagglutination-inhibition test was carried out with 2 agglutinating units (AU) of the test virus and a 1% suspension of chick red cells. The neutralization test was carried out by the usual method (Boulter, 1957), while the Ouchterlony method (1949) was used in the agar gel precipitation test.

RESULTS

Research on the laboratory diagnosis of smallpox carried out in this institute in 1970, with material taken from patients during the period 18 December 1969 to 22 October 1970, enabled us to confirm the presence of smallpox in a number of provinces of the Democratic Republic of the Congo (Dendale Kinshasa, Kalomy, Longo Ngaba Kinshasa, and Kabulo Kalukivu). The strains of variola virus isolated did not differ from the typical virus of natural smallpox.

During the same period a virus was isolated from material taken on 1 September 1970 in the village of Bokenda in the province of Basankusu from the child A. I. as already stated. On the basis of an examination of infected CAM after 48 hours and serological identification by means of the agar gel

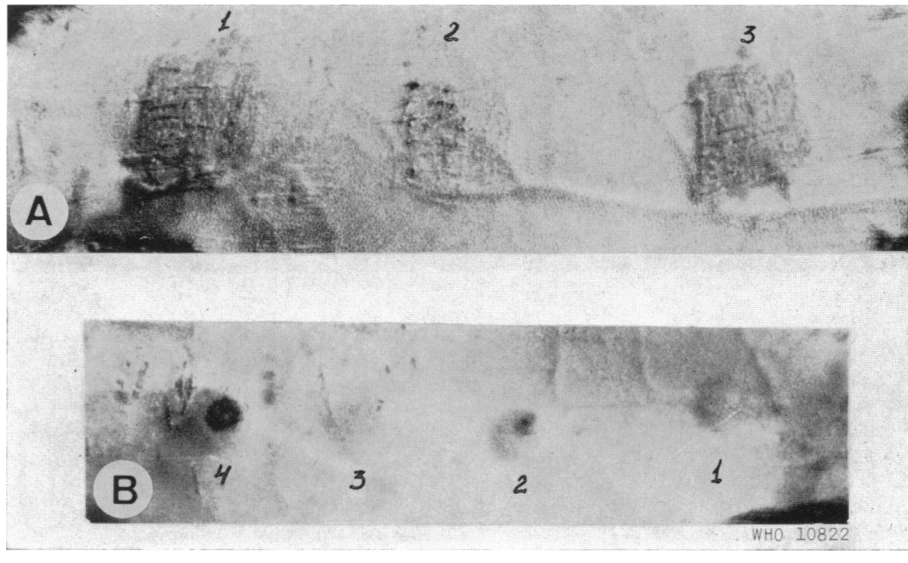

Fig. 1A. Lesions on scarified rabbit skin. 1, Congo-8 virus; B: variola virus, strain MK-60; 3, monkeypox virus, Copenhagen strain.

Fig. 1B. Lesions on rabbit skin after subcutaneous inoculation. 1, vaccinia virus Tanzania-3 strain; 2, Congo-8 virus, 3, variola virus, strain UK-60; 4, monkeypox virus, Copenhagen strain.

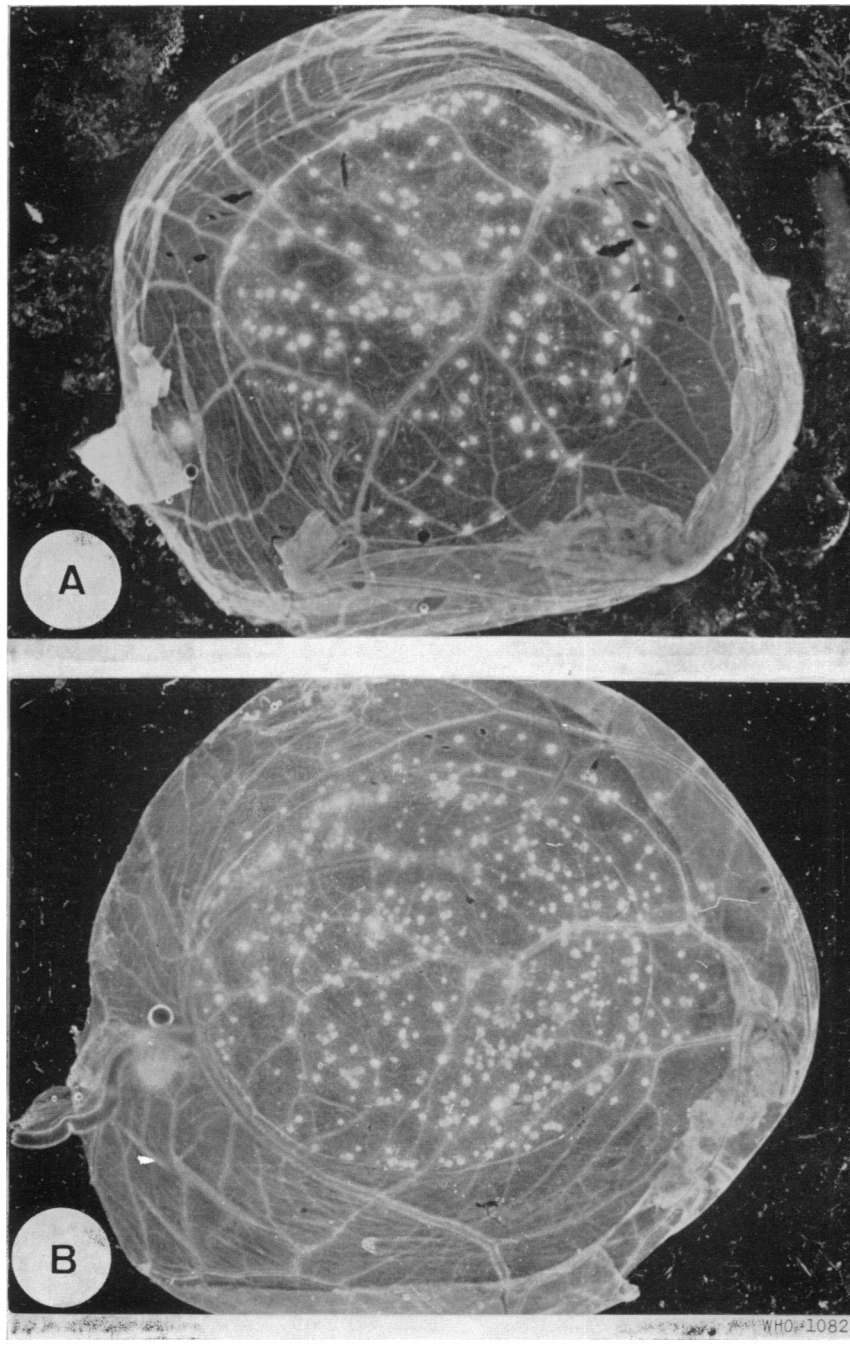

Fig. 2. Lesions on chick embryo chorioallantoic membrane; magnification × 2. A: Congo-8 virus; B: monkeypox virus, Copenhagen strain.

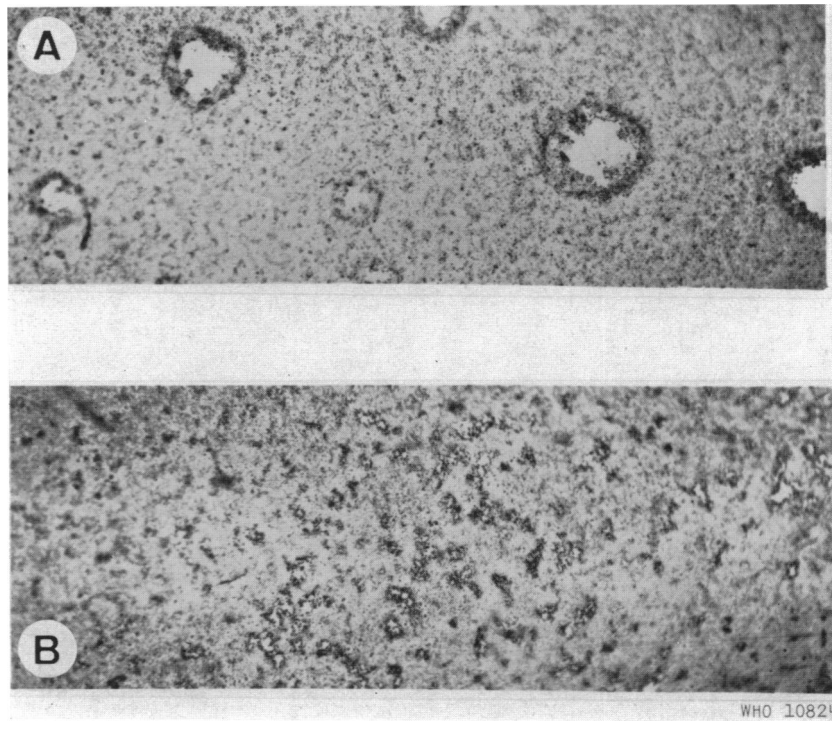

Fig. 3. Morphology of plaques in VERO cell culture 6 days after infection; magnification × 7. A: Congo-8 virus; B: variola virus, UK-60 strain.

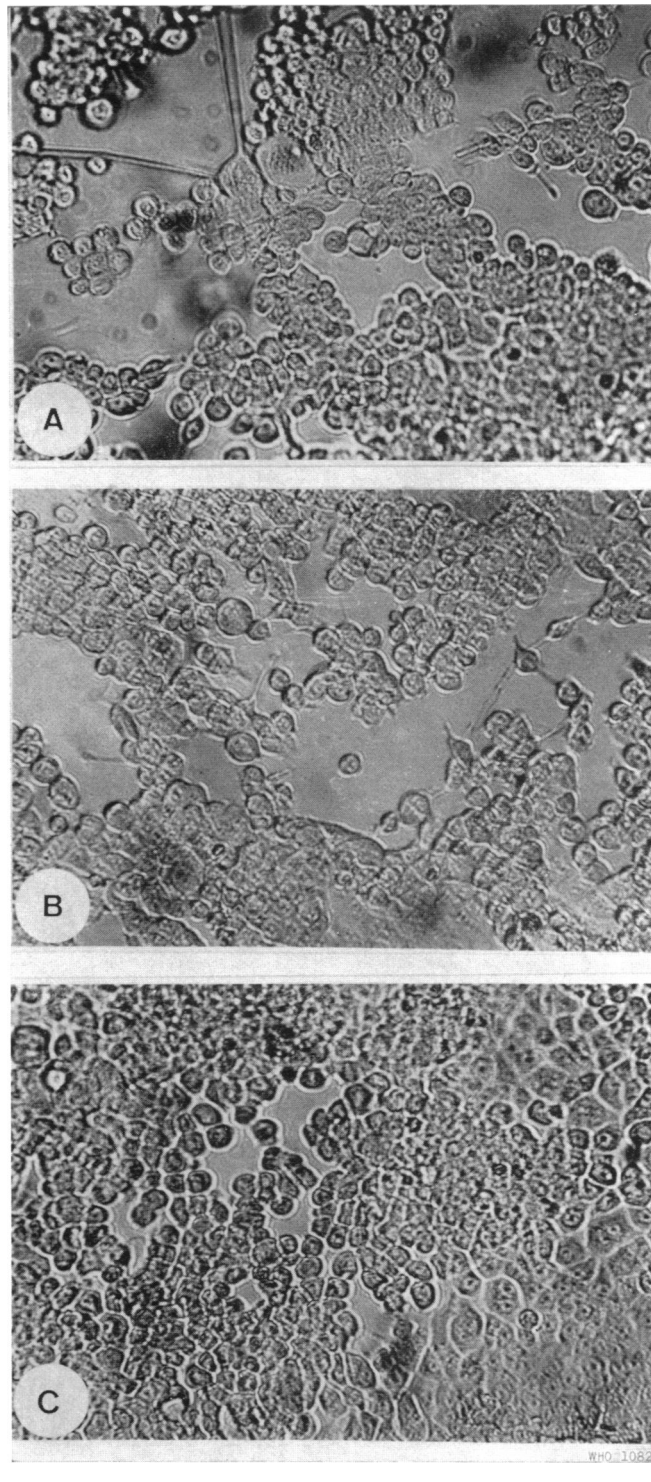

Fig. 4. Cytopathic effect in VERO cell cultures 5 days after infection; magnification 9 × 12.5. A: Congo-8 virus; B: monkeypox virus, Copenhagen strain; C: variola virus, MK-60 strain.

microprecipitation test, this virus was initially identified as variola virus (23 September 1970). However, the character of the pocks on CAM had changed sharply after 72 hours and this observation, together with results of haemagglutinating activity tests, suggested that the agent isolated was of a different nature. A detailed study was then undertaken and the virus was also re-isolated from material taken from the patient.[1]

Table 1 shows the results of isolating the virus from material taken from the patient and testing the basic properties of the strain. Investigations showed that the isolated virus (strain Congo-8) produced a peculiar type of pock on CAM, distinguishing the strain from both vaccinia virus and ordinary variola virus. This virus also showed a high degree of haemagglutinating activity and a marked tendency to cause lesions on scarified rabbit skin. On the third to fourth day after infection of the rabbit, the scarified area stood out above the healthy skin surrounding it as a result of the development of confluent papular eruptions without any marked induration of the underlying tissues. The confluent elements took the form of moist ridges along the lines of scarification (see Fig. 1).

When the rabbits were infected intradermally, necrosis developed in the centre of the dense infiltrated area that formed at the site of inoculation. The area of infiltration itself was slightly haemorrhagic. In rabbits infected by scarification and intradermally, the infection assumed a generalized character.

After incubation for 48 hours, as already stated the lesions on chick-embryo CAM differed little from those caused by ordinary smallpox virus. However, after 72 hours they had already become flatter and haemorrhages appeared in the centre of most of the pocks (Fig. 2). By that time the non-uniformity of the pocks in respect of morphology and size had become obvious; side by side with small pocks containing haemorrhages, a small number of large white pocks without haemorrhages could be observed. This feature was seen at an incubation temperature of 35°C. An increase in the incubation temperature by as little as 1 degC led to changes in the nature of the pocks. Pocks that developed on CAM showed no haemorrhage.

The unusual combination of properties and the close similarity of a number of the features of the Congo-8 strain to those of other viruses in the pox group made it necessary to carry out a detailed

Table 1. Isolation and identification of virus from patient A. I.

Material for infection of chick embryos	Type of lesion on CAM after:		Examination of the isolated culture				
	48 hours	72 hours	Microprecipitation in agar with vaccinia antiserum	Titre in the haemagglutination test	Infectivity for chick embryos (pock-forming units/ml)	In scarified rabbit skin	Intradermally in rabbits
suspensions of crusts in a dilution of 1 : 1 000	confluent, small immature, indistinguishable from smallpox lesions	flatter than smallpox lesions and slightly larger with haemorrhages in the centre; some large white pocks without haemorrhages	precipitation band identical with that formed by vaccinia virus	1 : 1 280	1.5×10^8	semi-confluent and confluent papules without marked infiltration of the subcutaneous tissue	area of dense infiltration 15 mm in diameter, with necrosis in the centre (6–8 mm)
smear on slide (washing)	small immature pocks of the smallpox type	small pocks with haemorrhages and occasional larger white pocks without haemorrhages	precipitation band identical with that formed by vaccinia virus	1 : 320	2.8×10^8	semi-confluent papules[a] without infiltration of the subcutaneous tissue	area of dense infiltration without necrosis[a]

[a] A smaller dose (10^4 pock-forming units/0.1 ml) was tested.

[1] The World Health Organization was informed of the true nature of the virus for the first time on 5 October 1970.

Table 2. Study of the test virus in a continuous culture of pig embryo kidney (PEK) cells

Virus	Strain	Characteristics		
		Cytopathic effect on administration of 10^4 pock-forming units [a]	Haemadsorption phenomenon with cytopathic effect	Infective titre TCD_{50}/ml [b]
variola	MT-60	++++	clearly marked	$10^{6.5}$
cowpox	Brighton	++++	clearly marked	$10^{6.5}$
monkeypox	Copenhagen	—	absent	$10^{2.6}$
test virus	Congo-8	—	absent	$10^{2.6}$
test virus	Liberia-1	—	absent	$10^{2.3}$
test virus	Liberia-2	—	absent	$10^{2.5}$

[a] The number of pluses indicates the intensity of the cytopathic effect; dashes indicate that no cytopathic effect was observed over a period of 7 days.

[b] For infection use was made of virus-containing suspensions of the virus strain with a titre between 6.5×10^7 and 6.6×10^8 pock-forming units/ml.

comparison of the virus in experiments with cowpox, monkeypox, variola, and vaccinia viruses. The strains of Liberia-1, Liberia-2, and V-70 1 266 from Liberia and Sierra Leone, which had been isolated from cases of variola-like illness in man, similar to that from which A. I. was suffering, were included in the same tests. The results of the comparative study showed that Congo-8, Liberia-1, Liberia-2, and V-70 1 266 did not differ substantially one from another according to the tests used, nor did they differ from the classical type of monkeypox virus of the Copenhagen strain (Tables 2 and 3).

Not only was these a close affinity between these four strains according to the tests already described (type of pock on CAM, skin lesions in rabbits, and haemagglutinating activity) but they all produced plaques of identical morphology and size, whether agar overlay was used (in chick embryo fibroblast cells) or not (Vero continuous cell line). Unlike ordinary variola virus, which under agar overlay produces small plaques less than 1 mm in diameter, monkeypox virus and the Congo-8, Liberia-1, and Liberia-2 isolates formed larger plaques with a visible internal structure and an uneven margin. In experiments without agar overlay, variola virus gave scarcely visible plaques with an intensely stained rim, whereas the isolates formed large plaques with a transparent centre and two peripheral zones: an inner reticular zone with an uneven edge and an outer intensely stained zone (Fig. 3). The differences in the type of cytopathic effect between these viruses and the variola virus were less marked than the differences in plaque formation. The isolates produced a cytopathic effect of focal type in the initial stages with breaks in the cell layer and a zone of cell proliferation on the periphery. The cells bounding the focus were rounded and highly refractive. In the centre of the focus were cells or groups of cells that had lost the capacity for process formation. At higher doses of the virus (over 10^5 TCD_{50}) there was a " diffuse " cytopathic effect throughout the cell layer (Fig. 4).

The study of a number of cell cultures infected with Congo-8, Liberia-2, and Copenhagen viruses showed that the most sensitive were VERO cells (titres of $10^{7.5}$ and $10^{8.2}$ TCD_{50}) compared with chick embryo fibroblast cells (titres of $10^{5.5}$–$10^{6.5}$ TCD_{50}) and the A-1 cells (titres of $10^{5.5}$–$10^{6.5}$ TCD_{50}). These viruses showed marked haemadsorption phenomena in chick embryo fibroblasts, monkey kidney cells, Vero cells, A-1 cells, and HEP-2 cells.

It has been shown previously (Marennikova, Gurvič & Šeluhina, 1970) that a feature of monkeypox virus that distinguishes it from variola and vaccinia viruses is its incapacity for active replication and the absence of the haemadsorption phenomenon in a culture of the continuous pig embryo kidney (PEK) cell line. Study of the behaviour of Congo-8, Liberia-1, and Liberia-2 strains showed that they behave similarly: their titre in PEK cells did not exceed $10^{2.6}$ TCD_{50}/ml and the haemadsorption phenomenon did not occur even when cytopathic changes had taken place (Table 2).

Determination of the ceiling temperature for the

Table 3. Haemagglutinating activity and pathogenicity of the test strains

Virus	Strain	Haemagglutination test titre	Pathogenicity				
			Chick embryos		White mice	Rabbits	
			Type of pock	LD_{50} (pock-forming units/0.1 ml)[a]	LD_{50} (pock-forming units/0.03 ml)[b]	Intradermally (10^6 pock-forming units)	On scarified skin (10^6 pock-forming units)
variola	MT-60	1 : 20	without haemorrhages	non-pathogenic in doses up to $10^{5.8}$	non-pathogenic in a dose of 10^6	pink infiltrate	no reaction
monkeypox	Copenhagen	1 : 160	the majority with central haemorrhages	$10^{4.5}$	$10^{4.1}$	infiltration with necrosis	papular eruption
test virus	Liberia-1	1 : 10	the majority with central haemorrhages	$10^{5.3}$	$10^{4.2}$	infiltration with necrosis	papular eruption
	Liberia-2	1 : 320		—[c]	$10^{3.1}$		
	Congo-8	1 : 320		$10^{4.2}$	$10^{3.2}$		
	V-70 1 266	1 : 2 560		—[c]	$10^{2.5}$		
cowpox	Brighton	1 : 20	haemorrhagic	$10^{3.8}$	$10^{2.7}$	haemorrhagic infiltration with necrosis	papulo-pustular eruption with haemorrhages and induration
vaccinia	Tanzania-3	1 : 320	without haemorrhages	$10^{2.4}$	—[c]	infiltration	papulo-pustular eruption

[a] 72 hours after infection.
[b] Following intracerebral infection.
[c] Not investigated.

development of pocks on chick embryo CAM showed that, unlike vaccinia and cowpox viruses, strains Congo-8, Liberia-1, and Liberia-2, as well as monkeypox virus, did not cause lesions at a temperature of 39.6°C following the administration of 100 and 1 000 pock-forming units. However, this group of strains and the monkeypox virus caused the development of pocks on CAM at a temperature at which no lesions developed following infection with the MT-60 strain of variola virus (38.6.39°C). The test strains differed from variola virus and cowpox virus in their higher haemagglutinating activity (see Table 3). The Congo-8 strain and the three other isolates tested proved highly pathogenic for chick embryos. Following intracerebral infection of white mice, these strains, like monkeypox virus, were considerably more pathogenic than variola virus for adult mice (Table 3).

In the serological studies use was made of hyperimmune rabbit sera against vaccinia and variola viruses. In the haemagglutination-inhibition test with both antisera, the test viruses produced titres identical with those produced by monkeypox virus (Table 4). In the agar gel precipitation test, all the viruses except cowpox virus were found to have an identical antigenic structure (Fig. 5).

In view of the fact that the experimental material obtained enabled the Congo-8 virus to be identified as monkeypox virus, it seemed useful to investigate monkeys and apes living in the area of the infective focus. In January 1971 Dr I. D. Ladnyj and Dr P. Ziegler trapped a group of monkeys of different species whose blood, serum, and organs (liver, spleen, and kidneys) were studied. The results of serological examinations of the animals are given in Table 5. Of 7 sera tested, 2 showed antihaemagglutinins in titres of 1 : 16 and 1 : 1 280. In these same sera (Nos 4 and 9), virus-neutralizing antibodies, in a titre of more than 1 : 40, and precipitins were discovered. The detection of antibodies to vaccinia virus shows that an agent of the poxvirus group is circulating among certain local species of monkey. Furthermore, the presence of precipitins and the high level of antihaemagglutinins found in a chimpanzee suggested that it had been infected. This view is supported by the isolation from the kidneys of the same chimpanzee of a variola-like virus. The results of this study are given by Marennikova et al. (1971).

Table 4. Serological identification of the test viruses in haemagglutination-inhibition tests

Virus	Strain	Titre	
		With antivaccinia hyperimmune rabbit serum	With antivariola rabbit serum
variola	MK-60	1 : 1 280	1 : 80
monkeypox	Copenhagen	1 : 640	1 : 40
test virus	Congo-8	1 : 640	1 : 40
test virus	Liberia-2	1 : 640	1 : 40
cowpox	Brighton	1 : 320	1 : 20
vaccinia	France	1 : 640	1 : 40

Table 5. Results of serological examination of monkeys and apes in the Basankusu region

No.	Species of animal	Antibody titre		Precipitation test [a]
		Haem-agglutination test	Neutralization test	
1	*Colobus polykomos* Zimm.	<1 : 2	<1 : 10	negative
2	*C. polykomos*	<1 : 2	—[b]	negative
3	*Cercopithecus mona wolfi* Meyer	1 : 2	—[b]	negative
4	*C. mona wolfi*	1 : 16	>1 : 40[c]	positive (+)
5	*Colobus badius* Kerr	<1 : 2	1 : 10	negative
8	*Cercocebus aterrimus* Oud.	<1 : 2	<1 : 20	negative
9	Chimpanzee	1 : 1 280	>1 : 40[c]	positive (+++)

[a] The number of pluses indicates the intensity of the precipitation bands in agar with antivaccinia serum.

[b] = Not investigated.

[c] The dilution 1 : 40 was the final dilution tested.

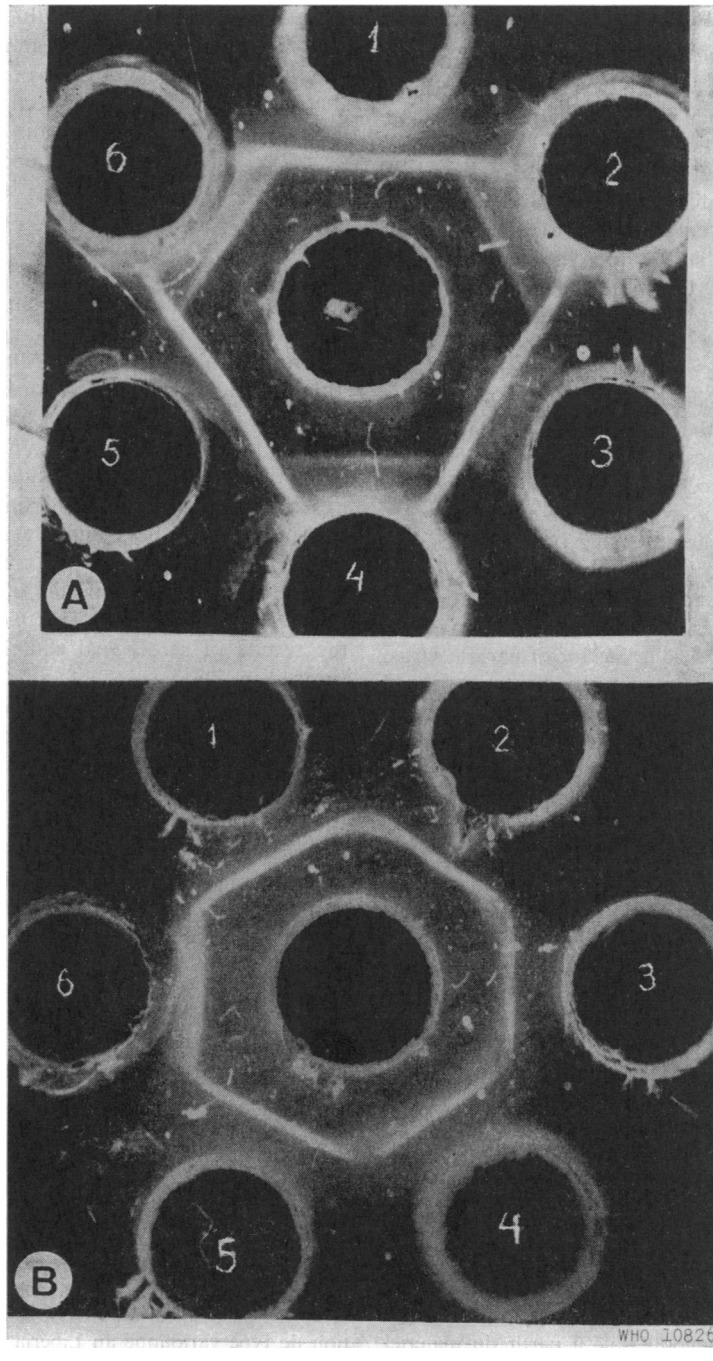

Fig. 5. Antigenic structure as found by the agar gel precipitation test. A: in the central well, hyperimmune anti-vaccinia rabbit serum. 1, Congo-8 virus; 2, 4, 6, cowpox virus, Brighton strain; 3, Liberia-2 virus; 5, variola virus, MT-60 strain. B: In the central well, hyperimmune antivaccinia rabbit serum. 1, 3, 5, Congo-8 virus; 2, variola virus, MT-60 strain; 4, Liberia-2 virus; 6, monkeypox virus, Copenhagen strain.

DISCUSSION

Experience gained in recent years in studying variola virus has shown that it is distinguished, like a number of other viruses, although to a lesser degree, by intraspecific variability or natural variation (Bedson, Dunbell & Thomas, 1963; Sarkar & Mitra, 1967; Marennikova & Šafikova, 1969). The differences found were related to the degree of pathogenicity of the strains for chick embryos and white mice, the ceiling temperature for pock development, and some other indices. However, not one of the strains of variola and alastrim viruses investigated proved capable of causing the development of lesions on scarified skin in rabbits. The Congo-8 strain isolated from the patient A. I. with an illness resembling smallpox caused a marked specific reaction when it was placed on scarified rabbit skin. This feature of the Congo-8 virus, combined with other characteristics (the haemorrhagic type of pocks on CAM, types of plaque in tissue culture, high degree of haemagglutinating activity, ceiling temperature for the development of lesions, etc.) makes it impossible for it to be considered as a variant of variola virus. On the other hand, the properties of Congo-8 proved to be indistinguishable from those of the classic representative of monkeypox virus (the Copenhagen strain), and provided good grounds for identifying it as a monkeypox virus itself. The evidence thus indicates that the monkeypox virus can cause disease in man.

The fact that examinations of numerous samples over a number of years of material from patients from different countries with suspected smallpox have not previously revealed an agent identical with, or close to, the Congo-8 virus in its properties, and that no such cases have been reported by other authors, may indicate that this infection has a low degree of contagiousness and a comparatively limited area of distribution. This view is supported particularly by the results of examinations of other material taken from patients in the Democratic Republic of the Congo. Except for Congo-8, the virus isolated in each case did not differ from the variola virus. However, the results of examining Liberia-1 and Liberia-2 strains, which according to the results we obtained did not differ substantially in their properties from the Congo-8 strain, suggest that monkeypox in man is not strictly limited in distribution since the Democratic Republic of the Congo and Liberia have no common frontier and are fairly far apart. It may be assumed that cases of monkeypox in man are caused by the presence of a reservoir of the virus in certain species of monkeys and apes in the areas concerned. This view is supported by the results of serological examinations of animals trapped in the focus of infection. The low degree of contagiousness of monkeypox to man or the complete absence of infectivity for other persons in the patient's household may be the reason why the disease was not discovered earlier. There is no doubt that the discovery of this infection was aided by the establishment of an epidemiological surveillance system sufficiently sensitive to discover single cases of illness.

RÉSUMÉ

ISOLEMENT ET PROPRIÉTÉS DE L'AGENT RESPONSABLE D'UNE NOUVELLE MALADIE RESSEMBLANT À LA VARIOLE (MONKEYPOX) CHEZ L'HOMME

Le but des présentes recherches était d'isoler, d'identifier et de soumettre à une étude comparative le virus responsable d'une affection ressemblant à la variole décelée au Zaïre chez un enfant de 9 mois non vacciné contre la variole. Le cas a été observé dans une région où aucune atteinte de variole n'avait été signalée au cours des deux années précédentes.

Le virus (souche Congo-8) isolé à partir du matériel (croûtes, sérosités) prélevé chez le jeune malade s'est révélé appartenir au groupe des poxvirus. Par plusieurs de ses propriétés, il se différenciait du virus de la vaccine et du virus variolique. Inoculé sur membrane chorio-allantoïde d'embryon de poulet, il produisait des pustules d'un type particulier; il témoignait d'une forte activité hémagglutinante et d'une aptitude marquée à provoquer des lésions sur la peau scarifiée du lapin. Une étude comparative, comportant une série de tests, a montré que la souche Congo-8 présentait pratiquement les mêmes propriétés que les souches Liberia-1, Liberia-2 et V-70 1 266, isolées à partir de cas humains d'une affection de type variolique au Libéria et en Sierra Leone, et que la souche Copenhague, souche de référence du virus du monkeypox. Comme le virus du monkeypox, et contrairement aux virus de la vaccine et de la variole, la souche Congo-8 se signalait par l'absence de réplication active sur culture cellulaire de rein d'embryon de porc.

L'isolement d'un virus du monkeypox chez un enfant a justifié une enquête sérologique parmi des singes capturés dans la région où le cas s'était produit. Sur 7 sérums examinés, 2 présentaient des titres d'anticorps inhibant l'hémagglutination de 1 : 16 et de 1 : 1280 et des titres d'anticorps neutralisants de 1 : 40 pour le virus vaccinal. On a isolé un virus de type variolique à partir des reins d'un des deux animaux positifs.

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