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Virus excretion in smallpox*

1. Excretion in the throat, urine, and conjunctiva of patients

J. K. SARKAR,[1] A. C. MITRA,[2] M. K. MUKHERJEE,[3] S. K. DE,[4] & D. GUHA MAZUMDAR[5]

Excretion of virus in the throat, urine, and conjunctiva of smallpox patients was studied daily for 2–3 weeks after the onset of fever. The virus titre in the throat and urine of haemorrhagic and confluent cases was higher than in discrete cases. The duration of virus excretion was also greater in confluent cases than in discrete cases. Conjunctival swabs from all 12 smallpox patients with conjunctivitis were positive for virus. The duration and titre of virus excretion in the throat, urine, and conjunctiva of patients were not related to their age or sex and did not depend on whether or not they had received a primary vaccination.

In the past vaccination and revaccination were thought to be sufficient to prevent and control smallpox in any area, and so a special effort to understand how infection spread was not thought essential. But since the World Health Organization embarked on a global programme of smallpox eradication in 1966, the number of countries reporting cases of smallpox has progressively fallen, and the prospects for complete eradication of the disease in the near future are fairly bright. This situation has demanded a critical study of the various factors responsible for the maintenance and spread of the infection. As a result, new knowledge and approaches have emerged, e.g., the recent records of monkeypox virus infecting man (WHO Expert Committee on Smallpox Eradication, 1972), the development of the idea that surveillance is more effective than large-scale vaccination for the control of smallpox (ibid., 1972), and the possibility of airborne infection in hospitals (Wehrle, 1970). The present work on the duration and extent of virus excretion in the throat, urine, and conjunctiva of smallpox patients was undertaken because it was thought that the findings might have an important bearing on our understanding of the epidemiology of the disease.

MATERIALS AND METHODS

All the subjects in the series were in-patients at the Infectious Disease Hospital, Calcutta, India, and were virologically positive, variola virus having been isolated from the blood in haemorrhagic cases and from vesicular or pustular fluid in non-haemorrhagic cases. Careful histories of the illness and of past vaccination were taken. The presence of a vaccination scar was taken as evidence of primary vaccination. No attempt was made to obtain the date of primary vaccination or a history of revaccination, as it was thought that the information would be unreliable. Patients with no vaccination scar were considered as unvaccinated. The day of illness was calculated from the date of onset of prodromal fever.

To denote the severity of the disease the cases were divided broadly into " haemorrhagic ", " confluent ", and " discrete " according to the criteria adopted by Sarkar & Mitra (1967). The haemorrhagic cases were characterized by early haemorrhages in the skin and mucous surfaces. Patients were extremely toxic and mortality was very high. In the confluent group, pocks on the skin were so plentiful that no healthy skin was visible between lesions, especially on the face and limbs. Toxicity and mortality were lower than in the haemorrhagic group. The discrete cases had far fewer lesions, with

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[1] Professor of Virology and Officer-in-Charge, WHO Research Project on Smallpox, Department of Virology, School of Tropical Medicine, Calcutta, India.

[2] Research Officer, WHO Research Project on Smallpox. Department of Virology, School of Tropical Medicine, Calcutta, India.

[3] Demonstrator in Virology, School of Tropical Medicine, Calcutta, India.

[4] Assistant Professor, Infectious Disease Hospital, Calcutta, India.

[5] Physician, Infectious Disease Hospital, Calcutta, India.

Table 1. Virus titre [a] in throat swabs from 32 smallpox patients on different days of the illness

Type of case [b]	Age (years)/ sex	Primary vaccination	Day of illness															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
H	32/M	—			10^5	10^5	10^5	E[c]										
H	42/M	—			10^6	10^5	NT[d]	10^2E										
H	8/M	—			10^5	10^5	10^4	E										
H	27/M	—			10^3	10^3	10^3	10^2	E									
H	19/M	—			10^5	10^4	10^4	E										
H	31/F	—		10^5	10^5	10^4	10^4	E										
H	11/M	—		10^4	10^4	10^3	E											
H	17/M	—			10^3	10^3	10^3	E										
C	28/M	+				10^5	10^5	10^4	10^3	NT	NT	O[e]	O					
C	12/M	—				10^5	NT	NT	10^3	10^3	NT	10^2	2×10	NT	O			
C	37/M	—				10^4	10^4	NT	10^2	10^2	10^2	10^2	NT	NT	10^2	NT	O	
C	57/M	+				10^5	NT	10^4	10^3	NT	10^3	NT	10^2	4×10	NT	10^1	E	
C	21/M	—				10^4	10^4	NT	10^3	10^2	NT	NT	10^2	10^2	10^2	E		
C	17/M	+				10^5	NT	10^4	10^4	10^4	10^3	NT	10^3	10^2	NT	10^2	E	
C	19/M	+			10^5	10^5	NT	10^4	10^4	10^3	10^2	10^2	10^2	NT	NT	10^2	E	
C	9/M	—			10^5	NT	NT	10^4	NT	10^3	10^2	NT	NT	10^2	10^2	10^1E		
C	11/M	—				10^4	10^3	10^3	10^3	NT	10^2	10^1	NT	NT	0	0		
C	24/F	+					10^3	NT	10^3	10^2	10^2	NT	0	0	0			
C	27/F	+					10^5	10^4	NT	10^4	10^3	10^3	10^2	NT	10^2	NT	0	0
C	17/F	—					10^4	10^3	10^3	10^2	NT	10^1	0	0	0			
D	24/M	+				10^3	10^3	10^2	10^2	NT	0	0						
D	11/M	+				10^3	10^3	10^2	0	0								
D	31/M	+				10^4	NT	10^3	10^3	3×10^2	10^2	NT	0	0	0			
D	12/M	+				10^3	NT	10^2	10^1	0	0							
D	47/M	—						10^1	10^1	0								
D	35/M	+						10^3	10^2	NT	0	0						
D	18/M	+						10^2	10^2	NT	10^1	0						
D	62/F	+					10^3	10^3	10^2	0	0							
D	37/F	+				10^4	10^3	10^2	10^2	10^2	0	0						
D	19/M	—					10^3	10^3	10^2	10^1	0							
D	10/M	—				10^3	10^3	10^2	10^2	10^1	0	0						
D	12/F	+					10^4	10^3	10^3	10^2	10^2	10^1	NT	0				

[a] Expressed as the number of pock-forming units per ml of swab-washed fluid (see text).
[b] H = haemorrhagic; C = confluent; D = discrete.
[c] E = expired.
[d] NT = not tested.
[e] 0 = absence of virus.

healthy skin between the pocks on both face and limbs. Patients were not toxic and mortality was almost nil. The haemorrhagic cases corresponded roughly to the " fulminating ", " malignant confluent ", and " malignant semi-confluent " cases of Dixon (1962); the confluent cases to his " confluent " and " benign confluent "; and the discrete cases to his " discrete ", " mild ", and " abortive " cases.

Collection of specimens began on the day of admission or the day following. Patients' throat and conjunctiva were swabbed with cotton swabs, which were first soaked in Hanks' basal salt solution (BSS) containing 0.5% bovine albumin and antibiotics (penicillin and streptomycin). Samples of midstream urine were collected aseptically from male patients. No specimen was collected from patients having smallpox lesions at the urethral opening.

All the specimens were immediately placed in ice containers and brought to the laboratory. After the swabs had been dipped in 1 ml of Hanks' BSS and squeezed, the fluid thus obtained was preserved at −20°C until inoculation. Egg inoculation and pock counts were performed in the standard manner as reported previously (Sarkar & Mitra, 1967).

RESULTS

Virus in the throat

This study was made with 8 haemorrhagic, 13 confluent, and 11 discrete cases. Throat swabs were positive in all the cases. Table 1 gives data on the cases and shows the virus titre in pock-forming units (PFU) per ml of swab-washed fluid on different days. It was found that (*a*) the haemorrhagic and confluent cases had a higher virus titre in the throat than the discrete cases; (*b*) the confluent cases excreted virus for a longer period than the discrete cases; (*c*) among the confluent cases, the patients who died had virus in their throats till the last day; (*d*) all the haemorrhagic cases were unvaccinated and the virus titre was high in all; (*e*) in the confluent and discrete cases there was no apparent relation between virus titre and vaccination status, age, or sex; and (*f*) the virus titre was highest in all 3 groups on days 3 and 4, after which it gradually diminished until days 7–9 in discrete cases and days 8–13 in non-fatal confluent cases.

Virus in the urine

Of the 39 patients studied, 21 (5 haemorrhagic, 18 confluent, and 16 discrete cases) showed virus in their urine, while urine samples from the remaining 18 (9 confluent and 9 discrete cases), all collected daily during the same period of illness, were negative. The virus titre in the urine of the 21 positive cases on different days of illness is shown in Table 2. It was found that (*a*) the haemorrhagic and confluent cases had a higher virus titre in the urine than the discrete cases; (*b*) the confluent cases had a more prolonged period of viruria than the discrete cases; and (*c*) previous vaccination, age, and sex of the patients did not appear to have any effect on virus excretion.

Virus in the conjunctiva

The study was made with 16 patients, of whom 12 had conjunctivitis. The 4 patients without conjunctivitis did not show virus in their conjunctiva. Table 3 shows the results of examination of swabs from the 12 positive cases.

The table indicates that (*a*) haemorrhagic and confluent cases had a higher virus titre than discrete cases; (*b*) confluent cases excreted virus for a longer period than discrete cases; and (*c*) previous vaccination, age, and sex did not seem to be related to virus excretion in the conjunctiva.

DISCUSSION

Virus excretion in the throat

It is now recognized that the most important mode of transmission of smallpox infection is by drop or droplet nuclei. Hence it may be expected that the degree of infectivity by this means at any time will depend on the titre of virus in the throat at that time. Table 1 shows that haemorrhagic and confluent cases, having a higher virus titre in the throat, are more infectious than discrete cases. But haemorrhagic patients, because they die earlier, have less opportunity to spread the disease than confluent patients. The epidemiological observation of Rao et al. (1968) that no first generation case became infected after day 13 of the disease in the primary case gains general support from the virological findings in this study, as none of the patients—except in fatal confluent cases—excreted virus in the throat beyond day 13. However, their observation that vaccinated patients transmit less infection than unvaccinated patients is not supported by our findings. Age and sex of the patients seemed to be unrelated to the virus titre in the throat. In the 5 fatal confluent cases, throat swabs were positive almost to the time of death.

Table 2. Virus titre [a] in the urine of 21 male smallpox patients on different days of the illness

Type of case [b]	Age (years)	Primary vaccination	Day of illness																		
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
H	32	—			10^5	10^5	10^3	E[c]														
H	42	—				10^3	NT[d]	10^2E														
H	8	—			10^4	10^4	10^3	E														
H	27	—			10^3	10^3	2×10^2	10^2	E													
H	19	—			10^5	10^4	10^3	E														
C	28	+					10^3	NT	10^3	10^2	10^2	10^2	NT	10^2	NT	NT	7×10^1	NT	NT	NT	2×10^1	0[e]
C	12	—			10^4	NT	10^4	10^3	NT	10^2	10^2	NT	NT	NT	10^2	NT	10^2	NT	10^2	0	0	
C	37	—				10^4	10^3	NT	10^2	NT	10^2	NT	10^2	NT	10^2	NT	7×10^1	0	0			
C	57	+				10^3	10^3	NT	10^2	NT	10^2	NT	10^2	NT	10^2	NT	E					
C	21	—					10^4	10^4	NT	10^2	NT	10^2	10^2	NT	10^1	E						
C	17	+					10^3	10^3	10^2	NT	10^2	NT	2×10^1	NT	10^1	NT	E					
C	19	+						10^4	NT	10^2	NT	10^1	NT	10^1	NT	NT	E					
C	9	—					10^4	10^3	10^3	NT	NT	10^2	NT	10^2	NT	NT	E					
C	11	—					10^3	10^2	10^1	10^1	NT	0	0									
D	24	+				10^2	0	NT	0													
D	11	+				10^2	10^2	0	0													
D	31	+					10^2	0	NT	0												
D	12	—						10^2	NT	10^1	10^1	NT	0									
D	47	+						10^2	NT	0	0											
D	35	+						7×10^1	0	NT	0											
D	18	+					2×10^2	10^2	0	0												

[a] Expressed as the number of pock-forming units per ml of urine.
[b] H = haemorrhagic; C = confluent; D = discrete.
[c] E = expired.
[d] NT = not tested.
[e] 0 = absence of virus.

Table 3. Virus titre [a] in conjunctival swabs from 12 smallpox patients on different days of the illness

Type of case [b]	Age (years)/ sex	Primary vaccination	Day of illness													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
H	32/M	—			7×10^4	NT[c]	3×10^3	E[d]									
H	42/M	—				4×10^3	2×10^2	7×10^1	E								
H	8/M	—			5×10^4	4×10^2	2×10^2	E									
C	28/M	+					7×10^4	NT	4×10^3	NT	4×10^2	7×10^1	NT	0	0		
C	12/F	—						9×10^3	NT	7×10^2	6×10^2	NT	0	0	0		
C	37/M	—					9×10^4	NT	7×10^3	NT	5×10^2	NT	2×10^1	0	0		
C	57/F	+						7×10^3	5×10^3	NT	4×10^2	0	0	0	0		E
C	21/M	—					4×10^4	4×10^4	3×10^4	NT	7×10^2	0	0	0	0	E	
C	17/M	+						4×10^3	NT	0	0	0	0	0	0		E
D	24/M	+				4×10^3	NT	0	0	NT	0	0	NT	0			
D	11/M	+					2×10^3	10^2	0	NT	0	NT	0				
D	31/M	+						10^3	2×10^2	0	0	NT	0				

[a] Expressed as the number of pock-forming units per conjunctival swab (see text).
[b] H = haemorrhagic; C = confluent; D = discrete.
[c] NT = not tested.
[d] E = expired.
[e] 0 = absence of virus.

Virus excretion in the urine

The duration and degree of viruria were greater in haemorrhagic and confluent than in discrete cases (Table 2), while confluent cases excreted the virus over a longer period than discrete cases. From the epidemiological standpoint, live virus in the urine may contaminate the patient's immediate environment. The titre of the virus in the urine was highest on days 5–6 of the disease and then diminished gradually. That haemorrhagic patients would excrete virus in their urine as long as they lived was expected because of the long persistence of viraemia (Mitra et al., 1966), but how virus excretion could continue for long periods in non-haemorrhagic cases is not known. Why 18 out of 39 patients did not excrete a detectable amount of virus in their urine, although they were comparable with the virus-positive cases, also remains unexplained. The duration or degree of viruria seemed to be unrelated to the vaccination status, age, or sex of the patients.

Recently the colleagues of S. S. Marennikova (personal communication, 1971) have also found virus in the urine of patients convalescing from smallpox.

Virus excretion in the conjunctiva

Conjunctival swabs from all 12 patients showing clinical conjunctivitis were positive for virus. In general, the trend of virus titre and the duration of virus excretion in the conjunctiva accorded with what was found in the throat and urine, i.e., a higher virus titre in the haemorrhagic and confluent cases than in the discrete, and a longer period of virus excretion in the confluent than in the discrete. As the virus titre in some cases was fairly high, patients' bedding could clearly be grossly contaminated from this source. The absence of virus in the conjunctiva of 4 cases without conjunctivitis indicates that the presence of virus there elicits inflammatory change. Kempe et al. (1969) isolated virus from the conjunctiva of 60 patients who developed conjunctivitis early in the disease, but not from 24 who developed the inflammation during convalescence.

CONCLUSIONS

Smallpox patients excrete virus in the throat and urine for varying periods. Clinically more severe (haemorrhagic and confluent) cases excrete more virus than less severe (discrete) cases and the period of excretion is longer in the first two groups than in the last. Virus is present in the conjunctiva of smallpox patients who show conjunctivitis. The duration and titre of virus excretion in the throat, urine, and conjunctiva seem to be unrelated to whether patients have had a successful primary vaccination or not. This does not mean that previous vaccination does not reduce the severity of the disease; however, if a vaccinated person undergoes a severe form of smallpox, the excretion of virus in his throat, urine, or conjunctiva will be like that of an unvaccinated person developing a similar illness.

RÉSUMÉ

EXCRÉTION DE VIRUS AU COURS DE LA VARIOLE: 1. EXCRÉTION PAR LA GORGE, L'URINE ET LA CONJONCTIVE DE MALADES

Des prélèvements de sécrétions pharyngées ont été effectués quotidiennement chez 8 patients atteints de variole hémorragique, 13 malades porteurs de lésions confluentes et 11 varioleux présentant une forme discrète bénigne de la maladie. La recherche du virus a été faite par inoculation à l'œuf. Dans les cas hémorragiques et confluents, les titres de virus, exprimés en unités formatrices de plages (PFU), étaient plus élevés (10^4-10^5 PFU /ml) que dans les cas bénins (10^3-10^2 PFU/ml). L'excrétion du virus s'est poursuivie jusqu'au décès, survenu du 5e au 7e jour, dans les cas hémorragiques; elle a persisté pendant une quinzaine de jours dans les cas de variole confluente et a cessé après une semaine dans les cas bénins.

La recherche du virus dans l'urine de 18 malades (9 cas confluents et 9 cas bénins) est restée négative. Chez 21 autres (5 cas hémorragiques, 9 cas confluents et 7 cas bénins), l'excrétion du virus dans l'urine a persisté pendant une durée atteignant 5-6 jours dans les cas hémorragiques, 15 jours dans les cas confluents et 1 semaine dans les cas bénins, ces derniers présentant par ailleurs les titres de virus les plus faibles.

Chez 12 malades atteints de conjonctivite (3 cas hémorragiques, 6 cas confluents et 3 cas bénins), les prélève-

ments de sécrétions ont montré la présence du virus pendant une période variable, plus longue dans les cas confluents. Les titres étaient les plus faibles dans les cas bénins. Chez 4 patients indemnes de conjonctivite, la recherche du virus est restée négative.

On n'a constaté aucune corrélation entre d'une part la durée et l'intensité de l'excrétion du virus par la gorge, l'urine et la conjonctive et d'autre part l'âge et le sexe des malades ou d'éventuels antécédents de primo-vaccination antivariolique.

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