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The Recovery of Smallpox Virus from Patients and Their Environment in a Smallpox Hospital*

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Attempts had been made in 1961 to recover smallpox virus by air sampling in smallpox wards and close to the mouths of smallpox patients, but these had been largely unsuccessful, possibly owing to the air sampling method used. Further attempts were therefore made in 1963, with a fluid impinger for air sampling and with Petri dishes placed below the orifice of the impinger to collect large droplets or particulate matter that the impinger might miss.

Air samples from near the patients' mouths yielded little virus, this being more readily recovered from the settling-plates. Patients' bedclothes sampled with the impinger yielded rather more virus, but again even more was obtained from the Petri dishes.

The results suggest that contamination of the air in the vicinity of smallpox patients is due to relatively large particles of infected dust from the patients' bedclothes rather than from fine droplets or droplet nuclei coming from the upper respiratory tract. Secretions from the mouth and upper respiratory tract appear to be responsible for the early contamination of pillows and bedclothes.

In a previous study carried out at the Infectious Diseases Hospital in Madras it was shown that smallpox virus could be obtained from patients' mouth washings most readily between the sixth and ninth days of illness (Downie et al., 1961). However, our attempts to recover virus by air sampling in the wards and in close proximity to the patient's mouth were practically all unsuccessful (Meiklejohn et al., 1961). It was suggested that this failure might have been due to the method of air sampling used—the drawing of air at a rate of 10 litres per minute through a small glass funnel containing packed, dry cotton-wool; after runs for various periods in the vicinity of the patient the contents of the cotton-wool were extracted with diluent, and this was then inoculated on the chorio-allantoic membranes of chick embryos.

In the spring of 1963, observations of this kind were repeated, using a fluid impinger for air sampling in the vicinity of the patient. At the same time, Petri dishes containing sterile fluid were exposed 2 inches (5 cm) below the orifice of the impinger while air samples were collected. It was felt that settling-plates of this kind might collect virus contained in large droplets or particulate material which might not be detected by the impinger. In addition, specimens were collected by means of moist cotton-wool swabs from the pillows and bed sheets, from the skin of the patient's back and from the skin around his mouth. The results are reported in this present paper.

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METHODS OF STUDY

Collection of air samples

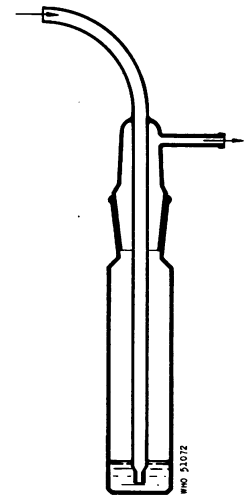
The fluid impinger used in most runs was of the Porton type (May & Harper, 1957; see the accompanying figure). Air was drawn through the impinger at a rate of 10 litres per minute. The impinger contained approximately 4 ml of sampling fluid—saline containing 20% broth, penicillin 300 units/ml and streptomycin 300 μg/ml. In many of the experiments, the opening of the inlet tube was a few millimetres above the surface of the collecting fluid and the rate of air-flow was such as to produce marked turbulence in the collecting fluid. In other experiments, the opening of the inlet tube was below the level of the collecting fluid, so that the incoming air caused vigorous bubbling. Before the fluid was removed for test, the vessel was rotated in the horizontal position to recover any virus which might have been deposited on the walls. In some of the experiments, after the air sample had been collected, the inlet tube of the sampler was washed through with diluent to obtain any virus which might have been retained within it. From 12 patients examined late in the study, 16 air samples were collected by the fluid impinger with a metal funnel attached to the inlet tube. In these instances, the funnel (8 inches —20 cm—in diameter) was held within a few inches of the patient's mouth while he was encouraged to talk.

The Andersen air sampler (Andersen, 1958) was used in eight runs on four patients. Only the plate with the largest perforations and the corresponding dish were used, the remaining sections of the apparatus being removed. The rate of air-flow was the same as with the glass impinger and fluid diluent was used in the dishes for the collection of specimens.

When air samples were being collected near the mouth of the patient, the inlet tube of the impinger or sampler was placed 2-12 inches (5-30 cm) from the patient's mouth. These samples were collected for a period of 10-15 minutes, and during this time the patients were encouraged to talk and asked to cough or gargle several times towards the end of the sampling period.

The fluid from the samplers was tested for the presence of virus within two hours of collection, by inoculating 0.2-ml samples of fluid on the chorio-allantoic membrane of 12-day-old chick embryos; 6-24 eggs were used in testing each fluid, depending on the number of eggs available on the day the specimen was collected. The eggs were examined for the presence of virus lesions three days later.

ALL-GLASS PORTON IMPINGER



When air samples were collected from the bedclothes of the patient, the inlet of the sampler was held a few inches from the bedclothes, which were frequently moved by hand during the 10-15 minute period of collection.

Setting-plates

The 3-inch (7.5-cm) Petri dishes containing approximately 2 ml of sampling fluid were left exposed during the collection of the air sample. These plates were placed on the bed, approximately 2 inches (5 cm) below the inlet-aperture of the air sampler. The fluid from the plates was treated in the same was as the fluid from the impinger, 5-12 eggs being inoculated from each settling-plate.

Collection of swabs from the pillow, bedclothes, skin of the back and around the mouth

Ordinary throat swabs were used. They were first wetted with diluent as used for the impinger. The area of the pillow swabbed was that near the patient's head and was often soiled with secretions from the patient's mouth or skin. The swabs from the bedclothes were generally taken from the sheets about the level of the patient's umbilicus. Unless the sheets had been recently changed they were often soiled from oil which had been applied to the patient's skin or from exudate from skin lesions. When collecting specimens from the lumbar region, swabs were rubbed gently over skin areas on which

there were several unbroken vesicles or pustules. On several occasions broken pustules were swabbed and these were all positive.

The specimens from skin around the mouth were collected by swabs moistened with sterile broth. Extraction from the swabs was done in approximately 1-1.5 ml of diluent and the fluid was used for the inoculation of the chick-embryo chorio-allantoic membrane.

Urine

The urine was collected as "midstream" specimens in large, wide-mouthed, sterile glass centrifuge tubes. In earlier experiments, the urine was centrifuged, and both the deposit and the supernatant fluid were examined for virus. However, because of the breaking of tubes during centrifugation at 5000 rev/min, this method was soon abandoned and most of the specimens listed in the following tables were not centrifuged. Penicillin and streptomycin were added directly to the urine, or urine was diluted 1/3 in sterile fluid containing these antibiotics. Occasional specimens seemed to be toxic for the chick embryo but a 1/3 dilution usually proved satisfactory. Approximately 1 ml of each urine was examined for virus by chick-embryo inoculation.

Specimens were examined from 46 patients or their environment. Some patients were examined on several occasions, although the whole range of specimens was not always collected at any one time. For example, swabs from the back and from around the mouth were not collected from the earlier patients and air samples were not necessarily taken on the day that urine was collected for examination. Many of the specimens were collected from patients in the general ward but in a number of instances the patients were placed in a separate room, with the doors and windows closed, to minimize air currents.

RESULTS

The 46 patients from whom specimens were collected were classified in relation to rash, as 5 mild discrete, 11 moderate discrete, 9 severe discrete, 16 confluent and 5 haemorrhagic.

Air samples and settling-plates near the mouth

Most of the patients had obvious lesions in the mouth, and, as noted above, patients talked for part of the time and they were encouraged to cough several times while the samples were collected.

It will be seen from Tables 1 and 2 that in spite of the large volume of air sampled in the impinger, only five of the 47 specimens were positive, whereas virus was recovered from 12 of 30 settling-plates in the same position. Apart from two positive plate results on the fifth day, no specimens were positive before the seventh day. Of the two patients from whom virus was isolated from the settling-plates on the fifth day of illness, the first represented a mild case of smallpox. Three and eight virus lesions were present in two of the seven eggs inoculated from the specimen obtained. One lesion was observed under the tongue of this patient. The impinger sample and swab from the pillow were negative at this time; a swab was not taken from the skin around the mouth. The specimen from the other patient gave only two specific lesions on one of six chorio-allantoic membranes. At the end of the 15-minute collection period the impinger and plate had been held 3 inches (7.5 cm) from the patient's mouth while she coughed several times. A swab from the pillow was negative on the fifth day but virus was recovered from the skin around the mouth and from the pillow on the seventh day of illness, by which time her rash was confluent and she had numerous lesions on her buccal mucosa.

Of the 13 patients from whom positive samples were obtained from impinger fluid or settling-plates, seven had confluent and six had discrete eruptions. The number of pocks produced on the chorio-allantoic membrane from 0.2 ml of inoculum was never more than 24.

The results of examination of air samples and settling-plates taken near the patient's mouth suggest that the impinger used for the collection of air samples in 1963 was more effective than the air sampler used in 1961. Moreover, the greater frequency of positive results in the settling-plates indicates that virus collected from in front of the patient's mouth was present in relatively large droplets or particles. Droplets of 18μ or greater in diameter are not collected by impingers of this type (May & Harper, 1957). Even when talking and coughing, patients with lesions in the mouth seem to eject very little virus in small droplets of the aerosol type.

We obtained little evidence that virus was held in the inlet tubes of the impingers. No virus was recovered from the washings of four impinger inlet tubes used to collect specimens near the mouth when the impinger fluid was also negative. The inlet tubes of seven impingers used to collect specimens

TABLE 1
RECOVERY OF VIRUS FROM IMPINGERS, SETTLING-PLATES AND SWABS

Source of sample		Day of disease														Total
		5	6	7	8	9	10	11	12	13	14	15	16	17	19+	
Impinger, mouth	+			1		1		1	1	1						5
	—	3	3	3	4	6	7	5	3	2	3	2	1			42
Settling-plates, mouth	+	2		1	1	2		2		2	1	1				12
	—		2	1	1	5	4	1	3			1				18
Circum-oral swab	+		3	4	5	8	7	7	4	1	2	1				42
	—	3	1		1		2	2		1	3		2	1		16
Pillow swab	+		2	3	4	6	7	6	4	2	3	3		1		41
	—	4	2	2	3	3	2	3		1	3	0	2	1		26
Impinger, bedclothes	+				1		1				1	2				5
	—					3	1		1	1				1	3	10
Settling-plates, bedclothes	+				1	1	2		2	1	1	2		1		11
	—					3	1		2						3	9
Bedclothes swab	+				1	2	1		3	2	2	1	1	1	1	15
	—	1														1
Back swab	+		1	2	3	3	4	6	3		2	1				25
	—	3	3	3	4	7	5	4	2	2	4	1	2	1		41
Urine	+		1	3	2	2	2	2	2	1	1		1			17
	—	1	2	2	2	1	2	1	3		1	1	1			17
Total	+	2	7	14	18	25	24	24	19	10	13	11	2	3	1	173
	—	15	13	11	15	28	24	16	14	7	14	5	8	4	6	180

from bedclothes were also examined for virus. Of four impingers that gave positive fluids, three tube washings were positive and one was negative. Two inlet tubes of three impingers whose fluid was negative yielded a small amount of virus.

Air samples and settling-plates from bedclothes

Although the number of specimens collected from near the middle of the bed by the impinger and settling-plates was not large, the settling-plates again showed a greater proportion of positives than did the impinger. In the five positive impinger samples (from three patients with confluent, and two with discrete, rashes), the amount of virus collected was small, as not more than three pocks were produced on the chorio-allantoic membrane of any of the inoculated eggs. The fluids from the 11 positive settling-plates (from four patients with confluent, and three with discrete, eruptions) also usually yielded small amounts of virus, although with two specimens all eggs inoculated showed more than 100 pocks on the chorio-allantoic membrane. The findings, once more, suggest that the infected material disturbed by moving the bedclothes was associated with relatively large particles which were less readily collected by the fluid impinger. The use of the funnel attachment with the fluid impinger did not apparently facilitate the recovery of virus. Of the 16 specimens collected in this way, none was positive. In some instances, at the end of the run the inside

TABLE 2
SUMMARY OF RESULTS OF VIRUS EXAMINATION

Source of sample	No. of patients	No. of specimens			Percentage of pos. specimens
		Pos.	Neg.	Total	
Impinger, mouth	29	5	42	47	11
Settling-plates, mouth	13	12	18	30	40
Circum-oral swab	32	42	16	58	72
Pillow swab	40	41	26	67	61
Impinger, bedclothes	9	5	10	15	33
Settling-plates, bedclothes	13	11	9	20	55
Bedclothes swab	11	15	1	16	94
Back swab	35	25	41	66	38
Urine	16	17	17	34	50

of the funnel was seen to be spattered with droplets of various sizes; washings from the funnel were not, however, tested for virus. Similarly, no virus was recovered from the eight samples collected with the Andersen sampler.

Circum-oral and pillow swabs

Swabs from the skin around the mouth taken after the fifth day of illness showed a high proportion of positive virus isolations. In many of the patients there were no obvious lesions on the area swabbed, but presumably this area of skin becomes readily contaminated from infected saliva. The percentage of positive results and their relation to the stage of illness were in general similar to those obtained by the examination of mouth washings in 1961.

The swabs from the pillow gave results which were parallel to those from swabs of the skin around the mouth, although the percentage of positives was rather less. This difference may have been due to the changing of pillow linen of some patients on the morning that the sample was collected. The amount of virus recovered from circum-oral and pillow swabs was often large, the infected egg membranes showing confluent takes.

Swabs from the skin of the back and bedclothes

Only a little more than a third of the swabs from the patients' backs yielded virus, although care was taken to rub the swabs over the surface of focal lesions. Indeed, it would appear that no virus is released from skin lesions of the patient unless the covering of the vesicles or pustules is ruptured by minor trauma. The crusts which separate from the skin during convalescence contain plenty of virus, as can be shown by disintegrating the crusts in saline and inoculating the resulting suspension in chick embryos.

The swabs from the bedclothes were taken mostly from the sheet on which the patient was lying. As these sheets were often somewhat soiled by exudate from ruptured lesions, it is not surprising that the majority of the swabs collected virus. The positive swabs often collected large amounts of virus, as shown by the number of lesions produced on egg membranes.

Urine

Approximately half of the specimens, taken at various stages of the illness, yielded virus, and, in some, large amounts of virus were present. However, the significance of these results is rather doubtful, as the specimens were not collected by catheter; and virus in the urine might have been due to the presence of lesions on the external mucosa or prepuce that were sometime seen in the male patients from whom the specimens were collected. In fatal cases of smallpox, small haemorrhages have sometimes been observed in the kidneys, and focal lesions with cellular infiltration have been observed in the subcortical region (Bras, 1952). It would be surprising, therefore, if in severe cases virus did not sometimes appear in the urine.

TABLE 3
DETAILED RESULTS[a] FROM PATIENT H, SUFFERING FROM CONFLUENT SMALLPOX

Day of disease[b]	Impinger		Settling-plates		Swabs		Urine
	Mouth	Bedclothes	Mouth	Bedclothes	Pillow	Sheets	
7	+5/14		+3/7	−1			+
9			−6	−5			
11	+3/10		−6	−5			+
12							+
14		+8/9		+6/6	+	+	

[a] The figure after the − sign shows the number of eggs inoculated without positive result. The figure after the + sign shows the number of eggs bearing lesions out of the total examined.
[b] Patient died at 15 days.

Tables 3 and 4 show detailed results from two patients. The specimens taken by impinger and settling-plates from the bedclothes of patient H (Table 3) the day before he died produced pocks on most of the eggs inoculated; but the impinger sample produced no more than three lesions on the chorio-allantoic membrane of any egg, while the fluid from the settling-plate produced more than 100 lesions on each membrane. This greater yield of virus on settling-plates in comparison with impinger fluid was frequently observed. Table 4 gives the results of a relatively mild case; the findings are typical of many others.

TABLE 4
DETAILED RESULTS FROM PATIENT N, SUFFERING FROM PROFUSE DISCRETE ERUPTION

Day of disease	Impinger mouth[a]	Settling plates mouth[a]	Swabs		
			Circum-oral	Back	Pillow
6	−6[b]	−6	−	−	+
9	−6[b]	−6	+	−	−
12	−6[c]		+	+	
16	−6[c]		−	−	−

[a] Number of eggs inoculated without positive result.
[b] Andersen sampler.
[c] Impinger with funnel.

DISCUSSION

The observations recorded indicate, as might be expected, that the bedclothes and pillows of smallpox patients are heavily contaminated with virus. The swabs from skin around the mouth gave approximately the same proportion of virus isolations over the same period of illness as did swabs from pillows, suggesting that both were probably infected from saliva. The results with mouth washings from smallpox patients in an earlier study (Downie et al., 1961) showed that virus was most frequently found from the sixth day of illness onwards and, as shown in Table 1 above, this was observed with pillow swabs and swabs from the skin around the mouth. Although the number of specimens collected was small, virus was isolated regularly after the fifth day from swabs from the bedclothes. Secretions from the mouth as well as material from ruptured skin lesions may have contributed to the virus contamination of the bed sheets.

The frequent failure to find virus in the air samples collected in the impinger was rather surprising. Even air sampled with the impinger held near the mouths of patients who had obvious mouth lesions and who talked or coughed during the period of collection was usually negative. In many of these patients, swabs from circum-oral skin and pillow yielded virus. Of the five positive impinger samples collected near the mouth, four were taken when no settling-plates were being used, so that on most of the occasions when settling-plates were positive, the impinger sample was negative. The type of impinger used has been shown to be effective in recovering vaccinia virus in 1-μ aerosols in cloud chambers (Harper, 1961; Westwood, 1962) and our results indicate that very little virus is discharged from the mouth of the patient in droplets or droplet nuclei of

TABLE 5
ISOLATIONS OF VIRUS FROM SPECIMENS IN RELATION TO SEVERITY OF ERUPTION

Source of sample	Nature of eruption				Total	
	Discrete		Confluent			
	Virus isolations					
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Impinger mouth	2	19	3	23	5	42
Settling-plates, mouth	5	8	7	10	12	18
Impinger bedclothes	2	2	3	8	5	10
Settling plates, bedclothes	4	3	7	6	11	9
Circum-oral swab	30	13	12	3	42	16
Pillow swab	23	20	18	6	41	26
Bedclothes swab	5	1	10	0	15	1
Back swab	18	30	7	11	25	41
Urine	8	14	9	3	17	17
Total	97	110	76	70	173	180

this order of size when he is breathing, talking or coughing.

The recovery of virus from impingers and settling-plates was not apparently related to the severity of the patient's illness, as shown in Table 5. The number of specimens is too small to permit an analysis of the results in relation to more detailed clinical classification of patients: but the figures do not show a higher proportion of virus isolations by impingers or settling-plates from the patients with confluent eruptions. The virus isolations from the circum-oral and pillow swabs, however, do suggest a higher content of virus in the mouth secretions of these patients.

A good deal of evidence, which has recently been reviewed by Hare (1964), seems to support the view that pathogenic bacteria in the respiratory tract are not often expelled during coughing, talking, or even sneezing, in sufficiently small droplets or droplet nuclei or in sufficient numbers to provide a likely source of true air-borne infection. As Hamburger & Robertson (1948) and others have shown with streptococcal carriers, these pathogens are mostly contained in relatively large droplets which rapidly fall to the ground. Similar observations have been made in relation to cases of diphtheria and pulmonary tuberculosis (Duguid, 1946a) and it has been stated that droplet spray is unlikely to give rise directly to true air-borne infection unless very large numbers of pathogenic organisms are present in the secretions of the anterior mouth (Duguid, 1946b). But in measles and smallpox the occurrence of lesions in the buccal mucosa ensures heavy contamination of the mouth with the causal viruses and Riley & O'Grady (1961) consider, on epidemiological evidence, that measles is spread by droplet nuclei. It has been frequently shown that pathogenic bacteria from the respiratory tract readily contaminate the environment of a patient or carrier and that disturbance of clothing or bedclothes may liberate large numbers of bacteria into the atmosphere. Such infected particles may be small enough to remain suspended in the air for half an hour or longer (Duguid & Wallace, 1948; Lidwell, Noble & Dolphin, 1959) and may be more important in the transmission of infection than droplets or droplet nuclei from the respiratory tract. However, the observations of Rammelkamp and his colleagues (Perry et al., 1957a, 1957b; Rammelkamp et al., 1958) seemed to show that viable haemolytic streptococci dried in dust or on blankets had very low infectivity for human volunteers. Our own observations suggest that virus was much more readily recovered from the infected bedclothes of

smallpox patients than from direct air sampling of expelled air during talking or coughing; and there can be no doubt that smallpox infection can be transmitted from infected clothes or bedclothes, as instanced by the infection of laundry workers from this source (Cramb, 1951).

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RÉSUMÉ

Dans le cadre d'études sur la variole effectuées à Madras, les auteurs décrivent les appareils qu'ils utilisent pour recueillir des échantillons d'air à proximité de la bouche des malades, pendant que ceux-ci parlent et toussent. Dans l'impacteur, type Porton, l'air circule dans l'appareil et passe à travers un milieu liquide, qui est ensuite inoculé sur membrane chorio-allantoïdienne de poulet.

Les prélèvements faits de cette manière ont donné très peu de cultures positives, même lorsque les malades présentaient des lésions caractérisées de la muqueuse buccale. En revanche, on a obtenu une plus forte proportion de résultats positifs après inoculation d'un milieu liquide contenu dans des boîtes de Petri ouvertes, devant lesquelles on avait fait parler et tousser les malades. On a également utilisé ces deux méthodes pour étudier le degré de contamination de l'air au voisinage de la literie. On a trouvé une proportion plus forte de cultures positives à partir d'échantillons d'air prélevés pendant que l'on agitait et secouait les pièces de literie à proximité de l'impacteur. Ici, comme précédemment, un taux plus élevé de résultats positifs fut observé avec la méthode des boîtes de Petri qu'avec l'impacteur.

Beaucoup de malades excrètent le virus dans la salive, comme l'ont montré de nombreux prélèvements positifs faits sur écouvillon, autour de la bouche ainsi que sur les oreillers. Il ne semble pas que les pustules, tant qu'elles ne sont pas ouvertes, soient responsables de contamination. En revanche, le liquide qu'elles contiennent et les croûtes qui les recouvrent lors de la convalescence sont très riches en virus.

Les résultats de ce travail autorisent à penser que la contamination de l'air, au voisinage des varioleux, est due à de grandes particules de poussière provenant de la literie, plutôt qu'à des gouttelettes de sécrétions provenant des voies respiratoires supérieures. Il semble que la contamination des oreillers et de la literie, dès le début de la maladie, soit due à la présence du virus dans les sécrétions de la bouche et des voies respiratoires supérieures.

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