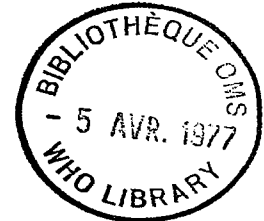




VIABILITY OF VARIOLA VIRUS IN CRUSTS
AT DIFFERENT TEMPERATURES AND HUMIDITIES

by

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Introduction

The ability of variola virus to remain viable and infective under certain conditions for long periods is well known (Dixon, 1962; Rhode & Van Rooyan, 1968). The viability of the virus in crusts will depend on various factors such as temperature and humidity. In such countries as Bangladesh where the confirmation of smallpox diagnosis is by isolation of virus and samples take several days to reach the laboratory, knowledge of the effect of various temperatures and humidities on the viability of variola virus is important.

Materials and methods

Crusts were collected from a single smallpox patient in a sterile bottle and were divided that day into several batches and stored under several different sets of conditions:

1. at incubator temperature (35°C);
2. (a) at room temperature;
(b) at room temperature in a dessicator;
3. (a) at refrigerator temperature (4°C);
(b) at refrigerator temperature (4°C) in a dessicator;
4. at deep-freeze temperature (-20°C).

The bottle caps were loosely screwed so that humidity would remain the same outside and inside the bottle. The temperature and humidity of the room, incubator and refrigerator were noted each day and the weekly average noted. Humidity inside the dessicator could not be measured due to difficulties in putting the hygrometer inside but it is presumed that the humidity was less than 10%. Each week three crusts were taken from each bottle and were titrated on egg chorioallantoic membrane (CAM) and the average titre noted. Gel precipitation was also done each week with each batch of crusts. The experiments in each group were terminated when for two consecutive weeks no virus was able to be grown and there was no evidence of gel precipitation.

The titre of virus at the end of each week was calculated and plotted as $\log P_0/P$, where P_0 is the original titre of the virus and P is the titre of the crust at any given week.

Results

The average initial titres of virus in the crusts were approximately 2.2×10^8 . At 35° with an average 65% to 68% relative humidity, viable virus could be isolated through the third week (Fig. 1). The gel precipitation test was positive for four weeks (Table 2).

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The average ambient room temperature remained between 25.8° and 26.4°C throughout the experiment and the relative humidity between 85% and 90% (Table 1). At this temperature and humidity no viable virus could be isolated after eight weeks (Fig. 1). The gel precipitation test was positive up to 10 weeks (Table 2). However, viable virus was found for up to 12 weeks in crusts kept in a dessicator at this temperature (Fig. 1) and gel precipitation was positive for 14 weeks (Table 2).

At 4°C with a relative humidity of 60% to 62%, virus could be isolated for 16 weeks but the titre decreased to 1.2×10^4 (Fig. 1). Gel precipitation was positive throughout this period (Table 2). The results obtained when crusts were kept in the dessicator at this same temperature were essentially the same. The experiment could not be carried out beyond 16 weeks as the stock of crusts was exhausted. At -20°C the titre at the end of 16 weeks was 3.1×10^5 (Fig. 1); gel precipitation remained positive throughout this period (Table 2).

Discussion

The viability of virus in crusts differed markedly under different conditions of temperature and relative humidity. At 35°C with 65% to 68% relative humidity, viable virus was found for only three weeks. This is higher than the average ambient temperature but the relative humidity was less. Notably, the antigenicity of the crust persisted even after no viable virus could be detected.

At ambient temperature and humidity the crusts showed viable virus for two months. The experiment was conducted during May, June and July, when average temperatures varied between 25.8°C and 26.4°C with relative humidities between 85% and 90%. Results obtained by MacCallum & McDonald (1957) were similar to our own. They found that virus in crusts kept at a temperature of 30°C and at a humidity of 84% could be detected for only two to three months. At a lower relative humidity, virus survival is somewhat longer. Unfortunately our experiments could not be repeated during the colder months of December, January and February. MacCallum & McDonald (1957), however, showed that at ambient temperatures of 20°-24°C and relative humidities of 55% to 75%, virus in crusts survived for up to 18 months. This corresponds roughly with the temperature and humidity in Bangladesh during the winter months.

At +4°C the virus is viable for a considerably longer time. In our experiment, studies could be carried out for only four months, but it is presumed that virus would remain viable in crusts for several years although with declining titre. Not surprisingly, virus titres of scabs kept in the deep-freeze were highest. The decline in titres of scabs kept at 4°C and -20°C appears somewhat surprising since virus kept at this temperature is usually very stable. A plausible reason for this decrease is that each week the same bottle was removed from storage and three crusts were removed. In this way, the crusts were thawed and thus exposed to room temperature and humidity once each week.

Summary

The viability of virus in crusts was observed at different temperatures and humidities. During the months of May, June and July when average ambient temperatures are between 25.8°C and 26.4°C and humidity between 85% and 90%, smallpox virus in scabs remains viable only for eight weeks. However, at low temperatures with lower humidity the virus survival is much longer.

REFERENCES

- Dixon, C. W. (1962) Smallpox, Little, Brown & Co., Boston
- MacCallum, F. O. & McDonald, J. B. (1957) Survival of variola virus in raw cotton, Bull. Wld Hlth Org., 16, 247-254
- Rhodes, A. J. & Van Rooyan, C. E. (1968) Text book of Virology, Williams & Wilkins Co., Baltimore, p. 318-331

TABLE 1. TEMPERATURE AND RELATIVE HUMIDITY BY WEEK

Month	Week	Average temperature (°C)	Average relative humidity (%)
May	3rd	26.0	86
May	4th	26.1	85
June	1st	26.0	88
June	2nd	25.8	88.5
June	3rd	26.1	89
June	4th	26.3	90
July	1st	26.1	86
July	2nd	26.4	87

TABLE 2. VIABILITY OF VIRUS AND POSITIVE GEL PRECIPITATION TEST FOR CRUSTS AT DIFFERENT TEMPERATURES AND HUMIDITIES BY WEEK

Temperature	35°C		25.8°C to 26.4°C			4°C		-20°C		
	RH ^a 65% to 68%		RH 85% to 90%		Dessicator	RH 60% to 62%		Dessicator		
Week of experiment	V ^b	GP ^c	V	GP	V	GP	V	GP	V	GP
1	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+
4	-	+	+	+	+	+	+	+	+	+
5	-	-	+	+	+	+	+	+	+	+
6	-	-	+	+	+	+	+	+	+	+
7	ND ^d	ND	+	+	+	+	+	+	+	+
8	"	"	+	+	+	+	+	+	+	+
9	"	"	-	+	+	+	+	+	+	+
10	"	"	-	+	+	+	+	+	+	+
11	"	"	-	-	+	+	+	+	+	+
12	"	"	ND	-	+	+	+	+	+	+
13	"	"	"	ND	-	+	+	+	+	+
14	"	"	"	"	-	+	+	+	+	+
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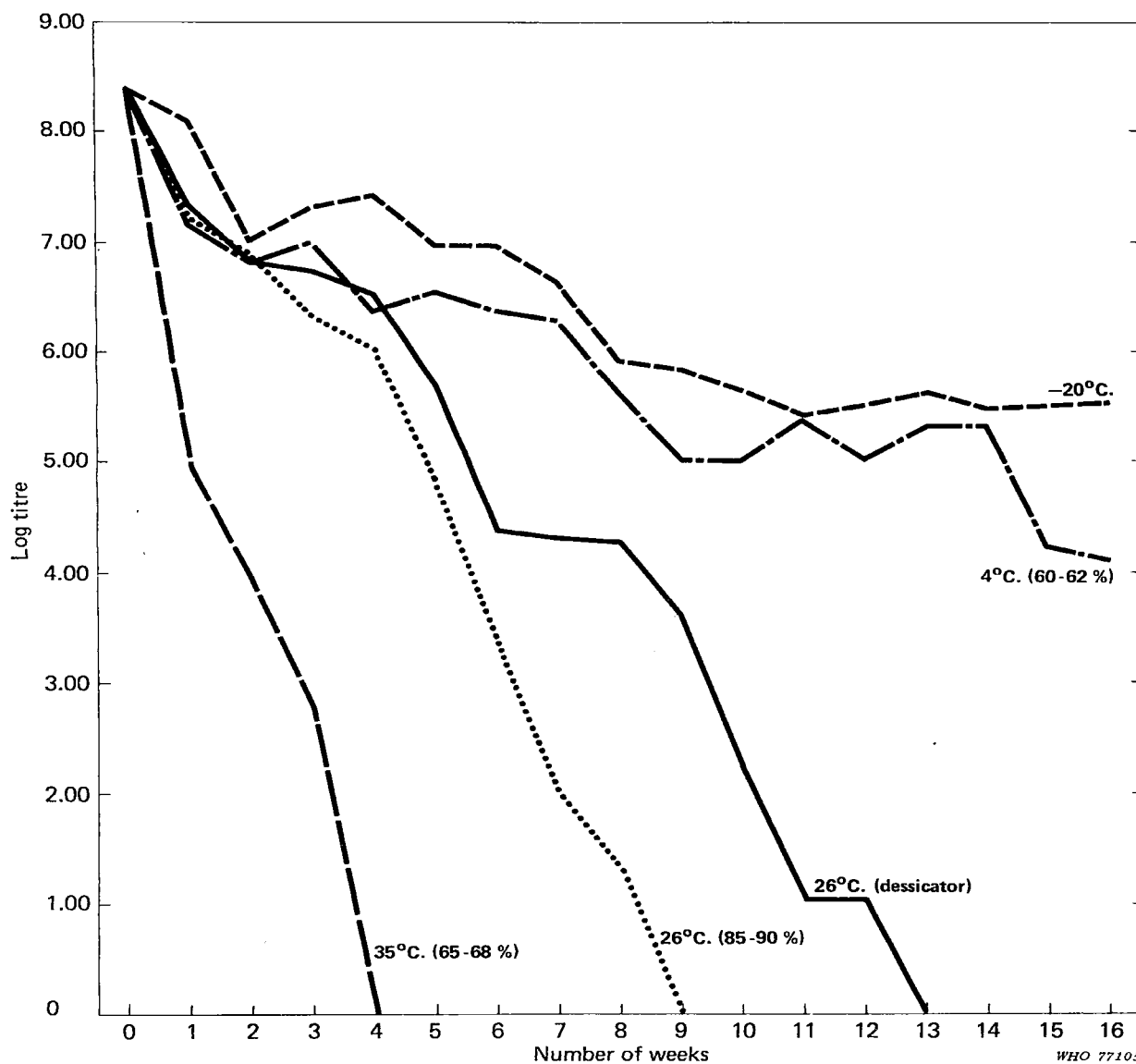
^a RH = Relative humidity.

^b V = Viability of virus on chorioallantoic membrane of egg.

^c GP = Gel precipitation test.

^d ND = Not done.

Fig. 1
WEEKLY DECREASE IN LOG TITRE OF VARIOLA VIRUS IN CRUSTS
AT DIFFERENT TEMPERATURES AND RELATIVE HUMIDITIES



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