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SURVIVAL OF VARIOLA VIRUS IN RAW COTTON

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SYNOPSIS

An investigation was carried out to establish the survival period of variola virus in relation to its importation into Great Britain in raw cotton. Under the conditions of the experiments described here, variola virus in scabs from a single patient survived for a maximum of three to four months at a relative humidity of 58, and for only two to four months at 30°C and humidities of 73 and 84. Exposed virus in the form of vesicle fluid in capillaries did not survive for three months at this temperature in any of these humidities.

These results suggest that variola virus in scabs or seeds in tropical climates—i.e., at temperatures of from 30°C to 40°C or higher—is unlikely to survive for as long as six months. Thus, if the period of storage of cotton were at least six months after ginning and before shipment from cotton-producing countries in the tropics where smallpox is endemic, the chances of importation of viable variola virus on raw cotton into areas free from infection would be very small. However, if cotton can become contaminated with smallpox scabs in temperate climates (20°-25°C) or is already contaminated when imported at this temperature, the experiments indicate that a few particles of virus may survive for as long as 18 months. The virus can, of course, survive for many years, ten or more, at from 4°C to 5°C in closed tubes or bottles, with little decrease in titre.

The possibility that the viruses of variola major or minor might be introduced into Great Britain on imported raw cotton has been recognized for nearly half a century. Suspicion was first raised in an outbreak which occurred in Stockport, Lancashire, in 1908 and subsequently on seven occasions between 1908 and 1952 when the first recognized cases, in outbreaks in Lancashire and Cheshire, were workers in the early stages of the cotton-handling process. The possibility was again considered after an outbreak of variola minor in Lancashire in late 1951 and early 1952, and also in 1953 when variola major occurred in some of the cotton-spinning towns of Lancashire. The measure of suspicion never amounted to conviction. The matter is discussed in annual reports for 1936, 1952 and 1953 of the Chief Medical Officer of the Ministry of Health.

The stability of variola virus in crusts or scabs, which was considered responsible in suspect cotton, has been postulated for many years. Blaxall [1] stated that the crusts remain potentially infective for years. This conclusion was based on epidemiological evidence, much of it of considerable age. More accurate data on this point became available in 1947 when Downie & Dumbell,[2] using the chorioallantoic membrane of fertile hen-eggs as culture medium, recorded recovery of virus from a pool of four scabs which had been stored in a tube loosely stoppered with cotton wool in the dark at room temperature (20°-22°C) for as long as 417 days, at which time the experiment was terminated because the supply of scabs was exhausted. In most other specimens tested by them, the survival period was shorter and there appeared to be little difference between those exposed in diffuse daylight and in the dark. The humidity was not stated but was probably within the range 35%-65% during this time (Downie, personal communication). Downie & Dumbell [2] also reported that variola-infected vesicle fluid suspended in 20% human serum, free from vaccinia antibody, and dried on glass slides kept in Petri dishes, survived for as long as 35 days in daylight and 84 days in the dark at the same temperature and humidity.

When the variola major outbreak was recognized in workers in cotton mills in 1953 in the same general area as had been affected with alastrim the previous year, it was decided to attempt to obtain further information on the mechanics of its possible importation, and on survival of the virus, using laboratory methods.

Inspection of the various stages of handling and processing the raw cotton in a mill indicated that the risk, if any, would be mainly in the early stage of processing. After removal of the hessian coverings in which the bales are enclosed, the cotton is passed through shredding machines, which obviously results in dissemination of many fine particles which might contain virus. The handling of waste and material recovered from the ventilators serving the first processing rooms would also carry risk of potential exposure. It appeared that little or no direct risk from the cotton was likely to be incurred by operators from the carding process onwards.

The size and weight of the bales vary from different countries; the weights range from 110 to 750 lb, and the density from 15 to 35 lb per cubic foot (about 50 to 340 kg, and from 0.24 to 0.56 kg per cubic decimetre). These factors, and the condition or state of the cotton, preclude the introduction of any reasonably economical and satisfactory method of sterilization either before or immediately after the bales are opened. The most appropriate method of dealing with the potential problem appears to be to improve public health conditions in the regions from which the cotton is exported, particularly by vaccination and, if necessary, by the elimination of workers with smallpox until all scabs have disappeared, for it is known that the very last ones shed may contain viable virulent virus enclosed in a scab or "seed". Meanwhile it was decided to obtain further information on the survival of

variola virus under conditions of temperature and humidity which simulate those obtaining in certain cotton-growing areas where the disease occurs.

In 1925 the late Sir Leonard Rogers reported [5] a close relationship between a low absolute humidity which is consequent on a low monsoon annual rainfall, and subsequent epidemic prevalence of smallpox, and vice versa in India; in a monograph on smallpox and the climate of India (Rogers [6]) he produced tables of temperature and humidity for different times of the year in different regions, together with smallpox mortality rates. After study of his tables it was decided not to embark on too extravagant an experiment, but to test the effect of a single temperature, 30°C, which was near the mean temperature recorded by Rogers for the spring months when the peak incidence of smallpox occurred, and three relative humidities, 58, 73 and 84, which were selected from the same table (Table A of the Appendix, Rogers [6]). These atmospheric conditions were chosen without any prejudice to the Indian continent, as cotton from other countries has also been suspected in the past.[3]

Methods and Materials

No large chamber was available for this work, but a small incubator, capacity about 2 cubic feet (about 0.06 cubic metres), adjusted to work at 30°C, was set aside for the experiments and was employed over the period for no other purpose.

The humidity tests were carried out by suspending virus material, wrapped in cotton, over saturated solutions of various salts of sodium or potassium in wide-necked, screw-topped one-pound jam jars, giving known relative humidities (RH) (O'Brien [4]).

The RH selected for the test, 58, 73 and 84, were provided by using jars containing saturated solutions of sodium bromide, sodium nitrate and potassium chloride respectively.

The raw cotton used to enclose the virus was obtained from a mill in Lancashire. Small samples were first tested for the presence of variola virus, with negative results.

Variola virus was provided by the scabs from a single patient. The first experiment commenced 19 days after receipt of the specimens, which in the interim were stored in a bottle with a screw cap at 4°C. They were divided into lots of 10-12 scabs of similar size. Three lots were taken for estimation of virus content two days later; 14 lots were placed in small bottles with screw caps for room temperature controls (20°-24°C), and one set of 14 lots was used for each humidity test. It was planned to test a bottle from each set—that is, one at room temperature and one from each humidity at 30°C, at 3, 6, 9, 12, 14, 15, 16, 17, 18, 21, 24, 27, 30 and 36 months.

Eventually the experiment was carried out in two parts because in the first test no virus could be recovered from the material at the two higher

humidities and 30°C after four months, and evaporation of most of the water had occurred by the fifth month at the lowest humidity. It was thought that a leak might have occurred at the point where the thread used for suspending the cotton made its exit between the lid and jar. In a second experiment using similar lots of scabs from the same patient, the cotton containing virus was fixed inside the lid of the jar with adhesive tape, and the join of lid to jar was sealed with paraffin wax.

At the same time as the first experiment, capillaries containing vesicle fluid and pus were wrapped in the same way as the scabs and suspended at the same humidities, but unfortunately no virus was recovered when they were first tested at the end of three months, and no material was available for repetition of the experiment.

The 14 bottles used as room temperature controls were exposed to diffuse daylight in a laboratory facing east.

When each lot of scabs was to be tested for virus it was ground with pestle and mortar, and grinding continued during the addition of 2.0 ml of 10% broth saline containing 100 units of penicillin and 100 μg of strepto-

TABLE I. SURVIVAL* OF VIRUS AT 30°C AND RELATIVE HUMIDITIES OF 58%, 73% AND 84% (FIRST EXPERIMENT)

Exposure (days)	Relative humidities		
	58%	73%	84%
97	20, 23, 24, 60	83, 21	4, 3, 2, 2
122 (4 months)	2, 1, 0, 0 P+	0, 0, 0, 0 P—	0, 0, 0, 0 P—
131	1, 1, 0, 0 P+	0, 0, 0, 0 P—	0, 0, 0, 0 P—
139	1, 2, 1, 1 P+	†	†
146	A dried-up bottle of RH 58 showed 60, 70 and 75 pocks on 3 CAM. The remaining RH 58 scab bottles were removed 10 days later, 5.0 ml of sterile distilled water were added to each, and the lids were replaced and paraffin-sealed.		
185	2, 10, 0 P+		
222 (7 months)	0, 0, 0, 0 P—		
229	0, 0, 0, 0 P—		
236	0, 0, 0, 0 P—		

* The figures refer to the number of pocks on each egg; P+ = positive on passage; P— = negative on passage

† Not tested

mycin per ml. 0.1 ml of this suspension was inoculated onto the chorio-allantoic membrane (CAM) of each of four 10- to 12-day-old fertile hen-eggs. The eggs were incubated for 72 hours at 36°-37°C; the whole CAM was then removed, and the pocks counted. If there were only single suggestive-looking or no obvious pocks, the membranes were ground, suspended in 1.0 ml per CAM, and passed to three more eggs. Passage of apparently negative membranes never revealed the presence of virus, although single specific pocks confirmed by passage were encountered on several occasions.

Results

Titration of four lots of 10-12 scabs from the pool used showed that there were about 500 000 viable virus particles in each lot. Three titrations were carried out at the beginning and one at the end of the experiment, on a sample stored at 4°C.

TABLE II. SURVIVAL * OF VIRUS AT 30°C AND RELATIVE HUMIDITIES OF 58 %, 73 % AND 84 % (SECOND EXPERIMENT)

Exposure (days)	Relative humidities		
	58 %	73 %	84 %
14	C, C, 70	C, 100, 32	SC, 102, ††
28	†	9, 30, 20	60, 40, C
31	C, C, C	†	†
42	SC, C, C	5, 4, 30	0, 0, 0, 0, 0, 0 P—
52	†	0, 0, 0, 0 P—	0, 0, 0, 0 P—
56	50, 10, 3, 52	200, 50, 20, 30	0, 0, 0, 2 P+
60	†	†	2, 4, 5, 5
70	15, 19, 34, 81	1, 1, 0, 0 P+	0, 0, 0, 0 P—
91	†	0, 0, 0, 0 P—	0, 0, 0, 0 P—
112	0, 0, 0, 0 P—	1, 0, 0, 0 P+	†
125	0, 0, 0, 0, 0, 0 P—		
	bottles finished		

* The figures refer to the number of pocks on each egg; P+ = positive on passage; P— = negative on passage; C = confluent growth; SC = semi-confluent growth

† Not tested

†† Not countable

In the first experiment, the results of which are shown in Table I, it was observed that very little virus could be detected at RH 73 and 84 after three months, and none could be detected at four months. Similarly, at RH 58 most of the virus had died by three months, but presumably conditions were compatible for survival of a few particles at the centre of certain scabs for a further month or two. As already stated, a small amount of evaporation probably occurred in all bottles in this experiment, and so end-points can be considered only as very approximate. Unfortunately all available capillaries containing infected material were used in this experiment, and none contained active virus after 90 days; thus only an upper limit of negative survival is available. This type of material is probably of less significance from a practical standpoint than scabs, as it is unlikely that patients will work during the vesiculo-pustular stage.

TABLE III. SURVIVAL* OF VARIOLA MAJOR VIRUS IN SCABS AT ROOM TEMPERATURE (20°-24°C), AND RELATIVE HUMIDITY OF 55%-75%

Exposure (days)	Results
97	C, C, C,††
216	7, 5, 3, 2
222	5, 8, 15, 24
302	0, 0, 0, 1 P+
317 (2 lots)	0, 0, 0, 0 P—
320 (2 lots)	4, 3, 11 P+
348	1, 0, 0, 0, 0, 0 P+
358	3, 2, 2, 2, 0, 0, 0, 0 P+
459	0, 0, 0, 0, 0, 0, 0, 0, 0, 0 P—
464	1, 1, 1, and 13 negative P+
471	1, 1, 1, 1, 1, 0, 0, 0, 0, 0 P+
530	1, 1, 2, 1, 1, 0, 0, 0, 0, 0, 0, 0 P+
Bottles finished	

* The figures refer to the number of pocks on each egg; P+ = positive on passage; P— = negative on passage; C = confluent growth

†† Not countable

In the second experiment the conditions of humidity were probably maintained satisfactorily. No titrations were carried out in the early stages, but the better survival of the mass of virus at the lowest humidity in these early stages is seen from the results in Table II. However, the final survival time was probably very similar at RH 58 and 73—about four months. The positive results obtained with some lots after negatives at earlier periods suggest the presence of an occasional scab with a thicker, harder surface, giving greater protection. At the same time the results suggest that there is little danger after six weeks at 30°C and RH 84, after two months at RH 73, and after three months at RH 58.

The control material (see Table III) was exposed not to direct sunlight but to indirect light at the temperature prevailing in this laboratory throughout the year (20°-24°C). The bottles were closed at the humidity in the laboratory, about 55%-75%. In the earlier tests only 0.1 or 0.2 ml were inoculated onto each egg, with a total of 0.8 ml out of 2.0 ml, but from 459 days onwards the entire suspension was placed on 10-16 eggs. The survival period was at least 18 months (530 days), although most of the infectivity was lost between the third and seventh month. The results suggest that if virus arrives in scabs in this country, an amount which may be an infective dose for man has a good chance of surviving storage here for many months.

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RÉSUMÉ

Des poussées de variole survenues en Angleterre dans des filatures ont appelé l'attention sur le danger d'importation du virus variolique avec les balles de coton provenant des régions d'endémie. Lors de l'enquête effectuée à la suite d'une poussée, en 1953, il apparut que les premiers stades du travail du coton étaient ceux qui comportaient les plus grands risques d'infection. En effet, le cardage met en suspension de fines particules et des poussières de coton pouvant renfermer le virus provenant de croûtes varioliques. Aux stades ultérieurs du travail, les risques sont presque nuls. Comme aucun procédé pratique de stérilisation n'est applicable, le meilleur moyen d'éviter la contamination consiste à améliorer les conditions de santé publique dans les pays exportateurs, en particulier par la vaccination, et d'éviter que la main-d'œuvre atteinte de variole ne participe au travail jusqu'à disparition des croûtes.

Les auteurs ont effectué des expériences afin d'établir la durée de survie du virus à divers degrés d'humidité relative (58%, 73%, 84%) à 30°C.

Les résultats permettent de conclure que, dans les régions d'endémie variolique, si le coton est conservé au moins six mois à la température de 30°C-40°C avant d'être expédié, les risques d'importation du virus dans les pays indemnes sont très faibles. En revanche, si le coton est contaminé dans des régions tempérées (20°C-25°C) ou qu'il

séjourne à cette température alors qu'il contient des croûtes virulentes, les particules infectantes peuvent subsister plusieurs mois, voire plus d'un an. Il est connu que le virus peut survivre des années à 4°C ou 5°C dans des tubes ou des flacons fermés.

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