

## The effect of temperature on the growth of pox viruses in the chick embryo

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### INTRODUCTION

In 1936 Burnet & Lush reported that pock formation by ectromelia virus on the chorioallantoic membrane (C.A.M.) of the chick embryo was prevented by incubation of the eggs at 39.5° C., and that a temperature of about 39° C. appeared critical for pock formation and serial passage with this virus. Despite this early observation there appears to have been no direct investigation of the possibility that other members of the group of mammalian pox viruses might each have their own characteristic critical temperature for pock formation. The likelihood of such a possibility has been supported by the recent observation of Nizamuddin & Dumbell (1961) that strains of variola major virus differ significantly from those of variola minor in this respect and that at 38.5° C. only variola major produces pocks on the C.A.M.

In the literature there are various references to the effect of temperature on the growth of individual pox viruses. From the work of Siim (1949) it is apparent that vaccinia virus grows on the C.A.M. at 40.5° C. Thompson & Coates (1942) studied three strains of vaccinia in minced chick embryo tissue cultures at different temperatures but did not distinguish between growth and survival. Marshall (1959) found that rabbit pox virus grew well on the C.A.M. at 39° C. and had some evidence that it was unable to do so at 41° C. Porterfield & Allison (1960) showed that both vaccinia and cowpox produced plaques in chick embryo monolayers at 39° C. while ectromelia did so at 35° C. but not at 37° or 39° C. Hahon, Ratner & Kozikowski (1959) give growth curves for variola virus in the C.A.M. at 35°, 37° and 39° C. and found growth to be inhibited at 39° C. The fibroma-myxoma group of viruses have also been studied in this respect (Thompson & Coates, 1942; Marshall, 1959; Kilham, 1959) but it is with cowpox, vaccinia, variola and serologically related mammalian pox viruses that this paper is concerned. Despite the many observations recorded, it is fair to say that there has been no detailed and systematic study of the effect of temperature on the growth of the viruses of this group.

In the present investigation we have examined a selection of these viruses and determined for each the highest temperature at which pock formation on the C.A.M. occurs. It will be convenient to refer to this temperature as the *ceiling temperature* for growth of the virus concerned. The first part of this paper deals with the results of this survey. It will be seen that interesting differences were found in the ceiling temperatures of the various pox viruses. In the following

section, the possibility has been examined that these differences might depend upon differences in sensitivity to thermal inactivation of the viruses concerned. Finally, an attempt has been made to see whether, in this group of viruses, the ceiling temperature of a virus may in any way be related to the virulence of the virus.

#### MATERIAL AND METHODS

##### *Virus strains*

Many of the viruses used in this work have been obtained through the kindness of colleagues in other centres. These sources are mentioned in the following list of virus strains. Those strains of which the origins and biological characters have been recorded in detail by Fenner (1958) are indicated by means of an asterisk.

Dermovaccinia	(1) The Lister Institute strain (2) The Connaught Laboratories strain* received from Dr E. A. Boulter of Porton. (3) The Lederle-7N strain* received from Dr Boulter.
Neurovaccinia	(1) The Hall Institute strain* received from Dr Boulter. (2) The Levaditi strain received from Dr F. Dekking of Amsterdam.
Rabbit pox	(1) The Utrecht strain* (Jansen, 1941) received from Dr Dekking. (2) The Rockefeller Institute strain* received from Dr Boulter.
Cowpox	(1) The Brighton strain* isolated from a human case in 1937 (Downie, 1939). (2) The Larkin strain isolated from a human case in 1959.
Monkey pox	(1) The Denmark strain (von Magnus, Andersen, Petersen & Birch-Andersen, 1959) received from Dr P. von Magnus of Copenhagen.
Ectromelia	(1) The Hampstead strain received from Dr C. H. Andrewes of the National Institute for Medical Research, Mill Hill. (2) The Moscow strain received from Dr Andrewes. (3) The Mill Hill strain received from Dr Andrewes.
Variola major	(1) The Harvey strain (Downie & Dumbell, 1947, Fenner & Burnet, 1957). (2) The Hinden strain (Downie & Dumbell, 1947).
Alastrim (Variola minor)	(1) The Butler strain isolated in the Rochdale, England outbreak in 1952 (Innes, 1953; Fenner & Burnet, 1957). (2) The Winkel strain isolated in Holland in 1954, received from Dr Dinger of Leyden.

*Eggs*

Fertile hens eggs incubated in a commercial hatching incubator for 12 days at 38.5° C. with hourly rocking were used throughout. They were prepared for chorioallantoic inoculation by a method in routine use in the department involving the use of a spring-loaded egg punch described by McCarthy & Dumbell (1961).

*Incubators*

After inoculation the eggs were incubated at the desired temperatures in incubators of cabinet or hot-room type. The air in these was maintained in continuous circulation by a fan and the heat input was controlled by a Sunvic TR-7 type thermostat. No capsule-controlled incubators were used. Temperatures were recorded by frequent direct observation of several thermometers placed in the immediate vicinity of the eggs. The incubators were not in general use and not therefore subject to frequent opening and closing and observed temperature fluctuations at any point were within  $\pm 0.25^\circ$  C. of the stated temperature. Some variation in temperature was observed from place to place within each of the incubators used. The internal fans reduced, but did not always eliminate this source of error and it seemed best to use only small numbers of eggs and to restrict these to a part of the incubator which was observed to be of even temperature. The direct observations of temperature were easier and possibly more satisfactory in the hot rooms than in the cabinet incubators, but in both it would obviously have been preferable to use remote-reading, continuous temperature recorders, had such been available.

*Virus suspensions*

Heavily infected c.a.m.s were harvested after 2 or 3 days incubation at 35° C., shaken with glass beads in McIlwaine's phosphate-citrate buffer (0.004M phosphate pH 7.2) and centrifuged to deposit cellular debris. The supernatant was mixed with an equal volume of sterile glycerol and stored at  $-20^\circ$  C. These were the stock suspensions for routine use.

For heat inactivation studies crude virus suspensions were prepared by grinding the infected membranes in a mortar pre-chilled to  $-20^\circ$  C. and extracting with 1 ml. per membrane of McIlwaine's phosphate-citrate buffer (0.004M phosphate; pH 7.2) containing 10% of nutrient broth. The resulting suspension was centrifuged to deposit cellular debris.

*Heat inactivation studies*

Only freshly prepared suspensions of virus were used thus obviating the effects of storage at 4° C. (Woodroffe, 1960). Virus suspensions were sealed in thin ampoules in 0.5 ml. volumes. Control ampoules were kept at 4° C. while test ampoules were immersed in a water bath at 55° C. On removal they were cooled immediately in ice-water and stored at 4° C. with the controls. Subsequently all ampoules were opened and titrated for virus in parallel. Titrations were usually made on the day of inactivation, but in some instances were delayed for a few days.

*Titration of virus*

All titrations of virus were made on the basis of pock counts on the C.A.M.s of groups of eggs incubated at 35° C. Suitable dilutions were made in 0.004M McIlvaine's buffer pH 7.2 and inocula were 0.1 ml.

## EXPERIMENTAL RESULTS

*Determination of ceiling temperatures*

All virus strains were capable of pock formation at 35° C. and control titrations were made at this temperature. In the first series of experiments a dose of about 50–100 pock forming units (p.f.u.) of virus, calculated on the basis of these titrations, was inoculated into each of a number of eggs. The eggs were then transferred in groups of 4–6 to a series of incubators at the required temperatures, a control group at 35° C. always being included. After incubation for between 40 and 72 hr. depending on the virus concerned, the membranes were excised and pocks counted. Characteristic specific pocks could usually be recognized without difficulty, but from time to time, on membranes incubated just above the ceiling temperatures, a few apparently non-specific lesions gave rise to difficulty. Membranes bearing such lesions were extracted with buffer and tested for virus by passage. In most instances membranes without pocks were also tested by passage and in no case were any of these membranes found to contain live virus. Had large inocula been used, a small proportion of viable virus from the inoculum might have been recovered after 48 hr. but with the small inocula chosen, this source of confusion was avoided. Small inocula were also of advantage because discrete lesions were easier to recognize than thin confluent thickenings.

Table 1. *Pock formation at different temperatures of incubation*

Virus	Number of strains	Temperatures of incubation (° C.)							
		35	37.5	38	38.5	39	39.5	40	40.5
Rabbit pox	2	+	.	.	+	.	.	+	+
Neurovaccinia	2	+	.	.	.	+	.	+	+
Dermovaccinia	3	+	.	.	.	+	.	+	+
Cowpox	2	+	.	+	+	+	.	+	0
Monkey pox	1	+	.	+	+	+	0	0	0
Ectromelia	3	+	+	.	+	+	0	0	.
Variola major	2	+	.	+	+	0	.	0	.
Variola minor	2	+	+	0	0	.	.	.	.

+, Pocks present; 0, pocks absent.

The results of tests on seventeen strains of virus are summarized in Table 1. No differences were observed between different strains of one virus and individual strains are not therefore shown separately in the table. It will be seen that the viruses may be arranged in a series by virtue of their ceiling temperatures. Rabbit pox, neurovaccinia and dermovaccinia were all capable of pock formation at 40.5° C. Cowpox produced pocks at 40° but not at 40.5° C., monkey pox and

ectromelia at 39° but not at 39.5° C. Variola major produced pocks at 38.5° but not at 39° C. and alastrim at 37.5° but not at 38° C.

The table does not show the numbers of pocks present at the various temperatures and thus gives no indication of the efficiency of pock formation at and below the ceiling temperature. The scale of the experiments was not sufficiently large to allow a reliable numerical expression of this efficiency. Nevertheless it was a constant finding that the efficiency fell markedly as the ceiling temperature was approached. Indeed only with rabbit pox and vaccinia were the pock counts at 38°–38.5° C. as high as at 35° C. With all other viruses pock counts were reduced at these temperatures and with variola major and alastrim some reduction was present even at 37.5° C. Control titrations were made at 35° C. as a matter of convenience. No attempt has been made to show that this temperature is, in fact, optimal for all the viruses tested.

It was also observed that individual pocks became smaller at temperatures near the ceiling temperature. It was expected that these smaller pocks would have a lower yield of infective virus and this was confirmed in several instances. In these experiments 10 individual pocks were excised from the membranes at each temperature, extracted with buffer and titrated for virus. Invariably it was found that, as the ceiling temperature was approached, the yield of infective virus per pock fell.

In addition to the experiments summarized in Table 1 an attempt was made to differentiate between rabbit pox, neurovaccinia and dermovaccinia in eggs incubated at 41° C. and above. At these temperatures difficulty was encountered since many of the embryos died. Nevertheless it was established that, at 41.5° C., only the Utrecht strain of rabbit pox produced pocks. The Rockefeller Institute strain of rabbit pox and strains of vaccinia produced pocks at 41.0° but not at 41.5° C. It is possible that there are further small differences in the viruses grouped at this end of the scale, but the poor survival of chick embryos at these temperatures makes these differences of little value.

Previous reports of the effect of temperature on the growth of pox viruses have been mentioned in the introduction. Our results do not seriously conflict with any of these, although many of the earlier observations were not designed to show the actual ceiling temperatures. The observation of Burnet & Lush (1936) that ectromelia ceases to grow at about 39° C. has been confirmed for all three strains. One minor discrepancy concerns the Utrecht strain of rabbit pox virus which we found to grow at 41.5° C. Marshall (1959) suggested that it would not do so at 41° C. but remained cautious about accepting his results because of the frequent death of embryos at this temperature.

These experiments have served to establish the ceiling temperatures for pock formation on the C.A.M. of the different pox viruses, and it is important that the individual strains of a particular virus do not appear to vary among themselves in this respect. It is clear, from the experiments involving passage, that above the ceiling temperature not only is pock formation prevented but also that multiplication of the virus does not occur.

*Heat inactivation studies*

In searching for an explanation of the different ceiling temperatures, an obvious possibility is that they may merely reflect differences in the resistance of the viruses to inactivation by heat. It is well known that pox viruses do vary in their resistance to heat. Fenner (1958) characterized a number of strains of vaccinia, cowpox and rabbit pox by their rates of inactivation at 55° C. and found this to be a valuable 'marker' character in studies of genetic recombination (Fenner & Comben, 1958; Fenner, 1959). However, it appears unlikely that differences in thermal stability will explain the ceiling temperatures, for Fenner (1958) found marked differences in thermal stability between the various strains of vaccinia. Thus vaccinia Lederle-7N was very much more sensitive to heat than all the other strains and yet in the present investigation it has shown the same ceiling temperature as other strains of vaccinia. Nevertheless, it seemed important to show beyond doubt that thermal stability and ceiling temperature are two separate and unrelated characters. We have therefore, made observations on the thermal stability of the viruses concerned, paying particular attention to those viruses not already dealt with by Fenner.

Table 2. *The reduction in titre ( $\log_{10}$  units) of pox virus suspensions after heating at 55° C.*

Virus	Strain	Period of heating		From
		20 min.	40 min.	Fenner (1958) 55° C. for 40 min.
Rabbit pox	Utrecht	—	1.5	0.9
Vaccinia	Lederle-7N	5.3	> 8.0	4.6
Vaccinia	Connaught Lab	1.2	3.1	1.2
Cowpox	Brighton	1.6	—	1.6
Cowpox	Larkin	2.3	—	—
Monkey pox	Denmark	2.4	—	—
Ectromelia	Moscow	3.2	—	—
Ectromelia	Hampstead	2.8	—	—
Variola major	Harvey	2.1	—	—
Variola major	Hinden	1.4	—	—
Variola minor	Winkel	5.2	—	—
Variola minor	Butler	5.1	—	—

Only twelve of the seventeen strains of virus have been examined in these experiments. For each strain duplicate estimations were made of the fall in titre of a suspension of virus heated for short periods at 55° C. The period was at first 40 min. but in subsequent experiments 20 min. was found to be more satisfactory. The results of these experiments are set out in Table 2 where the figure given is the mean of the two estimations. The methods used were based on those of Fenner (1958) and it was hoped that the results of the two series would be directly comparable. Fenner's figures for certain strains are also given in the table and it will be seen that inactivation was more rapid in our experiments. The difference is probably attributable to the different suspending media used.

Nevertheless, the relative sensitivities of the various strains to inactivation by heat are clearly shown. The results confirm the striking sensitivity to heat of

Vaccinia Lederle-7N when compared with other strains of vaccinia of which the Connaught Laboratories' strain was taken as an example. The results with the strains of smallpox are also of interest. The two strains of alastrim were both very sensitive to the action of heat and gave values like that of vaccinia Lederle-7N. On the other hand the two strains of variola major gave values which are within the range for cowpox and the ordinary strains of vaccinia. The values for strains of ectromelia and monkey pox are not particularly striking but it is to be noted that these viruses are rather more sensitive to heat than variola major.

It is quite clear from Table 2 that if the viruses are arranged in series on the basis of sensitivity to thermal inactivation at 55° C., the order is different from that in the series based on ceiling temperatures (Table 1). It may be concluded that these two characters, ceiling temperature and sensitivity to thermal inactivation *in vitro*, are separate and unrelated.

#### *Virulence of the pox viruses*

In the first section of this paper evidence was presented that the pox viruses differed in the temperatures which limit their growth on the C.A.M. In recent years much attention has been given to similar information about the effect of temperature on the growth of poliomyelitis viruses in tissue culture and great interest has been aroused by the finding that neurovirulence appears to be closely correlated with the ability to grow at higher temperatures (Lwoff & Lwoff, 1960). This in turn has revived interest in the general concept that the virulence of an organism may in part be related to its ability to grow at high temperatures (Lwoff, 1959; Bennett & Nicastri, 1960). It is natural therefore that our observations upon the ceiling temperatures of the pox viruses should lead to a consideration of their virulence.

In dealing with the question of virulence an immediate problem is presented by the wide range of hosts which may be infected by pox viruses. For many of the hosts existing information is sufficient to show that virulence is not related to ceiling temperature as determined in the chick embryo. But for the chick embryo itself there does appear to be a relationship between virulence and ceiling temperature.

The viruses have been compared by determining the mortality rates of 12-day chick embryos after inoculation on the chorioallantois. The methods used were similar to those described by Helbert (1957) in his comparison of the virulence of strains of variola major and alastrim. For each virus at least three separate doses of inoculum were used, a group of eight embryos being inoculated with each dose. The eggs were incubated at 35° C. and candled daily to determine the time of death in each. The observations were terminated on the 7th day.

The results of these experiments are too bulky to present in full and, in any case, a direct comparison of the viruses cannot be made from them because of unavoidable differences in the doses used. In order to extract the greatest information from the results and to make a comparison possible, the methods recently advocated by Bauer (1960) have been used. The harmonic mean survival time for each group of eggs has been calculated and plotted as a reciprocal against the log dose of virus inoculated. It is known that in many biological systems these

values show a linear correlation. The present instance is no exception and satisfactory linear plots were obtained for most of the viruses tested. Least satisfactory were the plots for the viruses of greatest virulence—vaccinia and rabbit pox. Here, even with the lowest practicable range of doses, most of the embryos were killed before the 3rd day. Because of the reciprocal time scale it was important to determine the time of death in this period more accurately than to the nearest day. When the eggs were observed 6 hourly during this period, satisfactory linear plots were obtained for these viruses.

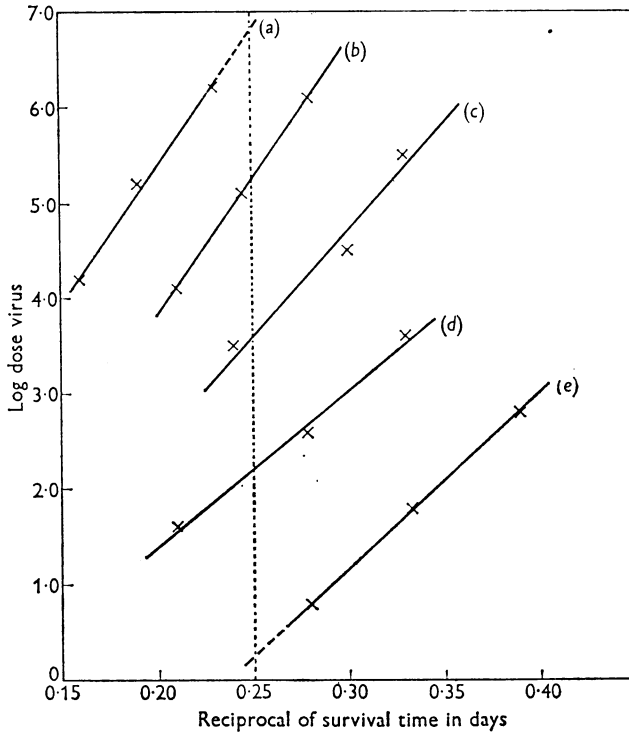


Fig. 1. Plots of log dose virus, in p.f.u. per egg, against reciprocal of the mean survival time in days obtained for one representative strain each of: (a) alastrim, (b) variola major, (c) ectromelia, (d) vaccinia and (e) rabbit pox.

Some examples of the plots are given in Fig. 1. In each case the line of best fit has been calculated by the method of least squares. All the data were obtained by daily inspection of the eggs with the exception of rabbit pox, for which the times of death were recorded at 6 hourly intervals between 1 and 3 days. It will be seen that the data fit the lines well and that the plots all have approximately the same slope. This is also true of the viruses not included in the figure. It is therefore possible to compare the viruses in their virulence by means of a single parameter. To do so by the log dose for zero mortality (Bauer's  $D_0$  units) involves considerable extrapolation and to avoid this we have used the log dose giving a mean survival time of 4 days. This value may conveniently be referred to as the  $D_4$  value and it is obvious that the more virulent the virus the smaller will the value



become. The scale of the experiments has not been large enough to allow very precise estimations of these values. Nevertheless, with a few strains replicate estimations have been made and show a reasonable correspondence and we believe that the accuracy has been sufficient for the purpose of placing the viruses roughly in the order of their virulence for the chick embryo.

Only twelve of the seventeen strains were tested because of the large numbers of eggs required. The  $D_4$  values are presented in Table 3 in which the ceiling temperatures of the viruses are also included for ease of comparison. It will be seen that the viruses may be arranged in four quite separate groups of virulence. The least virulent virus is alastrim and, as observed by Helbert (1957), it is quite separate from variola major. Monkey pox, ectromelia and cowpox form the third group, the virulence of which is intermediate between variola major and vaccinia. Strains of vaccinia and rabbit pox all have a high virulence. There appears to be a considerable range of virulence among the strains of vaccinia and this may be due to the diverse histories of the different strains of this virus. None of them achieved as low a value as the Utrecht strain of rabbit pox but the difference between it and the most virulent strain of vaccinia was small. Duckworth (1958) also observed that the Utrecht strain of rabbit pox was more virulent than either neurovaccinia or dermovaccinia. The  $D_4$  values have been calculated for the data of Helbert (1956) and Duckworth (1958). Their actual values, while maintaining the same relationship between the viruses, are a little higher than our own but it is probable that their experiments were made at a temperature nearer 36° than 35° C. That such a temperature difference would affect the results in this way is suggested by the work of Siim (1949) with vaccinia and by the results of recent experiments made with variola major and alastrim (Dumbell, Bedson & Rossier, 1961).

From Table 3 it is clear that over the whole series of viruses there is a very

Table 3. *Comparison of ceiling temperatures of pox viruses with virulence for the chick embryo*

Virus	Strain	Virulence		Ceiling temperature (° C.)
		$D_4$ value*	Group	
Rabbit pox	Utrecht	0.3	I	> 41
Vaccinia	Lederle-7N	0.8	I	41
Rabbit pox	Rockefeller Institute	1.1	I	41
Vaccinia	Connaught Laboratory	2.2	I	41
Cowpox	Brighton	3.5	II	40
Cowpox	Larkin	3.2	II	40
Ectromelia	Mill Hill	3.3, 3.6†	II	39
Ectromelia	Hampstead	3.3	II	39
Monkey pox	Denmark	3.3, 3.7†	II	39
Variola major	Harvey	4.6, 5.2†	III	38.5
Variola major	Hinden	5.5	III	38.5
Alastrim	Butler	6.8	IV	37.5

\* Log dose of virus giving harmonic mean survival time of 4 days.

† Replicate estimations.

reasonable correlation between ceiling temperatures and virulence for the chick embryo. It is true that there are minor discrepancies, (cowpox, for example, is no more virulent than monkey pox and ectromelia) but these do not reverse the trend from low to high virulence as one moves up the scale of ceiling temperatures. This correlation is valid only for the chick embryo. It is obvious that a consideration of virulence for man or for the mouse would place the viruses in quite different orders.

Table 4. *Comparison of normal host body temperature with ceiling temperature of those viruses which produce generalized infection in the natural host*

Virus	Ceiling temperature	Normal body temperature of natural host
Alastrim	37.5	37 (man)
Variola major	38.5	37 (man)
Ectromelia	39.0	38 (mouse)
Monkey pox	39.0	38-39 (monkey)
Rabbit pox	< 41.0	39 (rabbit)

A minor correlation of a different kind can be shown for those viruses—variola, monkey pox, ectromelia and rabbit pox—which produce generalized infections in their natural hosts. The normal body temperature of each of the host species concerned fall in the same order as the ceiling temperatures of these four viruses, and, as shown in Table 4, the ceiling temperature is, in each instance, higher than the corresponding normal body temperature. The significance of this is very doubtful for there is no certainty that ceiling temperatures determined in cells of these other species would parallel those observed in the chick embryo.

#### DISCUSSION

The basic information about the effect of temperature on multiplication and pock formation of the pox viruses is provided by the experiments reported in the first section. There is little that need be said about these experiments for they were simple in design and gave clear-cut results.

Evidence has been presented in the second section that the ceiling temperatures for pox viruses are not determined by thermal sensitivity of the intact virus particle before it is taken into the cell. This is further supported by the slow and rather uniform rates of inactivation shown by a number of the viruses in tests at the lower and more relevant temperatures of 35° and 40° C. (Bedson & Dumbell, unpublished). Heat is known to affect more than one function of the pox viruses since controlled inactivation at 55° C. may abolish infectivity and yet leave intact the capacity to be reactivated (Joklik, Woodroffe, Holmes & Fenner, 1960). Because of its greater heat stability the latter function has not been investigated in the present studies. Nevertheless, it is quite possible that this, or some other property of the virus may become more sensitive to heat in the altered conditions that obtain once the virus reaches the cell. An answer to this question must await further knowledge of the mechanisms which prevent the growth of viruses above their ceiling temperatures.

It is obvious that information of this kind is also necessary before the relation of ceiling temperature to virulence can properly be assessed. The correlation between the two for the chick embryo appears to be well established by the experiments reported in the third section. The meaning of this correlation is far from clear and it is obviously important to bear in mind that it does appear to be valid only for this one host.

One of the most obvious uses of the ceiling temperatures is to differentiate between these closely related viruses. Their value in distinguishing strains of variola major from those of alastrim has already been reported elsewhere (Nizamuddin & Dumbell, 1961), and it is likely that other diagnostic problems may arise in which they will be of use. It is interesting to note that by its ceiling temperature the Rockefeller Institute strain of rabbit pox is classed with the vaccinias and that it differs from the Utrecht strain. The origins of these viruses are obscure but it is possible that this arrangement has some significance, for there is a suggestion that the epizootic in the Rockefeller Institute arose from the accidental introduction of a vaccinal strain in use in the laboratories (Greene, 1935) while no source could be found in the Utrecht outbreak (Jansen, 1941). It is, therefore, by no means certain that the two strains of rabbit pox can be regarded as different strains of one virus, and certainly in no other instance has a difference been found between the separate strains of a particular virus. For diagnostic purposes it is obviously important that such differences should be exceptional and, furthermore, that the viruses should be relatively stable in respect of the ceiling temperature character. Evidence of this stability is given by the very limited success which has attended efforts to adapt viruses to altered ceiling temperatures. In these unpublished experiments serial passage has been carried out at suitable temperatures for upwards of twenty times without selecting strains with changed ceiling temperatures.

Another importance of the ceiling temperatures is that they provide an additional marker character in genetic studies. It is clear that they are quite independent of the T marker used by Fenner and his colleagues in their studies of genetic recombination (Fenner & Comben, 1958; Fenner, 1959). Moreover, the new character is particularly useful since it allows the chorioallantois to be made selective for strains bearing or receiving the marker necessary for growth at the chosen temperature of incubation.

Quite apart from their use in genetic studies we have found that the ceiling temperatures may be useful in studies of the phenomenon of reactivation. A more detailed account of this work is in preparation and will be published separately.

While the ceiling temperatures have useful applications to the problems considered above, it is obviously important to know more about the mechanisms which may be responsible for them. The importance of this information to the question of virulence has already been discussed and it is quite possible that such information will throw light on yet other problems of intracellular growth of pox viruses. A fuller understanding of any mechanism by which a virus infection can be aborted by a tolerable rise in host temperature is of obvious interest. Studies designed to elucidate the processes involved are now in progress.

## SUMMARY

The 'ceiling temperature' of a pox virus has been defined as the maximum temperature (to the nearest 0.5° C.) of incubation at and below which that virus will grow and produce pocks on the chorioallantois of 12-day-old chick embryos, and above which no pocks appear.

Ceiling temperatures have been estimated for: alastrim (2 strains), 37.5° C.; variola major (2 strains), 38.5° C.; ectromelia (3 strains) and monkey pox (1 strain), 39° C.; cowpox (2 strains), 40° C. Five strains of vaccinia and two of rabbit pox were all capable of pock formation at 40.5° C. Above this temperature difficulty was encountered because many embryos died. But the ceiling temperature for two strains of vaccinia and one strain of rabbit pox was probably 41° C. The Utrecht strain of rabbit pox produced some lesions at 41.5° C.—the highest temperature used.

The ceiling temperatures of the viruses used were not correlated with their thermal stabilities at 55° C. *in vitro*. Thus vaccinia strain, Lederle-7N, had a high ceiling temperature and a low thermal stability, while variola major had a low ceiling temperature and a high thermal stability. For this reason ceiling temperatures and thermal stability are regarded as distinct characters.

In experiments with twelve of the seventeen viruses of which the ceiling temperatures had been determined, the virulence for the chick embryo was then measured. It was found that, in general, the higher the ceiling temperature of a virus the greater was its virulence for the chick embryo.

The presentation of these results is followed by a brief discussion of their significance and potential use.

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