THE EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF *SERICESTHIS* IRIDESCENT VIRUS

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[*Manuscript received December* 3, 1965]

Summary

Development of *Sericesthis* iridescent virus (SIV) in larvae and prepupae of the wax moth *Galleria mellonella* is very sensitive to temperature. The optimum for SIV growth is 22°C in the wax moth, but it is probably slightly higher in the scarab *Sericesthis pruinosa*. SIV is not normally produced in wax moth prepupae at temperatures above 30°C and production of neither viral protein nor nucleic acid was detected at 35°C.

At 22°C SIV growth rate is maximal 7·1 days following infection, at which time 0·56 mg virus is produced per insect per day. At 16°C maximum production is at 8·2 days when 0·030 mg virus is being produced per day. If infected larvae are kept at 22°C for 10 days, and then moved to 28°C, virus increases slowly, whereas in those moved to 32°C virus already synthesized decreases in amount. Infection by SIV does not raise the temperature of wax moth larvae.

The nature of the "temperature lesion" in SIV development is discussed.

I. Introduction

The observation that *Sericesthis* iridescent virus (SIV) developed readily in the larva of the wax moth, *Galleria mellonella* (L.), at 22°C but poorly at 28°C (Day and Mercer 1964) poses questions about the growth of the virus under field conditions. Roberts (personal communication), who discovered SIV in northern New South Wales, has found that soil temperatures frequently rise above 28°C in pastures in which larvae infected by the virus have been located; an examination of the development of the virus under controlled laboratory conditions was therefore initiated as a preliminary to an investigation in the field.

Tanada and Tanabe (1965) have recently shown that the wax moth resists infection by *Tipula* iridescent virus (TIV) at a temperature of 30°C, whereas the larvae die of the infection when kept at 23 or 25°C. It seems likely that SIV and TIV behave similarly in response to temperature. Several examples of resistance to virus infection of insects at high temperatures were mentioned by Tanada (1963, 1965).

Recent work on the effect of temperature on virus infection has been summarized from different viewpoints by, amongst others, Viereck (1963) and by Lwoff (1962). It is apparent that virus synthesis may be greatly influenced by temperature and that the mechanisms by which temperature produces its effects may differ with different viruses. The wax moth is a particularly favourable subject for this work because it is poikilothermic and grows well in a range of temperatures, although it is best adapted to the temperature of the honey-bee hive, approximately 35°C.

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II. Materials and Methods

The characteristics of the virus used have been described (Day and Mercer 1964). Viral concentration was estimated on purified preparations by measuring absorbance at 260 mμ, in a Beckman DU spectrophotometer. The relation between absorbance and weight of virus is illustrated in Figure 1. A difference of 5×10^{-4} mg of SIV is equivalent to a difference in absorbance of 0·018. This difference between two preparations could be readily detected. Temperatures were measured with a calibrated platinum–constantin thermocouple and a Cambridge portable potentiometer.

Prepupae were infected by inoculation and kept in containers in incubators in which the temperature control was always better than ± 1 degC, and generally varied less than $\pm 0 \cdot 5$ degC.

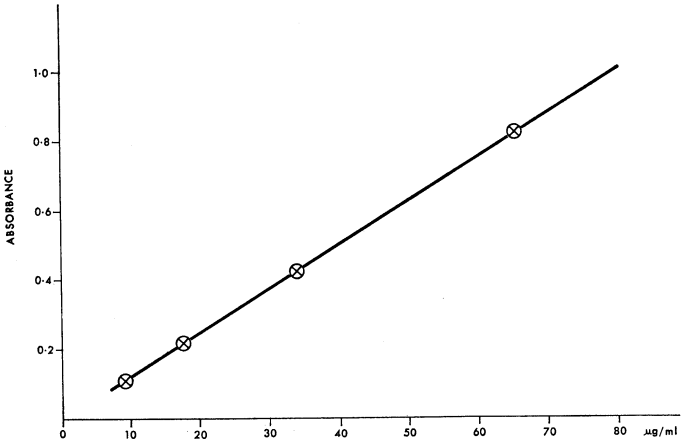


Fig. 1.—Relationship between absorbance at 260 mμ and weight of SIV per millilitre of aqueous solution.

III. Observations

(*a*) *Virus Growth at* 22, 20, *and* 16°*C*

In attempts to obtain growth curves of SIV in *Galleria* the first results were extremely variable. The data suggested the existence of a 2-day cycle in virus production, but sampling at 12-hourly intervals showed that the apparent cycle was not real. Three steps were taken to reduce variability: (1) Prepupae from the third or later generations of brother–sister matings were infected. (2) Larger inocula were used. (3) Prepupae were selected for uniformity in size, because larval size was found to constitute a major source of variability. These precautions resulted in decreased variability, and a more detailed experiment at both 20 and 16°C was then initiated.

A large number of prepupae was inoculated with a standard dose of virus. They were kept in groups of 10 at the appropriate temperature and the amount of virus in each prepupa in a group was measured at intervals from 6 or 7 days following infection. The results are plotted in Figure 2, the curves of which were fitted by the method described in the Appendix. Virus continues to be synthesized in the *Galleria* prepupae until virtually every susceptible cell contains masses of crystalline virus. *Galleria*, infected as prepupae, generally pupate; virus synthesis continues in prepupae (or

pupae) for about 4 weeks at 20°C. At 16°C the rate of synthesis is slower, and the total amount of virus produced is less than half that in prepupae held at 20°C. It was subsequently found (see below) that 22°C was near the optimum temperature for SIV production. The growth curve for SIV production at 22°C was therefore examined, and variability of results was reduced still further by relating the amount of virus produced to the weight of the inoculated host. These results are also presented in Figure 2. The differences in the amount of virus produced at 16, 20, and 22°C illustrate the marked temperature sensitivity of this virus.

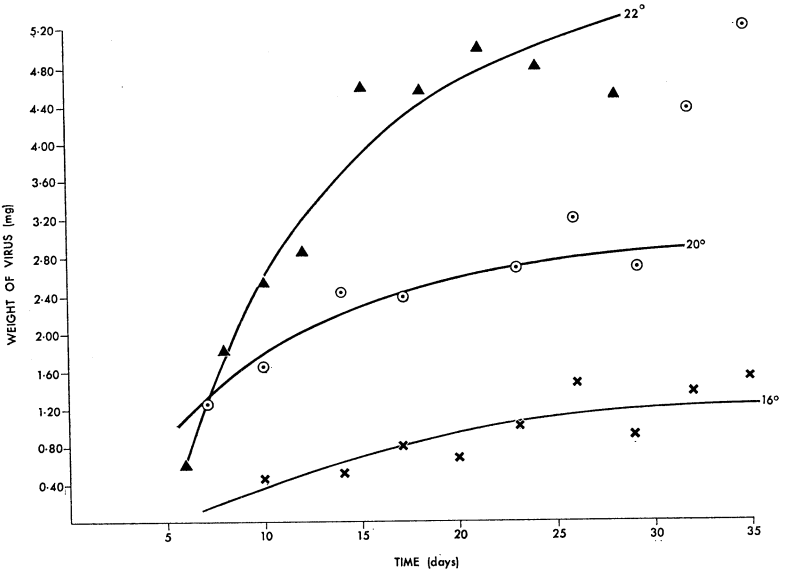


Fig. 2.—Growth of SIV in *Galleria* prepupae with time at 16, 20, and 22°C. Curve at 20°C fitted with omission of two high points. Each point is the mean weight of virus in each of 10 insects. For details of method of fitting the curves, see Appendix.

The question may now be asked whether the viral precursors synthesized at 22°C can be assembled, and thus increase the amount of virus in hosts after they have been transferred to higher temperatures. To obtain information on this point three groups of inoculated prepupae were held at 22°C for 10 days. Then one group was moved to 28°C, the second to 32°C, and a control group was kept at 22°C. At intervals the amount of virus in samples from each group was determined (Table 1). The amount of virus in infected insects increased less at 28°C than if the insects were held at 22°C, but the increase was greater than would have occurred if they had been held at 28°C since infection. In other words, virus assembly continued at the higher temperature. On the other hand, virus actually decreased after transfer to 32°C, suggesting that the infection is actually "cured" at this temperature. At 32°C assembly of viral precursors is inhibited, as well as the synthesis of these precursors.

These results suggest that there are temperature-sensitive steps in synthesis of virus. We can now ask the following question. If the temperature-sensitive step is

performed in one part of the insect, can the product move to another part, permitting enhanced virus production in that part? Prepupae were infected by inoculation through a proleg into the haemocoele and then placed in a cardboard diaphragm so that half the insect was in an incubator at 28°C, while the other half was at 16°C; virus developed only in the cooler end of the insect. With a smaller temperature difference, e.g. 26 and 22°C, in the majority of insects virus was produced in both ends, but in greater amount at the lower temperature. The temperature difference measured by thermocouples within insects held in the diaphragm, when the incubator and room

TABLE 1

AMOUNT OF SIV PRODUCED AT VARIOUS TIMES AT THREE TEMPERATURES

Group A was held at 22°C. Groups B and C were held at 22°C until day 10 then moved to 28 and 32°C, respectively

Days after Inoculation	Group A at 22°C	Group B at 22°C, then at 28°C	Group C at 22°C, then at 32°C
6	0·67±0·29		
8	1·26±0·29		
10	2·78±0·29		
15	5·21±0·29	4·26±0·31	2·75±0·31
20	4·62±0·29	3·60±0·29	1·74±0·33
24	4·59±0·33	3·92±0·33	1·65±0·46

temperature were 26 and 22°C respectively, showed that the actual internal temperatures in the two parts of the larva were 25 and 23°C. Even this difference in temperature was sufficient to be reflected in the extent of virus production; it is concluded that the products of the temperature-sensitive step (or steps) do not spread through the insect.

(*b*) *Optimum Temperature*

To determine the optimum temperature for SIV development, a solution of virus containing 1 mg/ml was diluted 10^4 times and inoculated into *Galleria* prepupae, approximately 1/60 μl into each. Ten insects were placed in each of the following temperatures: 16, 18, 20, 22, 25, 28, and 30°C. Virus production was measured after 28 days (Fig. 3). Only small amounts of virus were detectable in larvae kept at 28°C, and none at 30°C, but the pupae died during the experiment at these temperatures, and the results were therefore not included in the analysis. Many subsequent experiments have shown that SIV does not develop in *Galleria* kept above 32°C. The results of an earlier experiment in which virus production after 21 days was measured is also plotted (curve *A*). The hosts were not weighed in this experiment and the data were therefore not corrected for variations in host size, but the shape of the curve is substantially the same as for the 28-day measurements.

It appears that 22°C is close to the optimum temperatures for SIV development, and that there is a sharp reduction in virus synthesis above 25°C. The sensitivity to temperature of SIV development is clearly evident from Figure 3.

(c) *Occurrence of Thermosensitive Critical Event*

The question was then asked of whether there is, in the reproductive cycle of SIV, a thermosensitive event of the kind described by Lwoff (1962) in poliovirus multiplication. Several experiments were undertaken to examine this point. Prepupae, in groups of 10, were inoculated with SIV and immediately placed at 35°C. At daily intervals groups of prepupae (or pupae) were moved to 22°C. Virus was eventually produced in all groups, even after 6 days at 35°C. A similar observation was made by Tanada and Tanabe (1965) with TIV in *Galleria*. In our experiments after 7 days at 35°C adult moths emerged and no virus could be detected by ultraviolet absorbance in these. Thus, virus can remain in a state capable of producing infection for at least 6 days at 35°C, although it does not multiply at that temperature.

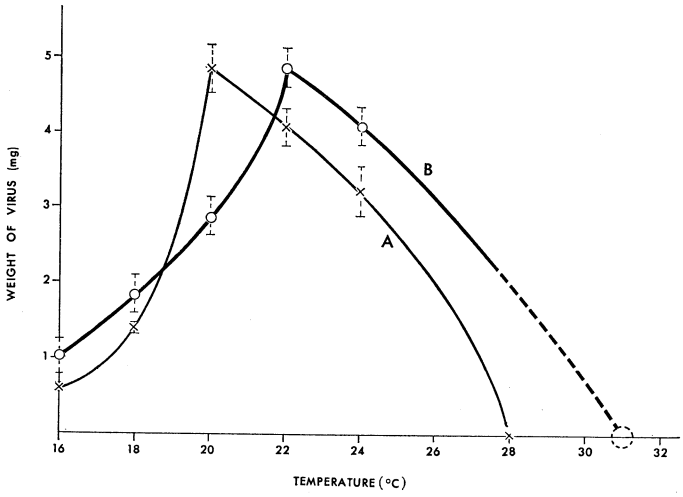


Fig. 3.—Amount of SIV produced in *Galleria* prepupae at various temperatures. Curve *A*, after 21 days; curve *B*, after 28 days, data corrected for variations in weight of prepupae. The temperature above which no virus was produced in curve *B* is between 30 and 32°C. The approximate point is indicated without indication of confidence limits.

When infected prepupae were held at 35°C, then moved to 22°C for 1 or 2 days, and then returned to 35°C, no development of virus was detectable. If, on the other hand, inoculated prepupae were kept at 22°C for varying periods, and the insects then moved to 28°C the following results were obtained:

Period (hr) at 22°C	Virus Produced at 28°C
6	None
18	A barely detectable amount in 1 larva out of 10
24	A larger amount in 6 larvae out of 10
48 or 72	Virus produced in all larvae

These results indicate that a period of about 24 hr at 22°C is sufficient time for the synthesis of all systems required for SIV development. An attempt was then made to shorten the period needed at 22°C for SIV development, by permitting any of the

early stages which could proceed at a temperature of 28°C (e.g. of viral absorption, uncoating, etc.) to do so during a 24-hr period following infection. Measurable amounts of virus were then produced at 28°C after a 6-hr exposure to 22°C, but the amount was very small. This suggested that the period of time at the lower temperature may determine the amount of virus produced.

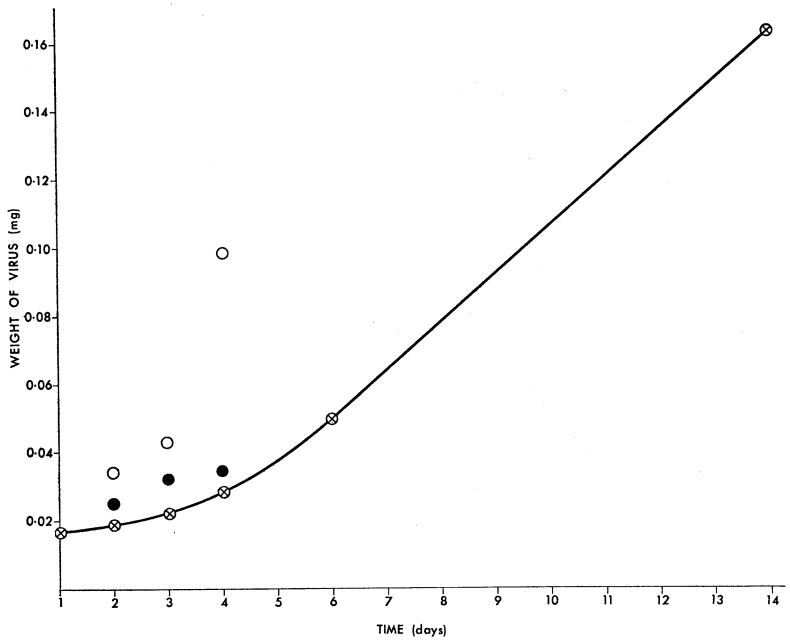


Fig. 4.—Amount of virus per prepupa produced at various times following several temperature treatments. ⊗ 1, 2, 3, 4, 6, and 14 days at 20°C followed by 14 days at 28°C. ● 3 days at 28°C, followed by 2, 3, or 4 days at 20°C, followed by 14 days at 28°C. ○ 5 days at 28°C, followed by the same schedule as the previous group.

Prepupae were therefore inoculated and moved between 20 and 28°C at various intervals. Figure 4 shows the amount of virus produced in each treatment. Results from this experiment lead to two conclusions:

(1) The longer the period at 20°C, the greater the amount of virus produced.

(2) Some stages of viral development (but very little SIV production) did occur at 28°C. Thus, 3 days at 28°C prior to treatment at 20°C increased the yield by about 30%, and 5 days at 28°C increased the yield about 50%. However, pretreatment at 35°C (rather than 28°C) did not increase yield of SIV.

If part of the viral development is able to proceed at the higher temperature, whilst another part is not, it should be possible to determine whether the temperature-sensitive part of the developmental cycle is concerned with nucleic acid synthesis, with protein synthesis, or with both. Prepupae were inoculated with SIV, and placed in groups of 10 at 20, 30, and 35°C. At intervals following the third day after infection,

the insects were examined for the presence of viral antigens in Ouchterlony plates. (The details of antigenic reactions of SIV were described by Day and Mercer 1964.) DNA was detected in sections stained by the Feulgen technique. The results (Table 2) demonstrate that neither nucleic acid nor protein synthesis was detectable at the higher temperatures before the 13th day. The evidence suggests that production of both protein and nucleic acid is inhibited, but the synthesis of nucleic acid is more sensitive to temperature than the synthesis of protein. Support for this conclusion comes from the electron-microscopic examination of sections of infected tissues kept at 28°C (Plate 1). Partly completed virus particles are far more abundant than they are in sections of tissues kept at lower temperatures (see, for example, Fig. 6 of Bellett and Mercer 1964). It is suggested that there are steps in the syntheses of both protein and DNA which do not proceed at temperatures above 30°C.

TABLE 2

DETECTION OF VIRAL ANTIGENS AND NUCLEIC ACID AT VARIOUS TIMES AFTER INFECTION AT THREE TEMPERATURES

Days after Infection	Antigen (Ouchterlony technique)			Nucleic Acid (Feulgen technique)		
	20°C	28°C	35°C	20°C	28°C	35°C
3	−	−	−	−	−	−
7	+	−	−	+	−	−
9	++	−	−	+	−	−
13	+++	+	−	+	+	−

(*d*) *Number of Thermosensitive Steps in SIV Development*

If a substance needed in viral synthesis is produced only at low temperatures, or if the final assembly of the SIV is controlled by temperature then it is possible that alternating low and high temperatures may result in a higher yield of virus than that resulting from insects kept at a constant "optimum" temperature. To test this hypothesis 150 larvae were inoculated and placed in groups of 10 at 20°C. These groups were alternated between 20°C and higher temperatures, three groups between 20 and 22°C, three between 20 and 25°C, three between 20 and 28°C, and three between 20 and 35°C. A further three groups were kept at 20°C as controls. The virus yields were estimated at 7, 14, and 21 days after inoculation and are given as means for 10 larvae in the following tabulation:

Days after Infection	Amount of SIV (mg) Produced per *Galleria* Prepupa				
	20°C	20⇌22°C	20⇌25°C	20⇌28°C	20⇌35°C
7	0·44	0·46	0·55	<0·10	<0·10
14	0·93	1·90	1·60	0·30	<0·10
21	1·96	1·95	3·42	0·72	<0·10

The results, although variable, suggest that alternating temperatures of 22 and 25 with 20°C does result in yields above those obtained at 20°C, but alternating between 20 and 28°C decreases the yield. Alternating between 20 and 35°C resulted in no viral production. In a second experiment performed to assess the effect of constant or fluctuating temperatures on SIV production, infected *Galleria* prepupae were either kept at constant temperatures of 20, 22, or 25°C, or moved at 24-hr intervals between these temperatures for 21 days. Results are given in the following tabulation in which each value represents the mean for 10 larvae adjusted to uniform initial weight:

Temperature (°C):	20	22	25	20⇌22	20⇌25
Virus production (mg/insect):	1·60±0·31	3·16±0·25	2·27±0·25	1·49±0·26	2·30±0·28

No evidence of an increase in virus yield due to fluctuating temperature was found. Although many insects are adapted to fluctuating temperatures, the native host of SIV, *Sericesthis*, may not be, for it lives beneath the soil where temperature changes are somewhat "buffered" against the fluctuations of air temperature.

TABLE 3

AMOUNTS OF SIV PRODUCED BY EIGHT HOST SPECIES AT DIFFERENT TEMPERATURES, AS A PERCENTAGE OF AMOUNT PRODUCED AT 22°C

Ten larvae of each species inoculated

Species	Temperature (°C):			
	16	18	22	25
Lepidoptera				
Gnorimoschema operculella (Zell.)	82	—	100	34
Ephestia kuehniella Zell.		66	100	98
Galleria mellonella (L.)	20	36	100	72
Pterolocera amplicornis Walk.	—	59	100	79
Bombyx mori (L.)	—	88	100	43
Coleoptera				
Tenebrio molitor L.	0	0	0	+*
Listroderes obliquus Gylh.	34	—	100	125
Sericesthis pruinosa (Dalm.)	75	—	100	122

* A trace of virus produced in a small percentage of larvae.

(e) *Temperature Optimum for SIV Production in Different Species*

One further question of interest is whether the temperature optimum for virus production is the same irrespective of the host. The wide host range of SIV makes it especially suitable for an investigation of this point. Eight host species were inoculated with SIV, and virus production at 16, 18, 22, and 25°C was estimated by absorbance at 260 mμ of virus purified for each species 21 days after infection (Table 3). In the majority of species the temperature of greatest virus growth was 22°C, but in the three species of Coleoptera studied, including *Sericesthis pruinosa*, the only species from which the SIV has so far been found in nature, virus production was higher at 25°C

than at 22°C. Roberts (personal communication) has shown that the temperature of greatest liveweight gain was about 25°C in *S. pruinosa*. It would thus appear that in its original host of SIV, the optimum temperature for virus development is close to the optimum temperature for its host.

It is noteworthy that the temperature response to SIV in *Antheraea* cells in tissue culture (Bellett 1965) is not very different from that reported in this paper in which intact *Galleria* is used as the virus host. The temperature optimum of SIV reported by Bellett was about 2 degC lower than that found in *Galleria*.

Two possible explanations suggest themselves to account for the observed differences in temperature optimum for SIV in different hosts. The first is that the system concerned in the thermosensitive step is coded by the host rather than by the virus, the second that the system functions optimally at different temperatures depending upon its cellular environment. The first is unlikely; for the alternative there are precedents in the literature of enzymology, but further work will be necessary to decide between the hypotheses.

(*f*) *Effect of Viral Infection on the Temperature of* Galleria *Larvae*

"Fever" is a frequent sign of viral infection of vertebrates, but this does not necessarily imply that infection of cells by virus results in an increase in their temperature.

If so slight an increase in temperature as indicated in Figure 3 is effective in "curing" SIV infection it would be interesting to know whether the host has evolved a mechanism for increasing body temperature in response to infection. A thermocouple was inserted into a number of SIV-infected and into uninfected *Galleria* larvae. The insects and the equipment were maintained in a constant-temperature room at 22°C. When care was taken to handle the insects only with non-conducting instruments no indication of increased temperature in infected larvae was observed, irrespective of the stage of the infection. The internal temperature of single larvae in a constant-temperature room does not vary significantly from the temperature of the laboratory. The temperature of aggregations of larvae may be raised several degrees, but infected larvae do not tend to aggregate more than normal larvae.

When larvae were infected with SIV, held at 22°C for 10 days, and then transferred to 32°C, SIV production ceased immediately upon transfer, and the virus decreased in the larvae with time. This suggests that the infection was "cured" at the increased temperature. Electron micrographs (Plate 2) of sections of tissues of *Galleria* prepupae reveal that after 21 days at 28°C all the virus particles are enclosed within membranes. Although virus particles have occasionally been seen in lysosome-like bodies at 22°C it seems clear that cellular mechanisms to isolate the virus from the cytoplasm of the cell are more effective at 28 than at 22°C.

(*g*) *Biochemical Mechanism of "Temperature Lesion"*

The rate of growth of many organisms (particularly bacteria) at extreme temperatures appears often to be limited by the rate of a single reaction. In these instances the addition of a particular metabolite may allow the organism to grow at a

temperature several degrees above the maximum at which it can grow with normal nutrition. The B vitamins or the amino acids, or sometimes metal ions, are the stimulants most frequently recognized in bacteria (Langridge 1963).

A preliminary experiment was undertaken to examine the possibility of determining the temperature lesion in SIV development, although it was appreciated that such a lesion would be difficult to identify in the intact organism. Half-grown *Galleria* larvae were inoculated with SIV and fed on various diets. Table 4 outlines these treatments and illustrates that SIV production was found at 30°C in larvae fed on high-protein diets, but not in larvae fed on the usual diet of beeswax.

It is thus possible that the temperature lesion in SIV may result from insufficient synthesis of one of the metabolites provided by the high-protein diet. Further work on this is in progress.

TABLE 4

SIV PRODUCED IN *GALLERIA* LARVAE FED ON DIFFERENT DIETS AT VARIOUS TEMPERATURES

Diet	Temperature (°C):			
	22	28	30	32
Honeycomb wax	+++	−	−	−
Farex*	+++	++	+	−
Casein	+++	++	+	−
Honeycomb+Farex	+++	−	−	−
Honeycomb+casein	+++	−	−	−

* A proprietary baby food containing carbohydrate 76%, protein 14%, fat 2%, minerals 4%, and a mixture of B vitamins.

IV. DISCUSSION

In spite of the low thermal optimum for SIV development, the virus is observed to multiply in the field. This is now understandable because soil temperatures even in midsummer fall below 22°C for some hours every day. Too few infected larvae have been located to draw any conclusions about seasonal distribution of the disease, but field experiments, at present under way, should yield information about its epidemiology. From the epidemiological viewpoint further work on thermal inactivation of SIV in soil is required. We already know that SIV that has been thoroughly dried over phosphorus pentoxide will withstand heating to 80°C for 3 hr, but the virus in the presence of water, and especially in the presence of Mg^{2+}, is more sensitive to thermal inactivation (Day and Mercer 1964).

There is a tendency to assume that the narrow temperature tolerance for viruses of vertebrates is an adaptation to homoithermy. It is therefore interesting to find that viruses of a poikilothermic insect also have a sharp temperature optimum and that this is at such a low temperature, even in the laboratory host (*Galleria*) which prefers a temperature roughly 10–15°C higher. SIV has a temperature optimum close to that of *Sericesthis pruinosa*, the host in which it occurs in nature.

"Ceiling temperatures" of the kind described for SIV have been reported for pox viruses by Bedson and Dumbell (1961). They believed the ceiling temperatures were the result of thermal denaturation. However, ceiling temperatures of SIV would seem to be related to temperature optima of the enzymes involved, because it does not seem reasonable to invoke thermal denaturation of proteins at temperatures below 30°C.

A great deal has been written about the effect of fever as a concomitant of viral infection in vertebrates, and of the effect of fever on curing of viral infections (see, for example, discussion by Bennett and Nicastri 1960). The fact that viral synthesis may be inhibited at temperatures above the physiological norm has tempted some writers to conclude that fever is a mechanism for enhancing resistance of the host. The absence of "fever" in *Galleria* infected with SIV, coupled with the low thermal optimum of the development of the virus, suggests that such considerations are probably irrelevant to poikilotherms. Transitory and slight increase in body temperature of *Periplaneta* infected by bacteria has been reported (Sauerländer and Köhler 1961), but nothing more is known of this phenomenon.

V. Acknowledgments

Thanks are due to Dr. E. H. Mercer for providing the electron micrographs, and to Mr. H. Irzykiewicz and Mr. S. Misko for technical assistance. The figures were drawn by Mrs. G. C. Palmer.

VI. References

Bedson, H. S., and Dumbell, K. R. (1961).—The effect of temperature on the growth of pox viruses in the chick embryo. *J. Hyg.* **59**: 457–69.

Bellett, A. J. D. (1965).—The multiplication of Sericesthis Iridescent Virus in cell culture from *Antheraea eucalypti* Scott. II. *Virology* **26**: 127–31.

Bellett, A. J. D., and Mercer, E. H. (1964).—The multiplication of Sericesthis Iridescent Virus in cell cultures from *Antheraea eucalypti* Scott. *Virology* **24**: 645–53.

Bennett, I. L., and Nicastri, A. (1960).—Fever as a mechanism of resistance. *Bact. Rev.* **24**: 16–34.

Day, M. F., and Mercer, E. H. (1964).—Properties of an iridescent virus from the beetle *Sericesthis pruinosa*. *Aust. J. Biol. Sci.* **17**: 892–902.

Dudzinski, M. L., and Mykytowycz, R. (1961).—The eye lens as an indicator of age in the wild rabbit in Australia. *CSIRO Wildl. Res.* **6**: 156–9.

Langridge, J. (1963).—Biochemical aspects of temperature response. *A. Rev. Pl. Physiol.* **14**: 441–62.

Lwoff, A. (1962).—The thermosensitive critical event of the viral cycle. *Cold Spring Harb. Symp. Quant. Biol.* **27**: 159–72.

Sauerländer, S., and Köhler, F. (1961).—Erhöhung der Körpertemperatur von *Periplaneta americana* L. im Verlauf zweier Bakteriosen. *Experientia* **17**: 397–8.

Tanada, Y. (1963).—Epizootiology of infectious diseases. Ch. 13. In "Insect Pathology". Vol. 2. pp. 423–75. (Ed. E. A. Steinhaus.) (Academic Press: New York.)

Tanada, Y. (1965).—Factors affecting the susceptibility of insects to viruses. *Entomophaga* **10**(2): 139–50.

Tanada, Y., and Tanabe, A. M. (1965).—Resistance of *Galleria mellonella* (Linnaeus) to the Tipula Iridescent Virus at high temperatures. *J. Invert. Path.* **7**: 184–8.

Viereck, E. G. (Ed.) (1963).—"Influence of Cold on Host—Parasite Interactions." (Arctic Aeromedical Laboratory: Fort Wainwright, Alaska.)

Appendix

Statistical analysis of regressions for growth of *Sericesthis* iridescent virus in *Galleria*

The growth data of *Sericesthis* iridescent virus in *Galleria* is asymptotic in character. An expression of the form

$$y = A10^{-B/(x-C)}$$

was fitted to the data, where y = number or weight of virus, x = age of virus (number of days), A = the asymptotic value to which y tends with increasing x (Dudzinski and Mykytowycz 1961), and B and C are constants. A and B were computed by "least-squares" from the linear form of the curve, viz:

$$\log y = \log A - B/(x-C).$$

C was determined by trial and error to give linearity. From this equation the age of maximum growth is $C+2{\cdot}3026B/2$, the relative rate of growth at any age (x) is $2{\cdot}3026B/(x-C)^2$, the maximum growth rate is $4y_{\max}/2{\cdot}3026B$.

Table 5

Parameters of the growth curves of SIV in *Galleria*

Temperature (°C)	A	B	C	Age of Maximum Growth	Maximum Growth Rate
22	7·106	3·09	3·0	6·6	0·559
20	3·621	3·05	0·1	3·6	0·294*
16	1·98	7·02	0·1	8·2	0·030

* Days 32 and 35 omitted from analysis.

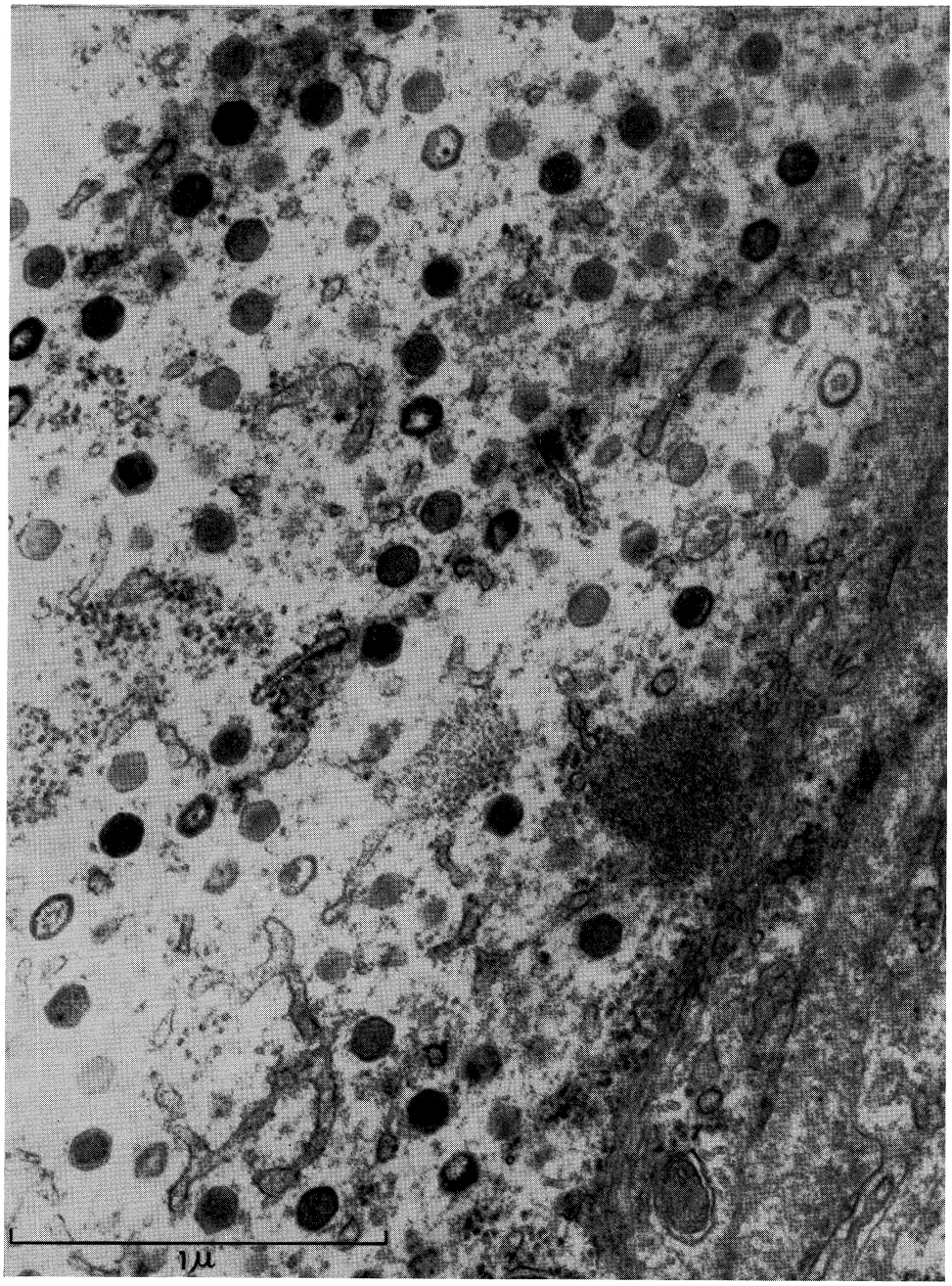
Growth curves of SIV at 16, 20, and 22°C are plotted in Figure 2 and estimates of A, B, and C, of the age of maximum growth, and of the maximum growth rate for these temperatures are given in Table 5. The statistical analysis of regression is given in Table 6.

A considerable reduction of variability of material can be achieved by adjusting for the initial weight of the larvae, e.g. the variance on absolute measurements in the 22°C material decreased from 0·7564 to 0·5605.

It should be stressed that the "trial and error" constant C does not, as far as is known, have special biological significance.

It should be noted that the final two points at 20°C in Figure 2 were not included in the analysis. It is believed that the high yields were due to the proximity to the heating element in the constant-temperature incubator. Alternating between 20°C and a slightly higher temperature could account for the anomalous results.

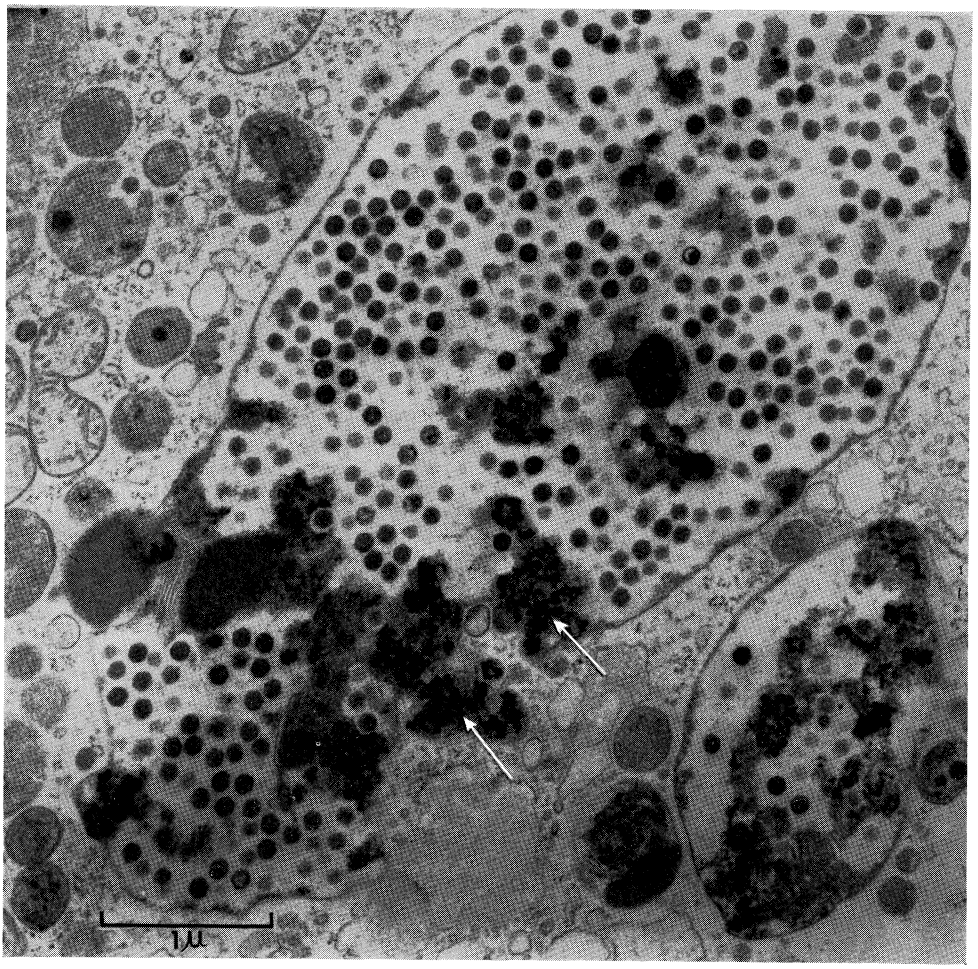
DEVELOPMENT OF *SERICESTHIS* IRIDESCENT VIRUS



Electron micrograph of section of fat-body of *Galleria* prepupa infected with SIV. The prepupa was held at 22°C for 3 days following infection with SIV, moved to 28°C for 14 days, and then fixed in glutaraldehyde followed by osmic acid. Tissue was embedded in Araldite and the section stained with lead acetate. A high proportion of virions lack the nucleic acid core characteristic of SIV seen in insects held at 22°C. Note absence of cytoplasmic DNA characteristic of SIV infection.

Aust. J. Biol. Sci., 1966, **19**, 481–93

DEVELOPMENT OF *SERICESTHIS* IRIDESCENT VIRUS



Electron micrograph of section of epithelial cell of *Galleria* prepupa. The prepupa was held at 22°C for 3 days following infection with SIV and then at 28°C for 21 days. Fixation and staining as in Plate 1. All virions are enclosed within membranes, some having the appearance of lysosomes. The material indicated by arrows is probably partially destroyed viral DNA.

TABLE 6

REGRESSION ANALYSIS OF THE GROWTH CURVES OF SIV IN *GALLERIA* (LINEAR FORM)

Source of Variation	16°C			20°C			20°C†			22°C		
	D.F.	M.S.	*F*	D.F.	M.S.	*F*	D.F.	M.S.	*F*	D.F.	M.S.	*F*
Regression‡	1	1·9879	22·08**	1	1·8661	31·00***	1	0·9608	68·33***	1	6·3695	454·64***
Departure from regression§	7	0·0901	2·57*	7	0·0602	4·30***	5	0·0104	<1	7	0·0169	1·21 (n.s.)
Variation between days	8	0·3274		8	0·2859		6	0·1688		8	0·8110	
Variation within days‖	77	0·0357		60	0·0140		51	0·0141		70	0·0140	

* $P<0·05$. ** $P<0·01$. *** $P<0·001$.

† Days 32 and 35 omitted from analysis.

‡ There is a highly significant relation between the number of days (age) and the weight of virus.

§ The variation of the means of daily observations is significantly greater at 5% only for 16°C, and uncorrected 20°C.

‖ Variability which can be reduced by adjusting for host size.