Reports

Induction of Polyhedral Bodies in Ovarian Tissues of the Tussock Moth in vitro

Polyhedral virus diseases in various lepidopterous larvae have been induced by varying the physical conditions under which the insects live (for example, by crowding them or by subjecting them to high relative humidity) (*1*) or by feeding them compounds such as nitrites (*2*) or acetoxime (*3*). The only study of a polyhedral virus in insect tissues grown in vitro is that of Trager (*4*), in which he observed multiplication of polyhedral bodies in the nuclei of cells from the ovarian lining of the silkworm (*Bombyx mori*).

In a series of experiments (*5*) primarily set up to test the effects of various concentrations of Eagle's basal medium (*6*) on the growth of the ovarian tissue of the tussock moth (*Hemerocampa leucostigma*), polyhedral bodies appeared in some of the cultures after a change was made in the media used. A polyhedral virus disease in larvae of the tussock moth was reported by Chapman and Glaser in 1915 (*7*).

The tissues were obtained from last instar larvae growing on sycamore trees. Usually the larvae were sacrificed within 2 to 3 hours after collection. No evidence of any disease was noticed at the time of collection or on examination of the organs and blood on dissection. After the larvae had been surface-sterilized with 70-percent alcohol, the ovaries were removed, freed of nonovarian tissue, and cut into pieces about 1 mm^3 in size. The explants were selected at random when the cultures were set up. Each culture consisted of one explant in 0.005 ml of medium set up in a hanging-drop. The cultures were incubated at 26°C.

Five media were used in the experiment. The control cultures were grown in Wyatt's medium (*8*) modified by Grace (*9*), to which had been added 3-percent plasma obtained from diapausing pupae of the promethea moth (*Callosamia promethea*). The four experimental media consisted of 2- and 3-percent Eagle's solution in (i) Wyatt's medium containing 3-percent plasma and (ii) Wyatt's medium without plasma. These four media will be designated 2Ep and 3Ep and 2E and 3E, respectively. Six cultures were set up in each medium. Three days later a new series of cultures was set up containing 10- and 20-percent Eagle's solution in Wyatt's medium with plasma (10Ep and 20Ep) and Wyatt's medium without plasma (10E and 20E).

On examination of the cultures 5 days after initiation, the cultures containing blood showed very good growth, but the growth was poor in the cultures with no blood. In order to save the latter cultures, the media 2E and 3E were replaced, respectively, with 10- and 20-percent Eagle's solution with blood. These cultures were designated 2E-10Ep and 3E-20Ep.

In all the cultures in which the medium had been changed, it was noticed 4 days later that many of the nuclei were filled with granules. The cytoplasm remained clear and free of these bodies. Within the next 3 days every cell had become infected and many nuclei had burst, liberating the granules into the medium (Fig. 1). When examined under the dark field of the microscope, the granules were observed to be highly refractile and polyhedral in shape and to resemble the typical polyhedral bodies found in other insect polyhedral diseases. All the other cultures continued to grow, and the cells showed no evidence of any nuclear granulation.

To determine whether these bodies would multiply in cultures not subjected to a change in medium, two cultures from each of the groups 3Ep and 20Ep were inoculated with granules from a culture 3E-20Ep. The remaining four cultures in each group acted as controls. Four days later, nuclei in each inoculated culture were filled with granules, and by the sixth day after infection every nucleus in every cell showed infection and many of the cells had burst. No infection was noticed in the control cultures.

Granules from these cultures were transferred to healthy cultures of the series 2Ep and 10Ep, and four cultures in each series were again used as controls. By the seventh day after introduction of the granules, every cell was infected. None of the controls showed any infection.

As is generally recognized, the various polyhedral diseases of insects are highly specific and only in a very few cases are they able to be transmitted to other (even closely related) species. It was therefore decided to test whether the granules were capable of multiplying in cells from the ovarian tissue of the promethea moth. Three cultures of this species, which had been growing for 20 days, were inoculated with the granules from one of the cultures 3E-20Ep. Three sister cultures were used as controls. In no instance were any granules observed within the nuclei, even 17 days after the inoculation.

It is necessary to emphasize the following points. (i) On gross and microscopic examination, both before and after dissection, no evidence of infection was seen in any of the larvae used. (ii) Explants were chosen at random when the cultures were set up. (iii) No evidence of infection was noticed in any culture prior to the change of medium. (iv) Only those cultures in which the medium had been changed showed infection; all other cultures continued to grow, and the cells remained healthy.

To account for the phenomenon observed, it is postulated that the larvae used in this experiment carried a latent infection of a polyhedrosis which became

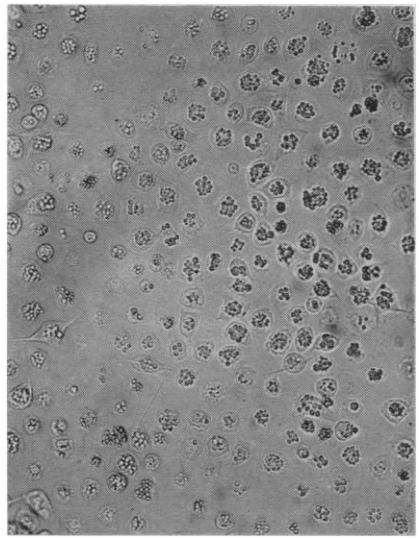

Fig. 1. Ovarian cells showing polyhedral bodies confined to nucleus, seventh day of infection. (About × 93) [Photograph by J. A. Carlile]

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manifest after the cultures were subjected to a "physiological shock" brought about by an abrupt change from one medium to another.

Because it was late in the season, it was not possible to inject some of the granules into larvae to observe whether symptoms of a typical polyhedral disease developed. Electron microscope studies of the granules are now in progress (*10*).

THOMAS D. C. GRACE*
Rockefeller Institute for Medical Research, New York, New York

References and Notes

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10. A paper describing these studies, as well as observations on the development of the disease in larvae, is in preparation.

* On leave of absence from the Division of Entomology, Commonwealth Scientific and Industrial Research Organization, Australia, February 1957–February 1959.

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Atomic Bomb Effect: Variation of Radiocarbon in Plants, Shells, and Snails in the Past 4 Years

The measurements reported in this paper were primarily designed for a study of the activity of recent organic and inorganic material in various environments. They also gave evidence, however, of a fast increase of the activity of C^{14} in the atmosphere due to atom bombs.

The first group of samples consisted of mussels from the Dutch Waddenzee (*Mytilus edulus*). They are indicated by *Ms* (shells) and *Mf* (Flesh). The second group consisted of land snails (*Helix pomatia*) from a snail farm in Valkenburg (province of Limburg). They are indicated by *W*. The third group consisted of fresh water molluscs (*Dreissenia polymorpha* Pallas) and an alga (*Tolypellopsis stelligera* (Bauer) Migula, family Characeae), collected by G. P. H. van Heusden in the lakes at Loenen (province of Utrecht). These samples are indicated by *Ls* and *Lf* for the dreissenia and by *Lp* for the plant. The lake water contained, on the average, 104 mg of HCO_3^{--} per liter and about 1 mg free CO_2 per liter. The recent calibration sample (*P*) consisted of peanut shells bought in March 1955. These shells probably grew in the summer of 1954. Because of the fast rise in the concentration of C^{14} in the past few years, it is also of importance to give the dates of collection of the other samples; *W*53, *M*, and *L* were collected in the second half of November, 1953; *W*56 on 15 Nov. 1956; and *W*57 on 18 June 1957. The snails collected in November were in a hibernating state.

The activities shown in Table 1 are given by their difference δ from the standard *P* in permillage of the activity of the standard. Each sample has been measured at least twice. The statistical error was between 0.2 and 0.3 percent. This is also the error in δ since the standard *P* has been measured several times.

As a check on fractionation during growth, and so forth, the concentration of C^{13} has been measured (*1*). The results are given in Table 1 by the deviation from the standard *P*. Though some of the measurements were certainly accurate to better than 0.1 percent, sometimes irregularities occurred. Since all measurements were duplicated at least once, the error in δ C^{13} is estimated to be less than 0.1 percent.

Recent increase of concentration of radiocarbon. In looking at the activity *Wf* of the flesh of *Helix pomatia*, which is in fairly fast exchange with the plants the animal lives on, a remarkable increase by 4.3 percent is observed between November 1953 and June 1957. This increase can be due only to production of C^{14} by atomic bombs. A similar effect has been observed in New Zealand (*2*) by a study of CO_2 samples immediately taken from the atmosphere. The increase of the activity of the shells (*Ws*) is only about 1 percent; obviously the carbonate in the shell does not exchange with the environment; the greater part of the shell was deposited in the period of lower C^{14} concentration, but this fraction is difficult to estimate. The owner of the snail farm claims that at least some of the snails may have been up to 10 years old, but a somewhat lower limit is more probable. The lower activity of *Ws*56 as compared with *Ws*53 may be due to a higher age of these shells; it is not possible to check this, however. Furthermore, the activity of *Ws*53 is also too low. These shells contain 1.6 percent more C^{13} than the flesh does. Thus they should contain 3.2 percent more C^{14} (see also below). This is not true at all for the more recent shells where the fast increase of atomic bombs has produced a fast increase of activity in the flesh. The flesh is even more active than the shell. But already by 1953 the flesh was only 1 percent less active, instead of 3.2 percent. This discrepancy has puzzled us for a long time; it probably means that the bomb had produced an increase of about 2 percent by the end of 1953. This is being checked by collection of more appropriate samples. Since the flesh of *Helix pomatia* has the same C^{13} content as the peanut shells, the two should have the same C^{14} content. The difference is probably due to the fast increase of C^{14} in the atmosphere between the two dates of collection, though it is not completely certain that geographic differences have not played a role.

The discrepancy between the enrichment of C^{14} and C^{13} in the shell does not occur for the mussels and the dreissenia. For the mussels this will be mainly due to the fact that the increase of C^{14} concentration in the ocean is much slower. New samples were collected in November 1957 from the same locality in order to obtain some information, which is very important for discussions on exchange between the ocean and atmosphere. The activity proved to be only 0.5 ± 0.3 percent above the activity in 1953. The dreissenia shells were at most 1 year old, and this may explain why no discrepancy occurred.

Isotopic fractionation. Organic and inorganic carbon in the same environment can have different isotopic composition by fractionation in chemical and physical processes. Generally the fractionation is small; then the enrichment of C^{14} should be twice the enrichment of C^{13}. It has been mentioned already above that this does not hold for *Helix pomatia*. The relation is nicely confirmed by the measurements on the three samples from the *Loenensche plassen* (*Ls*, *Lf*, and *Lp*) and the same is true for the samples *Ms* and *Mf*. It is of biochemical interest to note that the fractionation between shell and flesh is not the same for the three animals.

Difference of environment. It is obvious that the *Loenensche plassen* have a low C^{14} content; this had been expected because of the transport of old

Table 1. Summary of results. The notations for the samples are given in the text.

Sample No.	δ C^{14} (per mil)	δ C^{13} (per mil)
P	*0*	*0*
*Ws*53	−11	+16
*Wf*53	−21	0
*Ws*56	−17	*
*Wf*56	+18	*
*Ws*57	−2	*
*Wf*57	+22	*
*Ms*53	−13	+19
*Mf*53	−50	+4
*Ls*53	−44	~+25
*Lf*53	−94	~0
*Lp*53	−82	~+8

* The various batches of *Helix pomatia* (*W*) all gave the same concentration of C^{13}.