Table 1. Elimination of organic matter in *Orbulina universa* by Clorox treatment.

Time in Clorox (hours)	*Orbulina universa*			
	Before grinding		After grinding	
	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$
0	+3.44	+2.34		
	+4.65	+2.40		
48	+2.00	+2.43	+1.94	+2.26
	+1.99	+2.45	+1.90	+2.29
72	+1.98	+2.22	+1.89	+2.29
	+1.86	+2.38	+1.92	+2.28
120	+2.02	+2.26	+1.93	+2.33
	+1.87	+2.20	+1.91	+2.14

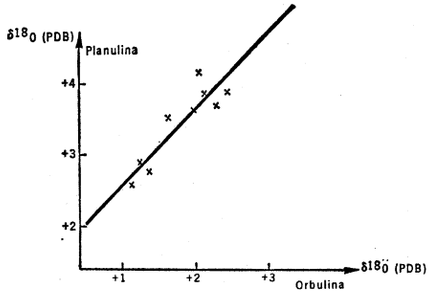

Fig. 2. Correlations between $\delta^{18}O$ for *Planulina* and *Orbulina* in the core.

calcite, when it is precipitated in the same conditions. The ^{18}O content increases 0.06 per mil for each mole percent of $MgCO_3$ in calcite. On the basis of the values given by Tarutani *et al.* (*7*), the isotopic effect might be explained if *Pyrgo* sp. contains 10 to 15 percent more $MgCO_3$ than is contained in *Planulina wuellestorfi*; a similar effect might be obtained for $SrCO_3$.

To determine if the isotopic differences noticed in our benthic Foraminifera were due to differences in chemical composition, we measured the magnesium and strontium content of the foraminiferal shells. The results show that the magnesium and strontium contents of the different species of benthic Foraminifera are of the same order as those of pelagic Foraminifera and that they are insufficient to account for the observed isotopic effect (less than 0.12 percent for $SrCO_3$ and less than 1.1 percent for $MgCO_3$).

From our results and on the assumption that the isotopic composition of seawater is +0.1 per mil for deep Atlantic water (referred to standard mean ocean water), the isotopic temperatures have been calculated. If this assumption is correct, none of the benthic species present in the first 35 mm of the core shows concordance with the environmental temperature, as the true temperature in this region is 2.8° to 3.2°C (*8*) and the calculated isotopic temperatures are 4.2°C for *Pyrgo* sp., 4.4°C for diverse benthic forms, and 6.6°C for *Planulina wuellestorfi*.

The only possible explanation is that the composition of the test is partly due to the biological activity of the animal. Craig (*9*) has shown that the isotopic composition of the carbon of foraminiferal tests does not reflect equilibrium with dissolved carbon but shows utilization of metabolic CO_2.

Assuming that metabolic CO_2 has an isotopic composition similar to that of the organic matter of the plankton, characterized by $\delta^{13}C = -14$ per mil, Craig concludes that 30 percent of the carbon in foraminiferal tests has a biological origin. If so, part of the oxygen could also have a metabolic origin, which would explain the observed small depletion of ^{18}O.

The contribution of metabolic CO_2 apparently varies with the different species, as is shown by the fact that we obtained different curves. The parallelism of the curves (Fig. 1) shows, however, that this contribution is constant for any given species. Apparently, it will be possible to calculate temperatures from the isotopic data only after a very careful study of the biological framework of the foraminiferal tests.

The oxygen isotopic composition of a benthic species (*Planulina wuellestorfi*) and a planktonic species (*Orbulina universa*) at each level of the core is compared in Fig. 2. The excellent correlation ($r = 0.889$) indicates that both benthic and pelagic Foraminifera are sensitive to the same phenomenon, most likely oxygen isotopic variations of the seawater (*6*).

This conclusion is substantiated by the fact that the slope of the regression line (Fig. 2) is very near 1. If the isotopic composition of seawater affects the isotopic composition of benthic as well as pelagic Foraminifera and if, in addition, temperature had an influence on the pelagic species, the slope of the regression line would be less than 1.

We conclude that temperature is not responsible for the variations of the isotopic composition of pelagic foraminiferal tests; instead, the variations of the isotopic composition of oxygen of the ocean are responsible for the variations affecting both the benthic and the planktonic species. As a result, the curves that show the variations of oxygen isotopic composition of foraminiferal tests all along the core give very useful paleoclimatological indications only if interpreted in terms of ice cap melting. Until more information is obtained on the participation of metabolic CO_2, it seems to us to be impossible to make a true correlation between $\delta^{18}O$ and temperature of crystallization of the foraminiferal tests.

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10. We thank Y. Le Calvez for help in identifying the foraminiferal species; Jacques Labeyrie for useful discussions of the problem; P. Martineau for help in the preparation of the samples; E. Brichet for the chemical analyses; and Professor Emiliani for his review of our text and his useful suggestions.

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Toxic Factor Produced by a Granulosis Virus in Armyworm Larva: Effect on Apanteles militaris

Abstract. *The internal parasitoid* Apanteles militaris *is affected by a proteinaceous toxic factor in the hemolymph of granulosis virus–infected armyworm larva. The hemolymph, after centrifugation to remove the virus particles and inoculation into the larval hemocoel, is still toxic to the parasitoids.*

Tanada and Hukuhara (*1*) separated the two strains of a granulosis virus of the armyworm *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae) on the basis of differences in synergistic property and virus particle size. The synergistic virus strain

Table 1. Effect of the synergistic and nonsynergistic strains of a granulosis virus on the development of parasitoids when virus infection and parasitization occurred simultaneously in armyworm larva.

Treatment	Healthy hosts with healthy parasitoids (No.)	Healthy hosts with dead parasitoids (No.)	Infected hosts with healthy parasitoids (No.)	Infected hosts with dead parasitoids (No.)	Hosts dead from other causes (No.)
		Experiment 1			
Control	9	1*	0	0	0
Synergistic	0	0	0	8	0
Nonsynergistic	0	0	9	0	0
		Experiment 2			
Control	9	1*	0	0	0
Synergistic	0	0	0	10	0
Nonsynergistic	0	0	9	1*	1*
		Experiment 3			
Control	9	0	0	0	0
Synergistic	4	0	0	6	0
Nonsynergistic	0	0	10	1*	0

* The dead parasitoids from these hosts did not exhibit the syndromes described for those from hosts infected with the synergistic virus strain.

(Hawaiian) was effective in enhancing the infectivity of a nuclear-polyhedrosis virus of the armyworm larva. The nonsynergistic virus strain (Oregonian) did not have this enhancing capacity (*2*). I have investigated the interaction of these two strains with an internal, gregarious parasitoid, *Apanteles militaris* (Walsh) (Hymenoptera: Braconidae), when the virus and parasitoids occurred together in their common host, the armyworm larva.

When the 4th-instar armyworm larvae were parasitized by *A. militaris* and fed the same day with the synergistic strain of the granulosis virus (1.82×10^8 virus capsules per seedling corn leaf), the parasitoids did not develop beyond the 1st instar and succumbed. Death of the parasitoids occurred when there was an active viral infection in the armyworm larva. The virus-infected hosts were maintained for 16 days and then dissected to ascertain the condition of the parasitoids. On the other hand, when 4th-instar armyworm larvae were parasitized by *A. militaris* and treated with the nonsynergistic strain at the same dosage, the parasitoids developed to maturity in 11 to 14 days even though the armyworm larvae were infected by this virus strain (Table 1). Thus, the infection of the host larva by the synergistic strain had a detrimental effect on the development of the internal, gregarious parasitoids.

Tests were conducted to determine whether the death of the parasitoid was caused by a virus infection. One hundred deranged parasitoids from virus-infected host larvae were washed in several rinses of 0.01*M* phosphate buffer (*p*H 6.8), triturated in the buffer, and centrifuged at 950*g* for 10 minutes. Ten microliters of the supernatant were inoculated through a proleg of a 6th-instar armyworm larva that contained 5-day-old parasitoids. The supernatant did not prevent the development of the parasitoids, and none of the host larvae were infected by the virus. This result indicated the absence of infectious viral material in the deranged parasitoids and suggested the presence of a toxic factor in the virus-infected host larva. However, this test does not exclude the possibility that the virus, if present, may have been in a noninfectious stage in the parasitoids. The following tests do not confirm this possibility.

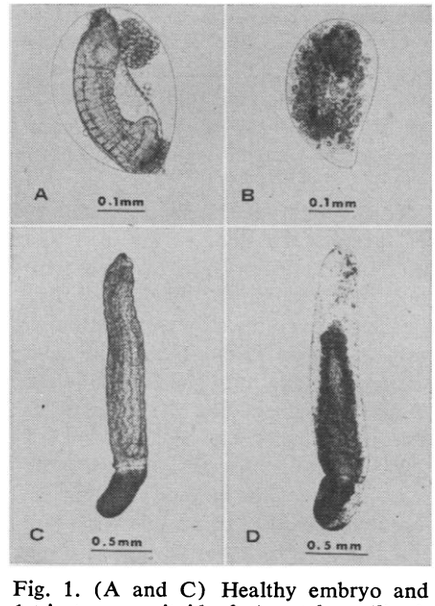

Fig. 1. (A and C) Healthy embryo and 1st-instar parasitoid of *Apanteles militaris* from uninfected armyworm larvae. (B and D) Deranged embryo and 1st-instar parasitoid showing the effects of a toxic factor present within the hemocoel of armyworm larvae infected with the synergistic strain of a granulosis virus.

In order to establish the presence of a toxic factor in virus-infected larvae, the hemolymph was obtained from 100 to 150 last-instar armyworm larvae which were infected with the synergistic strain of the granulosis virus. The hemolymph was centrifuged at 48,200*g* and at 67,300*g* for 1 hour each to remove the virus particles. Ten microliters of the supernatant of the centrifuged hemolymph were inoculated through a proleg of a 6th-instar larva which contained 5-day-old parasitoids. In three trials, 38 parasitized hosts were inoculated, and in each host, the parasitoids were not able to develop beyond the 2nd instar. They were apparently affected by a toxic factor in the supernatant of the centrifuged hemolymph. The hemolymph from healthy larvae and from larvae infected with the nonsynergistic virus strain was centrifuged as above and the supernatant inoculated into armyworm larvae that contained parasitoids. The parasitoids from 35 hosts inoculated with hemolymph from healthy larvae and 35 hosts inoculated with hemolymph from larvae infected with the nonsynergistic virus strain developed to maturity. In all cases, there were no signs or symptoms of virus infection in the armyworm larvae. Further tests showed that the hemolymph containing the toxic factor must be injected intrahemocoelically into the host to be effective against the parasitoids. The toxic factor had no apparent effect on the parasitoids when given orally to the host armyworm larvae.

The toxic or deleterious effect caused by the virus infection in the armyworm larva was evident in the embryonic and larval development of the parasitoid (Fig. 1). Parasitoid eggs deposited in virus-infected larvae did not complete their embryonic development. The initial pathology of parasitoid larvae present in virus-infected host was the cessation of growth, after which the tissues receded from the cuticle. Such deranged parasitoid larvae were still alive as indicated by their movements. The tissues of the parasitoids continued to separate from the cuticle, and the parasitoids eventually died. The parasitoids were seldom encapsulated by the host hemocytes. The parasitoids exhibited similar syndromes when the hemolymph, which had been extracted from virus-infected larvae

and centrifuged to remove the virus particles, was injected into the host larva. The host containing the dying parasitoids was not able to pupate and eventually died. However, the toxic factor obtained from the centrifuged hemolymph of virus-infected larvae had no detectable adverse effect upon unparasitized armyworm larvae when inoculated into the hemocoel.

The toxic factor responsible for the failure of the parasitoids to develop seems to be proteinaceous. This factor could be precipitated from the supernatant of the centrifuged hemolymph from virus-infected larvae with 50 percent ammonium sulfate. Ten percent trichloroacetic acid, on the other hand, destroyed its activity. The factor retained its toxic activity when kept frozen or at 4°C for 3 months. It was heat labile and lost its activity when heated above 50°C in a water bath for 10 minutes. The toxic factor was lost after an initial passage through a host larva, and this indicated that no multiplication of the toxic factor occurred. Possibly there was a breakdown or a dilution of the factor below the toxic concentration.

As far as I am aware, the present observation of a toxic factor within a virus-infected host has not been reported for invertebrates infected with viruses. However, toxins have been reported from viruses infecting mammals (*3*, *4*). From the results of the present study, I propose the hypothesis that the granulosis virus, in its interaction with the host, produces a toxic factor that affects the parasitoid but not the host.

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Steroid Hormones: Effects on Adenyl Cyclase Activity and Adenosine 3′,5′-Monophosphate in Target Tissues

Abstract. *The adenyl cyclases of chick oviduct and rat prostate were not stimulated by estrogen and testosterone, respectively, suggesting that growth and differentiation of these target tissues are not mediated by adenosine 3′,5′-monophosphate. Estrogen acutely activated adenyl cyclase in the castrate rat uterus, but this was prevented by administration of* DL-*propranolol, suggesting that the effect was mediated by catecholamines. Progesterone produced a delayed stimulation of oviduct adenyl cyclase preceding and concomitant with the induction of synthesis of avidin.*

The role of cyclic adenosine 3′,5′-monophosphate (AMP) in the mediation of the effects of steroid hormones is unclear, there being only one report of an increase in tissue 3′,5′-AMP after a steroid was administered (*1*). To clarify the role of 3′,5′-AMP in the stimulation of protein synthesis by steroid hormones (*2*), the effects on the adenyl cyclase system were investigated relative to (i) estrogen and progesterone in the chick oviduct, (ii) testosterone in the ventral prostate of hypophysectomized rats, and (iii) estrogen in the castrate rat uterus.

In our studies, adenyl cyclase activity was determined by a modification (*3*) of the method of Krishna *et al.* (*4*), with the addition of 100 to 150 μg of protein of the membrane fraction, obtained by sedimentation of homogenates at 2200*g*, to the reaction mixture in 25 μl of 0.05*M* tris-HCl (*p*H 7.4) buffer with 0.026*M* theophylline, 0.04 percent bovine plasma albumin, 0.013*M* $MgCl_2$, 0.014*M* mercaptoethanol, and 20 percent glycerol. The concentration of adenosine triphosphate (ATP) in the reaction mixture was 1.22 mmole/liter. Incubations were carried out for 20 minutes at 37°C. Adenyl cyclase activity varied among control groups by as much as 20 percent.

Tissue 3′,5′-AMP was assayed by the radiophosphate exchange method (*5*). With this assay, a linear standard curve was invariably obtained between 0.02 and 0.24 nmole of 3′,5′-AMP. Final recoveries varied between 20 and 35 percent. Protein was determined by the method of Lowry *et al.* (*6*).

Table 1 summarizes some of the effects of diethylstilbestrol (DES) and progesterone on adenyl cyclase activity and tissue 3′,5′-AMP concentrations in the chick oviduct. Given intravenously, DES did not acutely activate adenyl cyclase from 30 seconds to 120 minutes. In previous studies, DES given subcutaneously also failed to activate adenyl cyclase from 1 to 18 days (*7*), with a consistent fall in oviduct adenyl cyclase activity after DES was administered for several days. As we have found that DES caused no direct effect on adenyl cyclase activity in vitro, the decreased activity seen at day 5 may reflect (i) altered adenyl cyclase activity in the new cell populations which develop during prolonged estrogen administration,

Table 1. Effect of diethylstilbestrol and progesterone on adenyl cyclase activity and cyclic 3′, 5′-AMP concentrations in the chick oviduct. Each value represents the mean ± 1 S.D. of triplicate or quadruplicate determinations of results of a single representative experiment. Chicks were killed by cervical fracture; the oviducts were excised and frozen in liquid nitrogen within 10 seconds. Oviducts from Rhode Island Red chick oviducts (50 per group) were used for cyclic 3′, 5′-AMP determinations. For adenyl cyclase determinations, 12 oviducts per group were allowed to thaw at 4°C, minced, and then homogenized with 20 strokes of a motorized Teflon pestle; activity was measured as described. Diethylstilbestrol (5 mg in oil) was administered subcutaneously (s.c.) daily. Progesterone (5 mg) was given as a single subcutaneous injection in oil. For the intravenous administration (i.v.), DES or progesterone (6.5 μg per 100 g of body weight) were given in 0.1 ml of a 90 percent normal saline, with 10 percent ethanol carrier; control groups received carrier alone. Animals were killed at precise intervals after hormone was administered. All experiments were repeated at least three times with similar results, different animal groups being used. Adenyl cyclase activity is expressed as the numbers of picomoles of 3′, 5′-AMP accumulated per milligram of protein per 20 minutes.

Route	Interval after administration	Adenyl cyclase activity [pmole mg^{-1} $(20\ min)^{-1}$]	Tissue 3′, 5′-AMP (nmole/g, wet weight)
		Control	
		307 ± 17	1.28 ± 0.12
		Diethylstilbestrol	
i.v.	0.5, 2, 5, 10, 60, and 120 min	307 ± 17	
s.c.	1 day	308 ± 15.5	1.15 ± 0.20
s.c.	5 days	136 ± 10.5	1.28 ± 0.14
		Progesterone	
i.v.	0.5; 2, 5, 10, 60, and 120 min	307 ± 17	
s.c.	3 hours	473 ± 62	
s.c.	6 hours	785 ± 34	2.96 ± 0.65
s.c.	10 hours	926 ± 40	
s.c.	24 hours	1055 ± 150	2.73 ± 0.40
s.c.	48 hours	761 ± 68	