primarily DDT. Our data for the Bermuda petrel are entirely consistent with this pattern.

Observations of aggressive behavior, increased nervousness, chipped eggshells, increased egg-breakage, and egg-eating by parent birds of several of the above species (*3*, *6*, *13*) suggest symptoms of a hormonal disturbance or a calcium deficiency, or both. Moreover, DDT has been shown to delay ovulation and inhibit gonadal development in birds, probably by means of a hormonal mechanism, and low dosages of DDT or dieldrin in the diet of pigeons increased metabolism of steroid sex hormones by hepatic enzymes (*16*). A direct relation between DDT and calcium function has also been demonstrated, and these endocrine and calcium mechanisms could well be interrelated; DDT interferes with normal calcification of the arthropod nerve axon, causing hyperactivity of the nerve and producing symptoms similar to those resulting from calcium deficiency (*17*). Dogs treated with calcium gluconate are very resistant to DDT poisoning (*18*); female birds are more resistant than males (*19*), perhaps because of the calcium-mobilizing action of estrogenic hormones.

Of major importance, then, was the discovery that a significant ($P<.001$) and widespread decrease in calcium content of eggshells occurred between 1946 and 1950 in the peregrine falcon, golden eagle, and sparrowhawk, *Accipiter nisus* (*20*). This decrease correlates with the widespread introduction of DDT into the environment during those years, and further correlates with the onset of reduced reproduction and of the described symptoms of calcium deficiency. These multiple correlations indicate a high probability that the decline in reproduction of most or all of these birds, including *P. cahow*, is causally related to their contamination by DDT residues.

Other potential causes of the observed decline for the Bermuda petrel appear unlikely. The bird has been strictly protected and isolated since 1957, and it seems that human disturbance can be discounted. In such a small population, inbreeding could become important, but hatching failure is now consistent in pairs having earlier records of successful breeding, and deformed chicks are never observed. Furthermore, the effects of inbreeding would not be expected to increase at a time when the total population, and probably the gene pool, is still increasing. The population increase results from artificial protection since 1957 from other limiting factors, especially competition for nest sites with tropic birds (*21*).

It is very unlikely that the observed DDT residues in *P. cahow* were accumulated from Bermuda: the breeding grounds are confined to a few tiny, isolated, and uninhabited islets never treated with DDT, and the bird's feeding habits are wholly pelagic. Thus the presence of DDT residues in all samples can lead only to the conclusion that this oceanic food chain, presumably including the plankton, is contaminated. This conclusion is supported by reported analyses showing residues in related seabirds including two species of shearwaters from the Pacific (*22*); seabird eggs (*9*, *22*); freshwater, estuarine, and coastal plankton (*2*, *8*, *23*); plankton-feeding organisms (*2*, *8*, *9*, *22*, *23*); and other marine animals from various parts of the world (*8*, *22*). These toxic chemicals are apparently very widespread within oceanic organisms (*8*, *22*), and the evidence suggests that their ecological effects are important.

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Larval-Pupal Transformation: Control by Juvenile Hormone

Abstract. *Purified extract of juvenile hormone from the cecropia silkmoth prevents the transformation of larva to pupa in* Galleria. *The injected extract acts independently of the insect's own corpora allata. A morphological response occurs only when juvenile hormone remains in the body from the time of injection until the end of the period in which any cells are still sensitive to the hormone. The extent of the effect depends upon the age at which the larva receives the injection. The maximum effect (perfect superlarva) is produced when the extract is provided not later than in the first third of the instar.*

Juvenile hormone (JH), which is secreted by the corpora allata, controls differentiation during insect development (*1*). The titer of the hormone is high during larval stages and low during metamorphosis. Metamorphosis can be totally or partially prevented by implanting extra corpora allata; this results in the development of giant, but morphologically perfect, larvae (superlarvae) or intermediate forms having both larval and adult characteristics.

Similar effects have been demonstrated with lipoid extracts prepared from adult males of *Hyalophora cecropia* (L.) and related silkmoths, which are rich sources of JH (*2*). No particular difficulties have been encountered in impeding adult development in Hemimetabola or the pupal-adult transformation in Holometabola (*2*, *3*). On the other hand, attempts to suppress the larval-pupal transformation of holometabolous insects by the extract have failed (*3*) except in one series of experiments reported recently (*4*). In that study, however, pupal development was never completely prevented, and the findings were difficult to interpret because the insects were not deprived of their own corpora allata.

We examined the effects of the extract on the larval-pupal transformation in three aspects: (i) the role of the host's own corpora allata, (ii) the developmental stage of the host, and (iii) the dose of hormone applied. The study was made possible by the availability of considerably purified cecropia extract. In the past few years cecropia oil has been purified (*5*), nearly pure hormone has been isolated (*6*), and its chemical structure has been reported (*7*). In our experiments, hormone extracts having activities of 5×10^2 or 2×10^4 units per milligram were diluted with peanut oil to solutions containing 50 or 100 units per microliter [1 JH unit corresponds to 1 mg of standard crude cecropia oil and represents approximately 3 ng of pure JH (*6*)].

Waxmoths, *Galleria mellonella* L., were used since the effects of corpora allata implantation on the larval-pupal transformation in this species have been thoroughly studied (*8*). Thus we may compare the action of the JH extract with that of the implanted glands. Newly molted last-instar larvae were segregated from a stock culture, kept at 29°C, and fed on a modified Haydak medium (*9*). Only the larger larvae, which are usually females, were selected; this reduced variation in instar duration to 6 hours (*10*). Some of the larvae were allatectomized 1 day after the last larval ecdysis (*11*), allowed to recover for another day at room temperature, and then returned to the incubator. Allatectomy delayed the onset of the larval-pupal transformation by 1 to 3 days. To compensate for this delay, allatectomized larvae were considered to be 2 days younger than the intact larvae which had molted to the last larval instar at the same time. Larvae of a particular age group first were anesthetized by being submerged in water for 5 minutes, and then each larva received 1 or 2 μl of JH solution. The solution was injected through a fine glass capillary inserted into a small incision on the dorsal part of the fifth abdominal segment. After the next ecdysis, we examined insects for larval and pupal characteristics to determine to what extent metamorphosis had been blocked. A modified Piepho (*8*) scale was used to assess the effects of JH injections.

The effects of single injections of JH extract into intact or allatectomized larvae of various ages are recorded in Table 1. The extract was equally active in both normal and allatectomized insects. Thus, any major contribution of JH released from the corpora allata of the host can be discounted. The activity of the injected solutions can be attributed to their JH content since, in control experiments (not recorded in the table), injections of up to 3 μl of pure peanut oil never altered the normal development.

Injections of up to 100 units of JH into larvae on days 7 or 8 of the last larval instar (1.5 or 0.5 days before pupal ecdysis) had no effect. Similar injections performed 1 or 2 days earlier in the instar produced pupal-like intermediate forms; although 100 units of JH caused more pronounced effects than 50 units, none of the animals had a larval appearance. When, however, the injections were advanced to day 4, the resulting individuals showed many larval features. Finally, after injection on day 3, some morphologically perfect superlarvae emerged. These superlarvae had the internal and external structures

Table 1. Effects of single injections of juvenile hormone into last instar *Galleria* larvae. In the case of allatectomized larvae, the 3rd day after ecdysis was taken as the beginning of the last larval instar in order to compensate for delay in the development of these insects. Scale for evaluating the effects of juvenile hormone on the larval-pupal transformation in *Galleria*: —, normal pupa; (+), pupa with a patch of larval cuticle at the site of injection (only regenerated epidermis affected); +, pupa with patches of larval cuticle on the abdomen, wings and appendages shortened; ++, intermediate between larval and pupal forms with pupal cuticle on most body surface but with large areas of larval cuticle on the middle abdominal segments; +++, intermediate with pupal cuticle restricted to head, various proportions of thorax, and few last abdominal segments, wings everted in form of small buds; ++++, almost perfect larva but appendages partly metamorphosed; +++++, morphologically perfect "superlarva." The number of days in parentheses concerns the nonaffected individuals.

Time of injection		50 units of JH injected				100 units of JH injected			
Developmental stage	Days of the last larval instar	Insects (No.)	Affected (No.)	Effect (scale)	Duration of instar (days)	Insects (No.)	Affected (No.)	Effect (scale)	Duration of instar (days)
				Normal larvae					
Newly molted larva	1	4	0	—	(10)	2	0	—	(10)
Feeding larva	3					6	1	+++++ 6	(11)
Feeding larva	4					2	2	+++ 7	
Feeding larva	5	5	2	+ 8	(10)	5	5	+to++ 8	
Larva at onset of spinning	6	2	2	(+)and+ 10		4	4	(+) to+ 8	
Larva on well-formed cocoon	7	6	0	—	(9)	6	0	—	(9)
Pharate pupa	8	2	0	—	(9)	3	0	—	(9)
				Allatectomized larvae					
Newly molted larva	1	1	0	—	(12)	2	0	—	(11)
Feeding larva	3	4	0	—	(10)	10	2	++++and +++++ 8	(12)
Feeding larva	5	3	0	—	(13)	5	2	++and++++ 8	(11)
Larva at onset of spinning	6	3	2	(+) 12	(14)	3	2	(+)and+ 10	(12)
Larva in well-formed cocoon	7	2	0	—	(9)	2	0	—	(9)
Pharate pupa	8	2	0	—	(9)	3	0	—	(9)

and the behavior of normal larvae. Superlarvae were produced by injection of 100 units of JH, which are equivalent to a fraction of about 2×10^{-6} of larval body weight. Injections performed before day 3 were ineffective, and perfect pupae developed.

During two periods of the last larval instar, namely before the 3rd and after the 6th day, a single injection of 100 JH units had no effect. The sensitivity to JH in these periods was further tested by the following two experiments. Four larvae were injected with 100 units of JH on day 2 and again on day 4. Three of them molted into perfect superlarvae; one molted into a normal pupa. Hence, injections given early in the instar are effective as long as enough hormone is administered. In the other experiment, three larvae were supplied with 300 units on day 7, and another three larvae received the same dose on day 8. All of them molted into morphologically normal pupae except in the wound region at the site of injection. In animals of the 7-day group, the epidermis regenerated and produced larval cuticle. In the 8-day group, the wound failed to heal and remained open.

Different regions of the epidermis and internal organs are sensitive to a moderate concentration of JH at various periods of the last larval instar (*8*). Our results demonstrate the importance of the time of injection in eliciting a particular morphological effect. Thus, if extract is provided not later than the 3rd day of the last larval instar, the overall body epidermis will produce larval cuticle at the next molt. By the 5th day, however, the thoracic epidermis is already determined for the secretion of pupal cuticle and no longer responds to a moderate dose of JH, whereas large areas of abdominal epidermis are still sensitive to the hormone and will secrete larval cuticle. All epidermal cells become committed to pupal development by the 7th day and can be affected by JH only under exceptional conditions.

Only relatively large quantities of JH provided early in the last larval instar induced the development of perfect superlarvae. If not enough hormone was given, normal pupae emerged. Whatever the dose administered at the beginning of the last larval instar, either superlarvae or pupae resulted, but never intermediate forms. Later in the instar, smaller doses of JH were sufficient to affect those cells which were still sensitive to the hormone, and various intermediate forms were produced (Table 1).

Thus, we conclude that although the extent of morphological response to JH depends on the developmental stage of the insect, the hormone, once it has been injected, must remain in the body until the period of sensitivity for the whole insect is over. As long as any cells are still sensitive to JH, no change caused by the hormone is stable. Hence, sufficient JH must be injected to maintain a minimal amount of hormone throughout the period in which all cells become committed to a particular differentiation and the character of the next molt is firmly established. The later in the instar the hormone is applied, the shorter is the time it is required. Therefore injections of smaller doses are effective as far as the still-sensitive tissues are concerned.

If the hormone disappears from the blood before the sensitive period is over, a normal larval-pupal transformation ensues. Any development of larval characteristics which may occur before the hormone disappears is apparently labile and easily reversible. Unless the larval cells are continuously exposed to JH, they invariably change to a pupal program. The pupal program is firm and can be altered only under extreme conditions such as regeneration or exposure to a very high JH concentration. Thus, in the second half of the instar, large amounts of JH cause a more pronounced effect than doses just adequate to persist throughout the sensitive period (*12*).

These conclusions are consistent with earlier investigations on the effect of implanted corpora allata on the larval-pupal transformation in *Galleria* (*8*) and, moreover, provide some indications of the secretory activity of the implanted glands. Implantation of corpora allata is equivalent in effect to the injection of an adequate amount of JH in a droplet of peanut oil into larvae of the same developmental stage; the glands evidently start to secrete the hormone promptly after transplantation. Since the glands are effective if implanted at any time in the last larval instar and can induce several extra larval molts, they must maintain the secretion throughout the instar.

We now can understand why injected JH extract or JH-like compounds, unlike implanted glands, rarely prevent the larval-pupal transformation in holometabolous insects although they regularly affect adult development of both hemi- and holometabolous insects. In the larval-adult transformation of Hemimetabola and the pupal-adult transformation of Holometabola, the cells are sensitive to JH usually at the beginning of the particular instar, and this period of determination is short. For instance, experiments with corpora allata implantation in *Galleria* have indicated that the determination of adult development takes place within 24 hours after larval-pupal ecdysis, and after this period the cells are insensitive to JH (*13*). Studies of the effects of a JH mimic on the bug *Pyrrhocoris apterus* have revealed that adult development is determined between 24 and 48 hours of the last larval instar (*14*). To suppress adult development in both *Galleria* and *Pyrrhocoris,* JH or JH mimics are required for a short period of time, and small amounts applied at the beginning of a particular instar cause detectable effects. On the other hand, in the larval-pupal transformation of *Galleria,* determination proceeds between the 3rd and 6th day; that is, over an extended period of time and mainly in the second half of the last larval instar. If an amount of JH just adequate to produce an effect on day 3 is administered on day 1 of the last larval instar, the hormone will have no effect at all because it is metabolized too soon. This finding undoubtedly accounts for some of the reported failures of JH extract to affect the larval-pupal transformation.

It seems likely that many of the lipoidal substances that mimic JH (*15*) act in a similar manner to the cecropia hormone. If such a substance is to be tested for its effect on the larval-pupal transformation, the most sensitive response can be expected when the substance is administered in the last third of the last larval instar since small amounts of JH are then effective. This was indeed found to be the case when mosquito larvae were exposed to a mixture of JH-like compounds (*16*). Similarly, 2 μl of pure dodecyl methyl ether produced a marked effect in *Galleria* when injected on day 5 of the last larval instar, but had no effect when supplied before the 4th day. It is obvious that the time of application must be considered in any proposal to use JH-like substances as insecticides, especially against those insects which are sensitive to JH over an extended period of their development.

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Respiratory Inhibition in Chlorella Produced by "Purified" Polyethylene Glycol 1540

Abstract. *Polyethylene glycol 1540, added to culture solution in amounts sufficient to reduce the water potential to −10 atmospheres, inhibited respiration in* Chlorella *much more than mannitol solutions at −10 atmospheres. This occurred despite the purification of the polyethylene glycol by passage through exchange columns. The toxic properties, which developed some time after purification, increased with time of storage of solutions of polyethylene glycol 1540 at room temperature.*

Polyethylene glycols (PG) are now used extensively as osmotic agents to expose plants to a water deficit (among others *1*). Heavy metals may be present in the commercial polyethylene glycol ("Carbowax"), and most workers purify the PG either by dialysis or by passage over ion-exchange resins.

We first passed polyethylene glycol 1540 through the exchange resins Amberlite IR45 and Zeo Karb 225. The purified PG contained less than 0.3 parts of aluminum and 0.002 parts of chromium and copper (determined after ashing the carbowax and expressed as a percentage of the dry weight) per million. Water potentials were determined by psychrometer measurements (*2*).

Chlorella pyrenoidosa was treated with aqueous solutions of this purified PG1540, and effects on glucose- and acetate-induced respirations were compared with those of mannitol solutions at the same water potentials. Three days after purification, PG gave the same inhibition as mannitol, but after 3 weeks of storage PG1540 depressed the glucose-induced respiration more than mannitol did, and these specific effects increased with time of storage of the PG stock solution at room temperature (Table 1). In previous experiments (*3*) only freshly purified PG1540 was used for comparison with mannitol. After 6 months of storage of the PG, solutions of −10 atm gave "glucose respiration" at 3 percent of the control rates, this being considerably below the usual rate of endogenous respiration. Endogenous respiration is usually about 15 to 20 percent of "glucose respiration" and is not inhibited by mannitol solutions of −10 atm (*3*).

Table 1. Effects of time of storage at room temperature on the toxicity of "purified" PG1540 solutions. Glucose-induced respiration of *Chlorella* is expressed as a percentage of rates in control and mannitol solutions (−0.4 and −10 atm, respectively). A period of 70 to 200 minutes after application of the glucose and osmotic solution is used, since at 70 minutes control rates are nearly optimum and all experiments lasted for at least 200 minutes. Mannitol rates were assessed from other experiments done during the same series of tests. Temperature 25°C. Similar results were obtained for two different samples of PG1540, purchased in the United Kingdom and in Australia, respectively.

"Glucose respiration"	Time of storage				
	3 days	3 weeks	3 months	6 months	14 months
Control	70	40	18	3.5	4
Mannitol	100	56	26	5	7

The toxic effect was not confined to "glucose respiration." In *Chlorella*, both acetate-induced respiration and photosynthesis were inhibited much more by PG1540 stored for some months, than by PG1540 which was freshly purified by passage through an exchange column. Similar results were obtained for endogenous respiration of potato disks (Table 2). This latter experiment also showed that storage at 1°C prevented the formation of the inhibitor.

Psychrometer readings showed that the water potentials of PG1540 stock solution do not change after their original preparation. However, psychrometer readings would not be sensitive enough to detect the breakdown of some of the PG1540 molecules, which might have caused the toxic effect on *Chlorella*.

Fourteen months after purification of the PG1540, a sample of this purified solution was again passed through exchange resins. Its effect at −10 atm was compared with that of mannitol and of PG1540 purified only once, that is, 14 months earlier. The PG1540 that had been stored for 14 months severely inhibits "glucose respiration" of *Chlorella* (Fig. 1). Renewed purification completely removes the inhibitor. At the same time, PG1540 was purified only once and then used within 3 days of purification. The "glucose respiration" in the presence of this PG was the same as that induced by mannitol.

The chemical composition of the inhibitor is unknown, though it is presumably an ionized organic compound. The inhibitor might develop only in PG1540; for example, we found no