identical to that in intact animals. Peptide-induced degeneration appeared to be all-or-none and occurred with a threshold of about 0.1 unit of eclosion hormone. Sensitivity of the muscles to eclosion hormone appears on the last day (day 17) (*11*) of adult development. The ISM of abdomens isolated on the day preceding eclosion (day 16) showed only the slow atrophy typical of isolated abdomens, even when ten times the threshold dose of eclosion hormone was injected.

20-Hydroxyecdysone prevents many of the terminal events of adult development in insects (*12*), including release of eclosion hormone (*13*). Dissection of animals given this steroid revealed little or no degeneration of the ISM. When developing adult moths were given infusions of 20-hydroxyecdysone (30 ng/g-hour) (*14*) starting 2 days before eclosion (day 15), none of the animals emerged, and the ISM, when examined 5 days later, had retained their previous weights (Fig. 1B). A single injection of 20-hydroxyecdysone (25 μg per animal) into normal animals on the day preceding eclosion (day 16) permitted eclosion at the normal time on the following day, but the ISM continued to atrophy slowly rather than to undergo the expected rapid dissolution. Since 20-hydroxyecdysone can prevent the release of eclosion hormone, animals that had been given 20-hydroxyecdysone on the day before eclosion were given a supramaximal dose of eclosion hormone (1 unit), either on the day of expected emergence or 1 day thereafter. In both instances, the ISM failed to show rapid degeneration (Fig. 2). Thus the decline in 20-hydroxyecdysone appears to be essential for the development of sensitivity to eclosion hormone by the muscles.

The finding that the slow atrophy of the muscles was prevented when 20-hydroxyecdysone was injected 2 days before eclosion (day 15), but not when it was injected 1 day before eclosion, also suggested that the slow atrophy may be initiated by a decline in the endogenous ecdysteroid level below some threshold. The ecdysteroid levels in the hemolymph (*15*) declined from a mean ± standard error (S.E.) of 356 ± 61 ng/ml 3 days before eclosion to 86 ± 8 ng/ml 1 day before eclosion. A value of 20-hydroxyecdysone between these two values may represent the threshold necessary to initiate atrophy.

These data suggest a complex endocrine regulation of the fate of the ISM in *A. polyphemus*; the muscles either display a wasting atrophy, with fibers becoming very thin and eventually losing contractility, or a programmed breakdown occurs, with rapid degeneration of muscle. Late in adult development the ISM are maintained by an elevated titer of 20-hydroxyecdysone, which acts as a trophic factor for the muscles; a decline in this steroid appears to cause the muscles to atrophy. Similar steroid-dependent muscle maintenance in the levator ani muscle of rats requires the presence of testosterone for normal function (*16*).

The normal decline in 20-hydroxyecdysone also serves to make the ISM competent to respond to eclosion hormone. When 20-hydroxyecdysone titers are high, either earlier in adult development or as a result of exogenous hormone injection, the muscles do not degenerate in response to eclosion hormone. Thus, steroid withdrawal is necessary for peptide action in this system. The mechanism of this regulation is not known, but may be similar to that in rat myometrium, where a sharp decline in the levels of circulating progesterone before parturition induces the production of oxytocin receptors (*17*). After the decline of ecdysteroids in the silkmoth, eclosion hormone triggers the programmed cell death of the ISM, which is characterized by an increase in the number of muscle lysosomes, loss of myofibrillar proteins, and involution of the fibers (*3*). Thus, a peptide and a steroid interact with the ISM to regulate the mode of cell death.

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11. Animals were staged according to distinct morphological markers [D. R. Walters, *J. Exp. Zool.* **174**, 441 (1970)]. Two days before emergence (day 15), animals have an ochre face and two distinct orange bands across the wings. On day 16, most of the pupal endocuticle has been degraded and the wings are very dark. On day 17, developing adults have a thin, crispy exocuticle and have resorbed their molting fluid.
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Jumping Chickens: Relevance to Hazard in Humans

Sanderson and Rogers have reported the results of a study on the behavioral effects in chickens given "environmentally relevant doses" of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and they postulated that humans would be three times more sensitive to this herbicide than chicks (*1*). The conclusions drawn from this study do not reflect a valid evaluation of the data because (i) the doses were not environmentally relevant for bird eggs, much less for humans, and (ii) chick embryo data are not a suitable model for safety assessment in humans and are not used by any government agencies for this purpose.

Recent studies by Lavy *et al.* (*2*) have shown that the maximum exposure to

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2,4,5-T likely to be encountered in humans is about 0.1 mg per kilogram of body weight, which is orders of magnitudes less than in the chicken studies. Furthermore, such exposure would be likely to occur only in applicators who are using knapsack sprayers and wearing contaminated clothing or in mixers or loaders who are not careful to avoid contact with the concentrate and spray solutions. Measurable exposure to 2,4,5-T is extremely unlikely in bystanders or the general population (*3*).

In the study of Sanderson and Rogers, the doses ranged from 7 to 53 mg of 2,4,5-T per kilogram of body weight injected into the yolk sac of fertile chicken eggs on day 8 or day 15 of incubation, and from 75 to 225 mg/kg injected intraperitoneally into chicks on day 2 after hatching. The 2,4,5-T contained 0.03 part per million of the highly toxic contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which is representative of the level in 2,4,5-T produced worldwide today.

"Environmental relevance" was based on an estimated dose of 22 mg/kg for an egg contaminated with 1.1 mg of 2,4,5-T over the entire shell surface (area, 50 cm^2), presumed to be equivalent to spraying an 80 percent 2,4,5-T product (derivative not specified) at a rate of 2.8 liter/ha. The latter is equivalent to 224 mg of 2,4,5-T (or derivative) per square meter of horizontal surface area, not the entire egg surface, and 2,4,5-T is generally applied as a 2 to 5 percent solution in oil, oil-water, or water emulsion. Injection of 2,4,5-T into the egg was also assumed to be equivalent to contamination of the shell surface. However, only a small fraction of the herbicide applied to the surface of an egg penetrates the shell and reaches the chick embryo (*4*).

Sanderson and Rogers concluded that humans would be at least three times as sensitive to 2,4,5-T exposure as rats or chickens. This conclusion was based on studies by Piper *et al.* (*5*) and Erne (*6*), although neither study implied such a relationship. What happens as a result of the injection of a large dose of a chemical into an isolated egg is not representative of what might happen in a human fetus connected to its mother's efficient detoxification system, particularly for a chemical such as 2,4,5-T which is rapidly excreted in the urine of humans (*7*). Even more questionable is their extrapolation of effects from the injection of a nearly lethal dose of 2,4,5-T into newly hatched chicks to the potential effects from low-level exposure in adult humans. Unfortunately, reports of such studies done under highly exaggerated conditions continue to add to the confusion surrounding the use of beneficial agricultural chemicals such as 2,4,5-T.

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Several aspects of Leng's technical comment require correction. We found that, for chicken eggs, the most sensitive period to 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) was day 15 of incubation, when the injection of as little as 7 mg per kilogram of body weight caused subsequent behavioral abnormalities (*1*). This amount does not cause marked morphological abnormalities and is well below the LD_{50} of 53 mg/kg (the dose lethal to 50 percent of the animals tested) [see (*1*)].

The question of the amount of 2,4,5-T absorbed through the eggshell is, of course, controversial. If we accept that only 30 to 50 percent of an egg may be covered by the spray and if we use the concentration recommended by the government of Victoria, Australia, for spraying blackberries, an egg would receive 7 to 11 mg/kg. Even if only some of this is absorbed, it is still uncomfortably close to the amounts that caused behavioral abnormalities in our sample. Leng does not allow for a safety factor. Attempts to extrapolate from these results to the calculation of the "no-effect" dose in humans are complicated by individual variations in sensitivity to 2,4,5-T [see our jumping data in (*1*)].

We used the acid form of 2,4,5-T, which is less toxic than its esters, usually used for spraying. Moreover, we gave only one dose to each egg. Repeated dosing may well be toxic at lower daily rates of administration. Leng quotes a maximum exposure to 2,4,5-T of 0.1 mg/kg for knapsack sprayers but fails to say that this value was calculated for one operation lasting 180 minutes. Most sprayers are repeatedly exposed and for longer periods. The maximum exposure measured by Lavy *et al.* (*2*) was actually 1.85 mg/kg per spraying operation, and this did not include spray inhalation, which is probably a more important route of exposure, particularly in the vicinity of aerial spraying.

We agree that other species must be tested with 2,4,5-T. We have in fact shown that the oral dosing of pregnant rats with as little as 3 or 6 mg/kg on day 8 of pregnancy causes behavioral abnormalities in the pups tested several months later (*3*). This dose is one-hundredth of the LD_{50} in this species and one-tenth of the lowest dose reported to cause physical deformities (*4*). Phenoxyacetic acids themselves appear to be able to cause these effects; we have found similar effects with 2,4-dichlorophenoxyacetic acid (2,4-D), which does not contain 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (*3*).

Our statement that humans are likely to be more sensitive than rats and chicks is based on deductions from the plasma half-life, which for an oral dose of 2,4,5-T of 5 mg/kg is 23.1 hours in humans and 4.7 hours in rats (*5*). The results for chicks appear to be similar to those for rats (*5*). We therefore maintain that 2,4,5-T (and 2,4-D) may present risks to brain development and function in humans and other species.

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