plants exposed to ozone occurred after 15 days and the number of flowers increased linearly but at about one-half the rate exhibited by the control plants.

After 16 days there were roughly four times as many fronds in the controls as in the exposed plants. There were about six times as many flowers in the untreated plants as in the exposed plants. By the 24th day, after 8 days of ozone-free growth for both plant groups the gap between frond numbers had dropped slightly but the ratio of flower number between control and treated plants remained at about six to one.

Among treated plants, frond size was slightly reduced, and fronds tended to separate more readily when mature. There was a slight degree of yellowing which was not visible in untreated plants but no bleaching or other gross injury ever appeared.

These profound changes in population development and floral production occurred in the presence of amounts of ozone often encountered in the ambient air of the eastern seaboard during summer days. These effects coupled with observations on inhibition of pollen germination and tube elongation (*6*) suggest that low amounts of photochemical oxidants in the air environment may exert a selective effect upon plant populations.

WILLIAM A. FEDER
FLORENCE SULLIVAN
Department of Environmental Sciences, University of Massachusetts, Waltham 02154

References and Notes

1. For review article see H. E. Heggestad [*Phytopathology* **58**, 1075 (1968)] or L. C. Erickson and R. T. Wedding [*Amer. J. Bot.* **43**, 32 (1956)].
2. R. L. Engle and W. H. Gabelman, *Proc. Amer. Soc. Hort. Sci.* **91**, 304 (1967); H. A. Menser, H. E. Heggestad, J. J. Grosso, *Phytopathology* **56**, 466 (1966); H. A. Menser, H. E. Heggestad, O. E. Street, *ibid.* **53**, 1304 (1963); O. C. Taylor and F. M. Eaton, *Plant Physiol.* **41**, 132 (1966); W. A. Feder and F. J. Campbell, *Phytopathology* **58**, 1038 (1968).
3. E. Landolt, *Ber. Schweiz. Bot. Ges.* **67**, 271 (1957).
4. S. H. Hutner, in W. E. Loomis, *Growth and Differentiation in Plants* (Iowa State College Press, Ames, 1953).
5. W. S. Hillman, *Nature* **181**, 1275 (1957).
6. W. A. Feder, *Science* **160**, 1122 (1968).
7. I thank B. Udin for technical assistance and W. S. Hillman for the culture of *Lemna perpusilla* #6746. Supported in part by contract PH 22-68-30 from the National Air Pollution Control Administration.

11 April 1969; revised 13 May 1969

Cellular Response to Ecdysterone in vitro

Abstract. *Organ cultures of hindgut from diapausing tobacco hornworm pupae,* Manduca sexta *(Johannson), produce an abundance of migrant cells around the original explant. With time-lapse cinematography, these cells were seen to move slowly and tended to aggregate. The addition of ecdysterone (β-ecdysone) to these cultures stimulated a large increase in individual cell movements and the eventual disruption of cell sheets.*

The hindgut of a diapausing lepidopteran pupa is a narrow tube lined by a simple epithelium that is, in turn, surrounded by a mass of ill-defined tissue, probably composed of larval muscle fibers, deteriorated cryptonephric Malpighian tubules and sheaths, and hemocytes. During adult development, this sheath disappears, and the rectal sac enlarges and fills with waste material. Only a thin muscularis remains around the adult rectum (*1*).

The sequence of morphogenetic events involved in normal adult rectal development (*1*) has been reproduced by injecting molting hormone into diapausing pupae. We have now studied this effect in vitro.

We used diapausing pupae of the tobacco hornworm, *Manduca sexta* (Johannson), which were obtained by exposing the eggs and larvae to a short day photoperiod with 12 hours of light and 12 hours of darkness. After anesthesia with carbon dioxide, the hindgut or posterior portion of the hindgut was removed, rinsed with three changes of culture medium containing a small amount of phenylthiourea to inhibit phenolases, and then placed in a Rose multipurpose culture chamber (*2*). The explanted organ was held against the glass coverslip by a strip of dialysis tubing (*3*). The assembled chamber was injected with Grace's insect tissue culture medium (Grand Island Biological) (*4*) as modified by Yunker *et al.* (*5*), and placed at 27°C; the medium was changed every 2 to 3 weeks.

Two days after they were explanted, single cells began to migrate from the hindguts onto the cover glass. This migration continued for several days or weeks, depending on the condition of the explant, and the migrating cells frequently formed into locally dense sheets around the explant. These migrant cells flattened against the cover glass and extended long cytoplasmic processes that tended to join and form reticuli or contiguous sheets of cells (Fig. 1). When viewed under time-lapse photomicrography, the cells moved with slow, gliding motions, and their cytoplasmic processes exhibited little peripheral activity. Examination of stained sections of explants cultured for several weeks showed that the hindgut epithelium was intact and appeared similar to that of normal diapausing pupae. However, the sheath of tissue normally surrounding the epithelium was considerably reduced in thickness, and the migrant cells appeared to originate from the sheath.

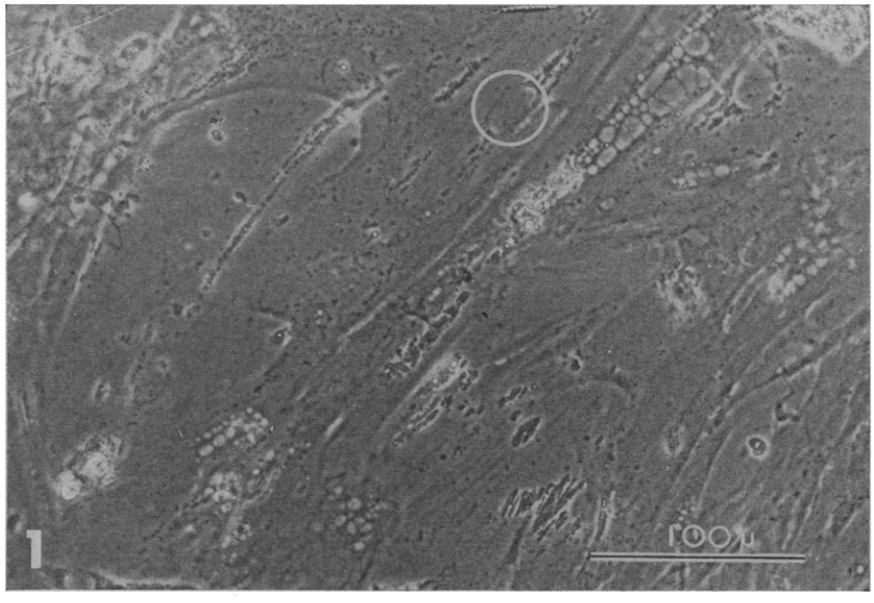
After several weeks in vitro, ten hindgut culture chambers were injected with ecdysterone (Mann Biochemical) dissolved in either sterile distilled water or methanol (nanograde, Mallinckrodt) (2 μg/μl); four control chambers received solvent alone. An additional series of four chambers was injected with 5 to 25 μg of 22-isoecdysone per milliliter (*6*), a compound with no known molt-stimulating properties in *Calliphora* (*7*). Tests on diapausing hornworm pupae whose brains had been removed showed that 22-isoecdysone was unable to induce development at concentrations up to 25 μg per gram of fresh body weight, but ecdysterone at a concentration of 1 μg/g stimulated adult development in similar pupae.

Positive responses in vitro were elicited by ecdysterone at concentrations of 2.5 to 25 μg per milliliter of culture medium as noted by a visible change in the behavior of the cells, whereas the controls remained unaffected.

The cultures were photographed continuously by time-lapse cinemicrography immediately before and for several days after injection. After a delay of from 8 to 20 hours after the addition of hormone, the normal, undulating movements of the membranes of the isolated migrant cells began to increase in speed and frequency, and the random movements of the cells over the coverslip became more rapid. This effect intensified for several hours and spread to the cells forming the dense sheets. Occasional connections sometimes formed between adjacent cells, but the general tendency was toward dissociation. Contiguous sheets of cells eventually dispersed into groups of "excited" single cells (Fig. 2) that retracted their elongate processes and tended to be-

come spherical. Highly excited cells extended and retracted short pseudopodia rather quickly and their peripheral cell membranes, particularly around the pseudopodia, exhibited small dentate projections. This increased membrane activity was often accompanied by the formation of pinocytotic vesicles in the cytoplasm. In hindgut cultures containing adherent segments of pupal fat body, the dense fat-filled cells comprising this tissue disaggregated and moved independently in the chamber. Enhanced cell activity persisted in some degree for several days and then gradually diminished to the original activity. No mitosis was ever observed in the migrant cells before or after hormone treatment.

Further experiments of this type were conducted with larval and pupal hemocytes from *M. sexta* and the Grace RLM-10 insect cell strain. In five test chambers, both the larval and pupal hemocytes responded to ecdysterone treatment with increased membrane activity and cell movement, whereas four controls injected with solvent showed no such response. The RLM-10 strain of cells showed no response to any of the hormone doses administered.



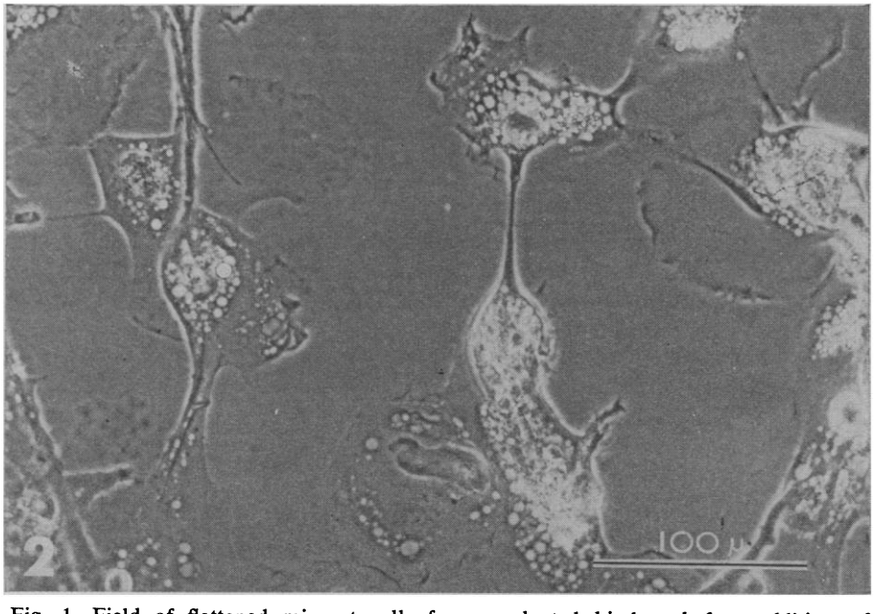


Fig. 1. Field of flattened migrant cells from explanted hindgut before addition of ecdysterone. Circle encloses one of the nuclei present in the field. Time in vitro, 35 days. Fig. 2. Same field as Fig. 1, 2 days after treatment with ecdysterone (12.5 μg per milliliter of culture medium). Time in vitro, 37 days.

The stimulation of migrant cells, fat body, and hemocytes in vitro may duplicate the response of these tissues in vivo to the elevated titer of molting hormone that terminates diapause and initiates adult development. The migrant cells observed in vitro bear strong resemblance to the sessile hemocytes that adhere to the surfaces of various internal organs during the pupal stage and which are stimulated to reenter circulation early in adult development (*8*).

Similarly, the dissociation of fat body cells in vitro appears to duplicate the condition in vivo of this tissue during early adult development. During the first week of adult hornworm development, I have observed the presence of large numbers of droplet-filled cells in the hemolymph. The fat body at this time is extremely friable and will partially dissociate, when teased with dissecting instruments, into individual droplet-filled cells identical to those in circulation. The dissociation of the pupal fat body in vivo and in vitro seems to be stimulated by molting hormones.

Also, the rectal epithelium in cultures treated with hormones undergoes considerable expansion and a degree of histological differentiation (*9*). This lends additional support to the contention that the effects obtained in vitro are related to normal in vivo development.

The total inactivity of 22-isoecdysone both in vivo and in vitro indicates that the target tissues for ecdysterone possess a strict hormone-specific mechanism involving the conformation of the 22-OH group.

KENNETH J. JUDY*
U.S. Agricultural Research Service,
State University Station,
Fargo, North Dakota 58102

References and Notes

1. K. J. Judy, thesis, Northwestern University (1968).
2. G. Rose, *Tex. Rep. Biol. Med.* **12**, 1074 (1964).
3. – → –, C. M. Pomerat, T. O. Schindler, J. B. Trunnell, *J. Biophys. Biochem. Cytol.* **4**, 761 (1958).
4. Mention of a proprietary product or company name does not necessarily imply endorsement by the U.S. Department of Agriculture.
5. C. E. Yunker, J. L. Vaughn, J. Cory, *Science* **115**, 1956 (1967).
6. 22-Isoecdysone was obtained through Dr. J. Siddall and the Zoecon Corp., Palo Alto, Calif.
7. A. Furlenmeier, A. Fürst, A. Langemann, G. Waldvogel, P. Hocks, U. Kerb, R. Wiechert, *Helv. Chim. Acta* **50** (8), 2387 (1967).
8. J. C. Jones, in *Physiology of Insecta*, M. Rockstein, Ed. (Academic Press, New York, 1964), vol. 3.
9. K. J. Judy, in preparation.

* Present address: Zoecon Corp., 975 California Ave., Palo Alto, Calif. 94304.

26 May 1969; revised 1 August 1969