lism. Several investigators (*18*) have previously demonstrated that progesterone can stimulate LH release in ovariectomized animals primed with estradiol. This LH release is associated with increased LHRH in mediobasal hypothalamus and can be linked to activation of a noradrenergic system of neurons (*19*). Our results confirm their data and show that in a 6-hour period progesterone increases LHRH concentrations in MBH-AHA-POA tissue and stimulates higher release of the hormone relative to tissue from animals in group 3. These three factors, LH, gonadal steroids, and the photoperiod might interact to set the appropriate conditions for neural processes triggering a complete and normal surge of LH.

V. D. RAMIREZ
D. DLUZEN
D. LIN

Department of Physiology and Biophysics, University of Illinois, Urbana 61801

References and Notes

1. J. W. Everett and C. H. Sawyer, *Endocrinology* **47**, 198 (1950).
2. J. A. Colombo, D. M. Baldwin, C. H. Sawyer, *Proc. Soc. Exp. Biol. Med.* **145**, 1125 (1974); V. D. Ramirez and C. H. Sawyer, *Endocrinology* **94**, 987 (1974); S. J. Legan and F. J. Karsch, *ibid.* **96**, 57 (1975).
3. L. Caligaris, J. J. Astrada, S. Taleisnik, *J. Endocrinol.* **55**, 97 (1972); W. F. Strauss and R. K. Meyer, *Science* **137**, 860 (1962).
4. J. T. Bradbury, *Endocrinology* **41**, 501 (1947); V. D. Ramirez and C. H. Sawyer, *ibid.* **76**, 1158 (1965).
5. C. E. McCormack and R. K. Meyer, *ibid.* **74**, 793 (1964); F. Döcke and G. Dörner, *J. Endocrinol* **36**, 209 (1966); L. Caligaris *et al.*, in (*3*); L. C. Krey, L. Tyrey, J. W. Everett, *Endocrinology* **93**, 385 (1973); K. Brown-Grant, *J. Endocrinol.* **62**, 319 (1974); I. Kraulis, H. Traikov, K. B. Ruf, F. Naftolin, *Endocrinology* **103**, 1829 (1978); D. R. Mann and C. A. Barraclough, *ibid.* **93**, 694 (1973); L. V. DePaolo and C. A. Barraclough, *Biol. Reprod.* **20**, 1173 (1979); R. L. Goodman, *Endocrinology* **102**, 142 (1978).
6. D. K. Sarkar, S. A. Chiappa, G. Fink, N. M. Sherwood, *Nature (London)* **264**, 461 (1976).
7. D. K. Sarkar and G. Fink, *J. Endocrinol.* **80**, 303 (1979).
8. We made two lateral cuts in the hypothalamic sulci, one anterior cut just anterior to the optic chiasm, and one posterior cut anterior to the mammillary bodies to a depth of about 1 to 2 mm, and then removed the MBH-AHA-POA, halved it at the midline, and placed it in Krebs-Ringer phosphate medium at 4°C. Histological examination of the remaining brain showed that the complete preoptic area region including the suprachiasmatic nuclei and the anterior hypothalamic area were removed together with the mediobasal hypothalamus. Six MBH-AHA-POA units per experiment were transferred to the bottom of a barrel of a 3-ml plastic syringe which constituted the superfusion chamber.
9. E. Gallardo and V. D. Ramirez, *Proc. Soc. Exp. Biol. Med.* **155**, 79 (1977); V. D. Ramirez, E. Gallardo, D. Hartter, *J. Endocrinol. Invest.*, in press.
10. S. L. Jeffcoate, H. M. Fraser, D. T. Holland, A. Gunn, *Acta Endocrinol.* **75**, 625 (1974); T. M. Nett, A. M. Akbar, G. D. Niswender, M. T. Hedlund, W. F. White, *J. Clin. Endocrinol. Metab.* **36**, 880 (1973). The LHRH antiserum (No. 48) was generously provided by T. Nett and used at a final dilution of 1:200,000. Sensitivity at 80 percent binding was 0.5 to 1 pg per tube.
11. Silastic capsules (Silastic medical grade tubing, 0.062 inch in inner diameter, 0.125 inch in outer diameter, and 10 mm in length) containing estradiol (235 μg/ml) in oil were incubated in physiological saline (37°C) for 24 hours before being implanted. Capsules were implanted immediately after ovariectomy in the dorsal neck region.
12. Data were analyzed by two separate two-way analyses of variance (time of day × hormone treatment). Fisher's least significance difference test was used for post hoc comparisons with $P < .05$ required for significance. Mann-Whitney U tests were used to evaluate the differences between a.m. and p.m. values for serum LH concentrations (Table 1).
13. In a separate control experiment, intact 30-day-old female rats were decapitated in the morning (0930 to 1030 hours), afternoon (1600 to 1700 hours), and night (2000 to 2100 hours) to determine LHRH concentrations in MBH-AHA-POA tissue before subjecting it to superfusion. The rats for these control experiments were kept under the same conditions as those used for the other experiments. The values (mean ± standard error, expressed in picograms per milligram; five animals per group) were 197.4 ± 36.8, 110.4 ± 30.2, and 131.5 ± 23.7, respectively. None of these values were significantly different, though LHRH concentrations tended to be lower in tissue from animals killed in the afternoon.
14. R. L. Eskay, R. S. Mical, J. C. Porter, *Endocrinology* **100**, 263 (1977).
15. S. R. Ojeda and V. D. Ramirez, *ibid.* **84**, 786 (1969).
16. M. A. Davis, F. Fraschini, L. Martini, *ibid.* **78**, 35 (1966); J. L. Turgeon and C. A. Barraclough, *ibid.* **98**, 639 (1976).
17. M. Molitch, M. Edmonds, E. E. Jones, W. D. Odell, *Am. J. Physiol.* **230**, 907 (1976); M. E. Molitch, M. Edmonds, J. C. Hendrick, P. Franchimont, W. D. Odell, *Neuroendocrinology* **29**, 49 (1979).
18. S. P. Kalra and S. M. McCann, *Endocrinology* **89**, 289 (1975); S. P. Kalra, P. S. Kalra, L. Krulich, C. P. Fawcett, S. M. McCann, *ibid.* **90**, 1168 (1972); L. Caligaris, J. J. Astrada, S. Taleisnik, *ibid.* **89**, 331 (1971); L. V. DePaolo and C. A. Barraclough, *Biol. Reprod.* **21**, 1015 (1979); M. Kawakami, J. Arito, Y. S. Visessuvan, T. Akema, *Endocrinology* **103**, 752 (1978).
19. S. P. Kalra, P. S. Kalra, C. L. Chen, J. A. Clemens, *Acta Endocrinol.* **89**, 1 (1978); J. W. Simpkins and S. P. Kalra, *Brain Res.* **170**, 475 (1979).
20. We thank G. Jackson and S. Carter-Porges for reviewing this manuscript. Supported by NSF-PCM 77-04656 to V.D.R. and by a fellowship from NIH (t32-HD 07028-0181) to D.E.D. We thank the National Institute of Arthritis, Metabolism, and Digestive Diseases and A. Parlow for the generous supply of rat RIA kits. Send reprint requests V.D.R.

7 January 1980; revised 21 February 1980

Red Cochineal Dye (Carminic Acid): Its Role in Nature

Abstract. *Carminic acid, the well-known red dyestuff from cochineal insects (*Dactylopius *spp.), is a potent feeding deterrent to ants. This deterrency may be indicative of the natural function of the compound, which may have evolved in cochineals as a chemical weapon against predation. The behavior of an unusual predator is described—the carnivorous caterpillar of a pyralid moth* (Laetilia coccidivora)*—which is undeterred by carminic acid and feeds on cochineals. The animal has the remarkable habit of utilizing the ingested carminic acid for defensive purposes of its own.*

Before aniline dyes came into use in the latter half of the 19th century, one of the most important red dyes in the textile industry was carminic acid (Fig. 1C), an anthraquinone extracted from scale insects (Coccidae) of the genus *Dactylopius*, the so-called cochineals (*1*). Although in commercial use, no biological function had been demonstrated for this substance (*1-3*). Other quinones, such as the benzoquinones and naphthoquinones discharged from the defensive glands of certain insects, millipeds, and opilionids (Fig. 1, A and B), are potent feeding deterrents to predators, including predaceous insects such as ants (*4*). Assuming that an anthraquinone might be similarly defensive, we undertook bioassays which showed that carminic acid is indeed potently deterrent to ants. The results of these tests are presented, together with an account of the behavior of a predaceous caterpillar that is undeterred by carminic acid and feeds on cochineals, and is able to put the ingested anthraquinone to defensive use of its own.

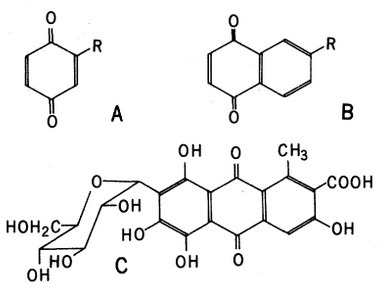

Fig. 1. (A) 1,4-Benzoquinones [for example, R = H, CH_3, C_2H_5 in defensive secretions of certain beetles (*25*)]. (B) 1,4-Naphthoquinones [for example, R = H, CH_3 in defensive secretion of an arachnid (*26*)]. (C) Carminic acid, the anthraquinone of cochineal insects.

Our observations were made on *Dactylopius confusus*, a cochineal species commonly found on prickly pear cacti (*Opuntia* spp.) in Florida. Aggregations of *Dactylopius* are conspicuously white (Fig. 2A), as a result of the fluffy investiture of waxy powder and silken threads that characteristically cloaks their bodies (*5*). This "wool" is thought to be defensive (*1*, *6*). Newborn cochineals and males (the only winged form) are devoid or nearly devoid of wool (*7*) and are distinctly red because of the carminic acid in their bodies (Fig. 2B). Cloaked individuals have similar red bodies, as is evident when they are plucked bare with forceps. Carminic acid is present in the blood and muscles of the immature insects and adults, as well as in the eggs and embryos within gravid females (*1*). The carminic acid content of reproduc-

0036-8075/80/0530-1039$00.50/0

tive females, males (both normal and with wings removed), and newborn of *D. confusus* was determined. Groups of 10 to 50 insects were extracted with methanol, and the carminic acid (*8*) was quantified by high-performance liquid chromatography (HPLC) (*9*). The values ranged from 1.5 to 3.0 percent (*10*).

The bioassays with ants (*Monomorium destructor*) (*11*) were intended to determine whether carminic acid is potentially defensive at the concentrations in which it occurs in *Dactylopius*. Feeding preference experiments were set up in which the ants were offered a choice between $10^{-1}M$ aqueous sucrose (a highly acceptable food) and sucrose solution with added carminic acid. The tests were carried out near a natural colony of the ant, at a location baited with honey droplets to which the ants had laid a network of foraging trails. For an individual test, a rectangular plastic plate with eight conical feeding depressions filled to the brim with test solution was placed in or beside a trail (Fig. 2C). Four depressions were filled with sucrose solution (controls), and the other four (experimentals), which alternated with the controls, were filled with sucrose solution containing carminic acid (*12*). Fifteen minutes were allowed for the ants to locate the depressions, after which counts were made, at 2-minute intervals for 20 minutes, of the total number of ants at the four control and four experimental depressions. Means were obtained for each set of 11 values (*13*), and the acceptability rating of the experimental solution, expressed as percent of control, was calculated [(mean of experimentals/mean of controls) × 100]. Tests were replicated five times for each of four carminic acid solutions in the range of 10^{-4} to $10^{-1}M$. At a concentration of $10^{-1}M$, which corresponds to a carminic acid content (about 5 percent) only slightly higher than that of cochineals, deterrence is essentially

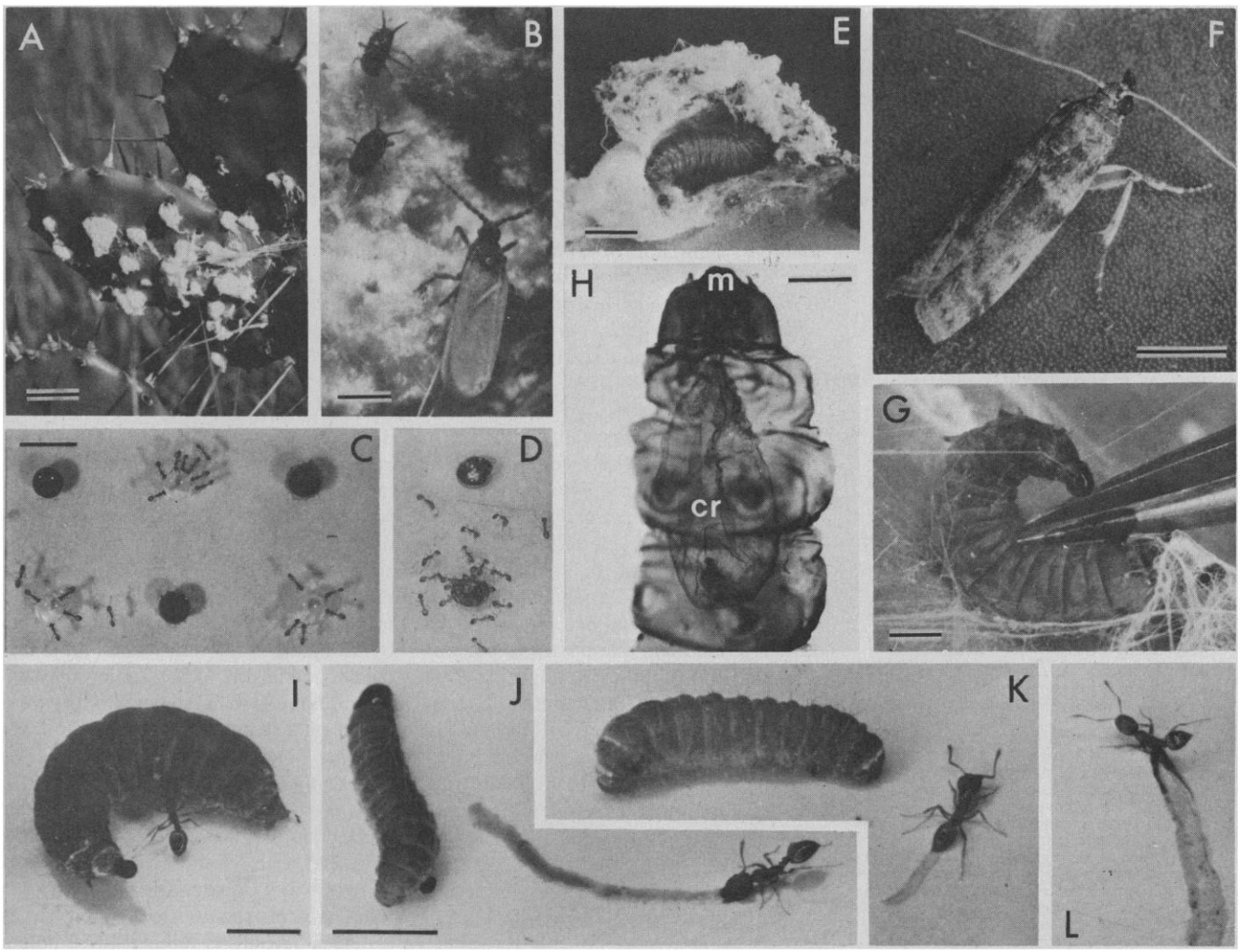

Fig. 2. (A) Prickly pear cactus (*Opuntia* sp.) infected with the cochineal scale insect *Dactylopius confusus*. The white "wool"-covered female cochineals are clearly seen. (B) Winged male cochineal and newborn young on surface of woolen investiture of the female parent. (C) Plastic plate with feeding depressions used in bioassays with ants (*Monomorium destructor*) (only six of eight depressions in the plate are shown); ants are seen drinking at the sites laden with control solution ($10^{-1}M$ sucrose) and ignoring the experimental sites ($10^{-1}M$ carminic acid in $10^{-1}M$ sucrose). (D) Detail of plastic plate similar to preceding; ants are feeding on the mealworm remains (control) and ignoring the upper depression baited with squashed female cochineals. (E) Mature larva of *Laetilia coccidivora*, exposed by lifting up the side of the cocoon it had spun in anticipation of pupation. (F) Freshly emerged adult of *L. coccidivora*. (G) Larva of *L. coccidivora* responding to pinching with forceps by rotating its front end and regurgitating a droplet of fluid upon the forceps; the forceps are already wetted from a previous drop emitted by the larva. (H) Front end of larva (treated by prolonged immersion in aqueous potassium hydroxide and consisting of cuticular portions only) showing the capacious crop (*cr*) that leads inward from the mouth (*m*). (I) Larva, under attack by ant (*Monomorium destructor*), revolving front end just prior to deposition of droplet of regurgitated fluid (shown) on the ant. (J) Ant that has been wetted by the larva shown, dragging its contaminated head as it backs away, leaving a conspicuous trail in its wake. (K and L) Comparable to preceding, showing alternative drag-cleaning behaviors of the ant. In (K), the ant is moving forward and dragging its wetted rear; in (L), it is moving sideways and dragging two contaminated legs. Reference bars: (A) 3 cm; (B and H) 0.5 mm; (C and E) 5 mm; (F, G, I, and J) 2 mm.

absolute (Fig. 3). In order to rule out the possibility that the ants had discriminated against the experimental solutions on the basis of their red color, we conducted several tests ($10^{-1}M$ carminic acid) in darkness, and monitored them only by brief intermittent illumination in red light [to which ants are reportedly insensitive (*14*)]. Carminic acid proved comparably deterrent under such conditions (*15*).

Feeding tests, similar to the above, in which one set of depressions was laden with body contents of freshly killed *Dactylopius* females (*16*) and the other (controls) with blood and tissues squeezed from decapitated mealworms (*17*), showed the *Dactylopius* themselves to be strictly unacceptable. Whereas the control depressions were always tightly encircled by feeding ants for as long as the bait lasted, the cochineal remains drew no more than fleeting attention from an occasional inspecting ant (Fig. 2D).

Exactly 100 years ago Comstock described a moth (*Laetilia coccidivora*) that is carnivorous as a larva (as lepidopteran larvae rarely are) and subsists exclusively on scale insects (*18*). While we were examining *Dactylopius* colonies, we found a number of these larvae feeding on their cochineal prey (Fig. 2E). These larvae readily consumed cochineals in the laboratory and grew to adulthood (Fig. 2F). Disturbance of the larvae, as by prodding or gentle pinching with forceps, showed a characteristic defensive behavior. When stimulated they revolved their front end and brought the mouth to bear on the affected region, at the same time emitting a droplet of fluid from the mouth. The responses were quick, and the offending instrument was always wetted by the liquid (Fig. 2G). The red color of the effluent suggested that it was of enteric origin and derived from ingested cochineals. Dissection showed that the anterior portion of the gut (the foregut) consisted of a capacious crop (Fig. 2H), replete with the liquified red remains of cochineals, and enveloped by compressor muscles. Contraction of these muscles, and simultaneous closure of the narrow junction (stomodaeal valve) by which the foregut communicates with the tubular next section of the gut (the midgut), could presumably effect the discharges. Carminic acid appears to be restricted largely to the gut of the larva. Other internal organs are white or at most faintly pink in appearance.

The larval discharge contained chemically unaltered carminic acid at potentially defensive concentrations. Individual larvae, medium-sized to full-grown (6 to 24 mg), were "milked" to depletion by pinching them repeatedly with forceps and causing them to regurgitate directly on weighed pieces of filter paper held beside the tips of the forceps. The wetted papers were promptly weighed and extracted with a mixture of methanol and aqueous acetic acid, and the extract was analyzed for carminic acid content by HPLC (*9*). The collected effluent (2 to 7 percent of body weight) contained 2.2 to 3.3 percent carminic acid (mean = 2.7 ± 0.3; $N = 11$), a concentration slightly higher even than that in the cochineals themselves (*19*).

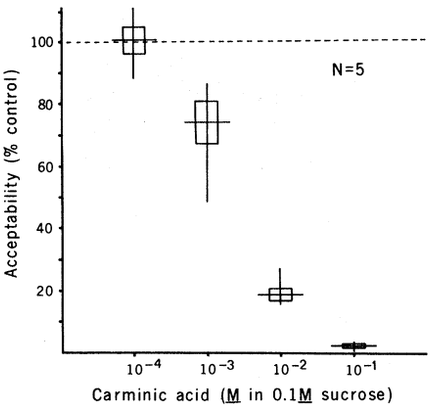

Fig. 3. Acceptability of carminic acid solution to ants (*Monomorium destructor*), expressed as percent of acceptability of control ($10^{-1}M$ sucrose). Horizontal lines indicate means, vertical lines give ranges, and bars indicate one standard error on each side of mean. Details in text.

Exposure to *Monomorium destructor*, one of many species of ants that could figure among *Laetilia*'s natural enemies, revealed that the larvae were well protected against such predators. Released singly near trails of the ants, they were soon encountered, and quickly subjected to mandibular biting and attempted stinging by individual ants. As soon as this occurred, a larva swung its front end toward the site attacked and regurgitated a droplet upon the ant (Fig. 2I). The latter released its hold and moved away, wiping the wetted regions of its body against the substrate and leaving a conspicuous red streak in its wake (Fig. 2, J to L). Larger droplets drenched the ant, weighing it down and hindering it mechanically by causing legs and antennae to stick temporarily to one another and to the substrate. But even a mere wetting of the mouthparts or of individual appendages sufficed to cause an ant to terminate its assault. Whether such lesser dosages wrought their effect chemically rather than mechanically, and specifically by the chemical deterrency of the carminic acid itself, cannot be said. In one context, however, the fluid did seem to act chemically. After an attack, a larva was always left visibly contaminated by residual fluid, both at the site of attack and on and around the mouthparts. Moreover, by writhing vigorously during an attack and rolling the body fully around one or more times, the larva (especially the younger ones) often succeeded in spreading the fluid over considerable areas of its body. On no occasion were ants seen to press their attacks upon such contaminated regions, whether these were wet or had already dried. They inspected the sites with their antennae in the usual fashion, but were always deterred on contact.

Given the proven defensive potential of carminic acid, and its apparent defensive use by *Laetilia*, it seems likely that the substance serves also for protection in cochineals and that it evolved in that capacity in these insects. Maximal benefit from possession of the substance is likely to be derived by the male and newborn cochineals, unprotected as these are by the coating of "wool." One wonders whether carminic acid might also be distasteful to predators other than ants, and in particular to visually oriented vertebrate predators. If so, the dye might also function, by virtue of its red color, as an aposematic deterrent. Although tests with vertebrates have not been done, it is perhaps significant that cochineals are said to be bitter to the taste in humans (*1*).

Laetilia is to be envisioned as an animal which, through evolutionary specialization, has managed to "crash" through the defensive chemical barrier of its host, while at the same time appropriating the weaponry for protective purposes of its own. Comparable cases could be cited, mostly of herbivores that utilize chemical deterrents from plants (*20*), but also of predators that obtain their defenses from animal prey (*21*). *Laetilia* is noteworthy because it exhibits the mode of employ of its acquired defense with such clarity. Defensive regurgitation is common in caterpillars (*22*), most of which are, of course, herbivorous. While it seems plausible that the oral fluid of such herbivores derives its effectiveness from plant metabolites, this remains to be shown.

Several anthraquinones, including kermesic acid (possibly the most ancient dyestuff on record), ceroalbolinic acid, erythrolaccin, 7-hydroxyemodin, deoxyerythrolaccin, and the laccaic acids (*3*, *23*) are known from other scale insects. It seems likely that these compounds are also defensive. Some, in fact, might also be used by *Laetilia*, which is not host

specific but feeds on a variety of scale insects. One wonders also whether a defensive role can be assigned to the many anthraquinones from plants (*3*). Interesting in this connection is the claim that anthraquinones in the heartwood of teak offer some protection against termites (*24*).

THOMAS EISNER
STEPHEN NOWICKI
Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853
MICHAEL GOETZ, JERROLD MEINWALD
Department of Chemistry, Cornell University

References and Notes

1. F. L. C. Baranyovits, *Endeavor (N. S.)* **2**, 85 (1979).
2. E. Lederer, *Biol. Rev.* **15**, 273 (1940).
3. R. H. Thomson, *Naturally Occurring Quinones* (Academic Press, New York, ed. 2, 1971).
4. R. J. Dean, thesis, Cornell University (1977); T. Eisner, *Nat. Hist.* **75**, 42 (1966); ——, A. F. Kluge, J. E. Carrel, J. Meinwald, *Science* **173**, 650 (1971).
5. A. C. Chibnall, A. L. Latner, E. J. Williams, C. A. Ayre, *Biochem. J.* **28**, 313 (1934); J. Meinwald, J. Smolanoff, A. C. Chibnall, T. Eisner, *J. Chem. Ecol.* **1**, 269 (1975).
6. G. H. Walter, unpublished data cited in D. P. Annecke and V. C. Moran, *J. Entomol. Soc. South Afr.* **41**, 161 (1978).
7. J. Mann, *U.S. Nat. Mus. Bull.* **256**, 1 (1969).
8. Initial identification of the carminic acid in the extract was by thin-layer chromatography (TLC), by HPLC, and by comparison with an authentic sample purified by droplet countercurrent chromatography (DDC). The chromatographic conditions were: TLC, cellulose F (butanol, acetic acid, water; 10:4:7) (10 percent acetic acid); HPLC, μ-Bondapak CN (methyl cyanide, water, acetic acid; 80:10:1; 1.3 ml/min; ultraviolet detection at 280 nm), μ-Bondapak C_{18} (methanol, water; 2:1; 0.8 ml/min; ultraviolet detection at 280 nm); DCC (Tokyo Rikakikai Co.) (chloroform, methanol, acetic acid, water; 7:12:1:8) (ascending mode).
9. The chromatographic conditions were as specified under HPLC in (*18*).
10. Mean individual weight and the carminic acid content (percent) were as follows: newborn (sample 1), 0.022 mg, 3.0 percent (N = 50); (sample 2), 0.016 mg, 2.6 percent (N = 50); male with wings, 0.059 mg, 1.8 percent (N = 10); male bodies only, 0.046 mg, 2.3 percent (N = 10); female, 3.446 mg, 1.5 percent (N = 15). The higher percent value in dealated as opposed to normal males reflects the fact, already visually apparent, that carminic acid is restricted to the bodies and absent from the wings of males.
11. *Monomorium destructor* co-occurs with *Dactylopius* in Florida and is a major insectivore.
12. The carminic acid (Pfaltz and Bauer, Inc.) was purified by Sephadex LH20 gel filtration (methanol) followed by recrystallization.
13. The values in any set varied little, due to the relatively constant turnover rate of the ants at the depressions.
14. R. Kretz, thesis, Zürich University (1977); R. Wehner and F. Toggweiler, *J. Comp. Physiol.* **77**, 239 (1972).
15. Further tests ($10^{-1}M$ carminic acid) in uninterrupted darkness for the duration of the experimental period revealed at the end of the period that only the control depressions had been partly depleted of fluid.
16. These females were denuded beforehand by plucking away their "wool" with forceps.
17. Mealworms are larvae of the beetle *Tenebrio molitor*. They are commonly used as acceptable items in palatability tests with insectivores.
18. J. H. Comstock, *North Am. Entomol.* **1**, 25 (1879).
19. The higher concentration of carminic acid in the larval effluent could be attributed to the apparent lack of absorption of the dye from the gut, which would result in its postingestive enteric buildup.
20. L. P. Brower, W. N. Ryerson, L. L. Coppinger, S. C. Glazier, *Science* **161**, 1349 (1968); L. P. Brower, P. B. McEvoy, K. L. Williamson, M. A. Flannery, *ibid.* **177**, 426 (1972); P. R. Ehrlich and P. H. Raven, *Evolution* **18**, 586 (1965); T. Eisner, J. S. Johnessee, J. Carrel, L. B. Hendry, J. Meinwald, *Science* **184**, 996 (1974); R. B. Kinnel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3576 (1979).
21. T. Eisner, K. Hicks, M. Eisner, D. S. Robson, *Science* **199**, 790 (1978); O. C. Glaser, *J. Exp. Zool.* **9**, 117 (1910).
22. T. Eisner, in *Chemical Ecology*, E. Sondheimer and J. B. Simeone, Eds. (Academic Press, New York, 1970), pp. 157–217.
23. J. Banks, *Va. Polytech. Inst. State Univ. Res. Div. Bull.* **127**, 51 (1977).
24. P. Rudman, *Chem. Ind. (N.Y.)* (1960), 1356; ——, E. W. B. DaCosta, F. J. Gay, A. H. Wetherly, *Nature (London)* **181**, 721 (1958).
25. W. R. Tschinkel, *J. Insect Physiol.* **21**, 753 (1975).
26. D. F. Wiemer, K. Hicks, J. Meinwald, T. Eisner, *Experientia* **34**, 969 (1978).
27. Supported in part by NIH grants AI 02908 and AI 12020, NSF grant PCM 77-25807, Hatch projects NY(C) 191405 and 191406, and a fellowship (to M.G.) from the Fonds National Suisse de la Recherche Scientifique. We thank J. Eidens, M. Eisner, R. Grant, and P. Haine for assistance; the staff of the Archbold Biological Station, Lake Placid, Florida, for hospitality during our stay in the field; and W. L. Brown, Jr., J. G. Franclemont, and D. R. Miller, for identifying respectively the ant, moth, and cochineal. Paper No. 65 in the series *Defense Mechanisms of Arthropods*.

11 December 1979; revised 15 February 1980

Regional Assignment of Genes for Human Esterase D and Retinoblastoma to Chromosome Band 13q14

Abstract. *The expression of human esterase D was evaluated quantitatively and qualitatively in five persons with partial deletions or duplications of chromosome 13. The results showed that the locus of this enzyme is at band 13q14. Deletion of this same band in other subjects has been found previously to indicate a predisposition to the development of retinoblastoma, which was present in the four individuals in this study who had partial deletions of chromosome 13. Because of this close synteny, esterase D evaluation should aid in the diagnosis and genetic counseling of retinoblastoma.*

Esterase D (E.C. 3.1.1.1) is an enzyme found in most human tissues but whose biological function is unknown. Its relative specificity for methylumbelliferyl esters as substrates and its electrophoretic mobility distinguish it from other esterases. Electrophoretic polymorphism for esterase D results from two common alleles, types 1 and 2. Somatic cell hybridization studies have assigned the genetic locus for this enzyme to chromosome 13 (*1*, *2*). With development of a quantitative assay for esterase D, we have been able to evaluate the regional assignment of this locus by analyzing both the qualitative and quantitative expression of this enzyme in five patients from unrelated families with structural abnormalities of chromosome 13 (Table 1); four of the patients have a specific eye tumor, retinoblastoma, associated with partial deletions of the long arm of one chromosome 13.

Chromosome analyses were carried out by standard techniques; family 4 was also studied by the high-resolution banding technique with amethopterin cell synchronization (*3*). Electrophoresis of esterase D was performed according to a modification of the method described by Hopkinson *et al.* (*4*). For esterase D activity, the fluorescent method described by Sparkes *et al.* (*5*) was used. Normal enzyme activities have been found for patients who have retinoblastoma and normal chromosomes; age-matched con-

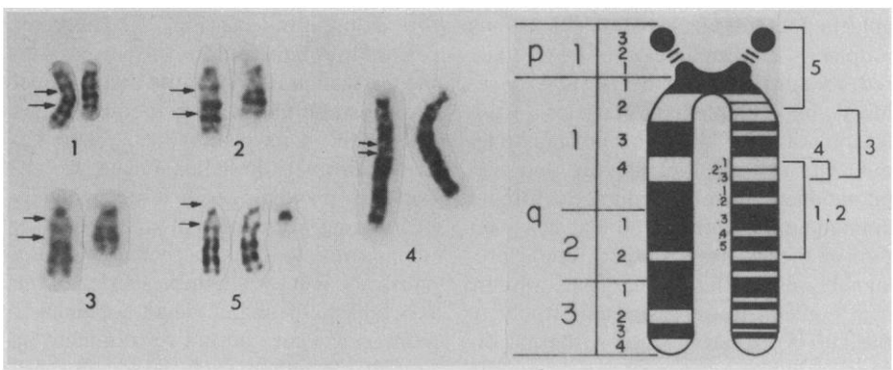

Fig. 1. The G-banded chromosomes 13 from the five probands. The number under each chromosome pair refers to the proband (Table 1). Arrows to the left of the normal chromosomes 13 identify the deleted or duplicated segment of the abnormal chromosomes. The diagram to the right summarizes these chromosome changes and shows the shortest region of overlap (middle of band q14).

0036-8075/80/0530-1042$00.50/0