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*).

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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 G. H. Walter, unpublished data cited in D. P. Annecke and V. C. Moran, *J. Entomol.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*.

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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-

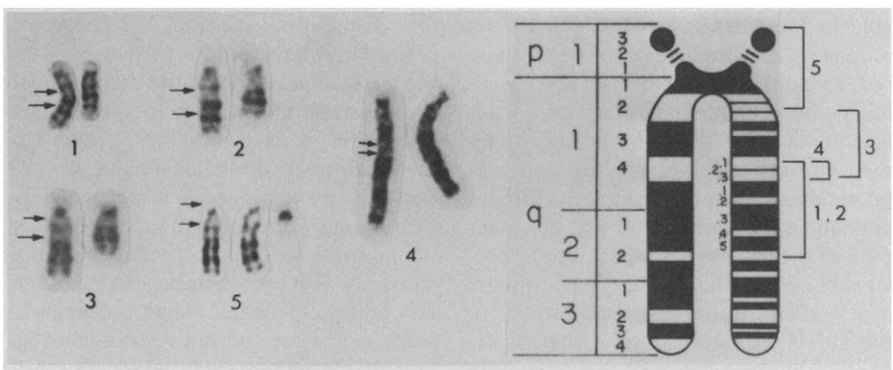

Fig. 1. The G-banded chromosomes 13 from the five probands. The number under each chromosome pair refers to the proband (Table 1). Arrows to the left of the normal chromosomes 13 identify the deleted or duplicated segment of the abnormal chromosomes. The diagram to the right summarizes these chromosome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

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trols for the patients described below; and for patients with another chromosome abnormality, trisomy 21. Esterase D activities in normal red blood cells are 68.9 ± 6.7 (1 standard deviation) units, and a range of 24.5 to 32.5 units has been found in six normal fibroblast cultures.

The proband of family 1, a female, has mild mental retardation, short stature, retinoblastoma, no dysplastic features, and a deletion of chromosome segment 13q14 → q22 (*6*) (Fig. 1). Her esterase D activity is approximately half the normal level (Table 1), which is compatible with hemizygous expression of the enzyme and assignment of its genetic locus to the deleted segment, 13q14 → q22.

The proband of family 2, a female, is developmentally mildly retarded; her developmental quotient at 9 months of age was estimated to be 75 to 80. Her only other abnormality was retinoblastoma of the left eye. She showed chromosome mosaicism of the type 46,XX/46,XX,del(13)(q14 → q22) in cultured lymphocytes, while the fibroblast culture demonstrated only the partial deletion 13 (Table 1). This proband showed heterozygous expression of esterase D in red blood cells (electrophoretic type 2-1) and the total esterase D activity was normal. In contrast, the fibroblasts showed half normal enzyme activity compared to a normal fibroblast culture and expression of only allele type 2, suggesting that an allele (type 1) has been lost in the chromosome deletion (q14 → q22) (Table 1).

The proband of family 3 is a male, whose defect was ascertained during a cytogenetic survey of patients with retinoblastoma (*7*); he is patient 3 in that report. When he was 4 months of age he was diagnosed as having a unilateral retinoblastoma, which was successfully treated. He is an attractive boy with short stature, frontal cranial prominence, a long face, large ears, epicanthal folds, a high arched palate, and an I.Q. estimated at 70. His chromosome analysis revealed a deletion of the q12 → q14 region of one chromosome 13. His esterase D activity is half normal which is compatible with the esterase locus being in the deleted part, q12 → q14.

Family 4 was previously described by Yunis and Ramsay (*8*). The proband, a female, has bilateral retinoblastoma and a small deletion of chromosome 13 which involves the middle of band q14(14.2). Since it was estimated that approximately half of band q14 is missing, the adjacent subbands q14.1 and q14.3 may be partially deleted also. Assay of esterase D (Table 1) shows the proband with half the activity found in the parents, who have normal activity. These findings suggest the esterase D locus is in the middle of band q14.

Table 1. Chromosome and esterase D findings in five families with chromosome 13 abnormalities. The esterase D activity in red blood cells (RBC) is expressed as 10^{-7} mole of methylumbelliferyl acetate metabolized per hour per gram of hemoglobin. Activity in fibroblasts is expressed as 10^{-7} mole of methylumbelliferyl acetate metabolized per hour per gram of protein.

Family	Relation	Chromosome findings	Esterase D		
			Tissue	Electrophoresis	Activity
1	Proband	46,XX,del(13)(q14→q22)	RBC	1-0*	33.4
	Mother	46,XX,inv(13)(q12→q22)	RBC	1-1	71.4
	Father	46,XY	RBC	1-1	64.5
2	Proband	46,XX,del(13)(q14→q22)†	Fibroblasts	2-0*	11.2‡
			RBC	2-1	59.5
	Mother	46,XX	RBC	2-1	60.9
	Father	46,XY	RBC	2-1	53.8
3	Proband	46,XY,del(13)(q12→q14)	RBC	1-0*	38.4
	Mother	46,XX	RBC	1-1	68.2
	Father	46,XY	RBC	2-1	67.3
4	Proband	46,XX,del(13)(q14)	RBC	1-0*	30.8
	Mother	46,XX	RBC	1-1	61.4
	Father	46,XY	RBC	1-1	62.1
5	Proband	47,XY,+13(pter→q12)	RBC	1-1	71.0
	Mother	46,XX,t(13;22)(q12;q13)	RBC	1-1	79.7

*The interpretation of the electrophoretic patterns as 1-0 or 2-0, rather than 1-1 or 2-2, is based upon reduced activity as seen by both electrophoresis and quantitative assay of the enzyme. See Fig. 2 for examples of the esterase D electrophoresis. †Peripheral blood lymphocyte culture showed 13 normal cells and 17 with the partial deletion 13q12→14. Twenty-six metaphases from cultured fibroblasts were analyzed and all of them showed the partial deletion. ‡Activity from a normal fibroblast culture grown and processed simultaneously gave an activity of 25.9 and electrophoretic type 2-1.

The proband of family 5, a male, has short stature, short neck, shield-shaped chest, and mental retardation. He has a partial duplication for the proximal portion of chromosome 13 (13pter → q12) resulting from a 13/22 translocation in the mother (*9*). The esterase D results are compatible with normal enzyme expression, while a patient with full trisomy 13 used as a control showed approximately 1.5 times the normal enzyme activity (*10*). These results suggest that the esterase D locus is not located in the 13pter → q12 region.

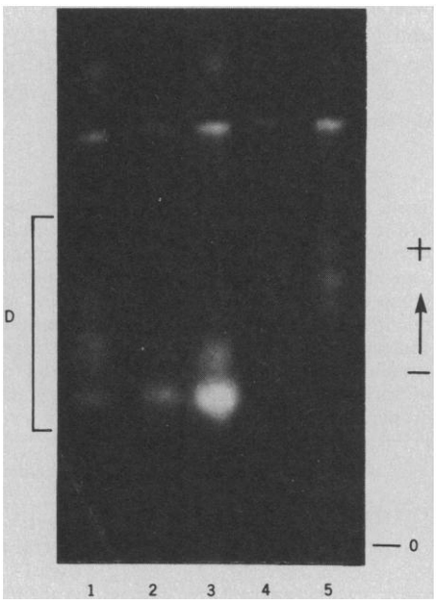

Fig. 2. Electrophoretic patterns of esterase D; *0* is the origin; *D* refers to the esterase D bands. The esterase D phenotypes for the channels are: 1, type 2-1; 2, type 1-0; 3, type 1-1; 4, type 2-0; and 5, type 2-2.

Taken together the results from these five families are mutually compatible and, on the basis of a gene dosage effect, suggest regional mapping of the esterase D locus to band 13q14 (Fig. 1 and Table 1).

Robson *et al.* (*11*) reported linkage analysis between esterase D and chromosome 13 short arm variants and between esterase D and the centromere of chromosome 13. They did not demonstrate close linkage and concluded that the esterase D locus is excluded from the proximal region of the long arm of chromosome 13, including "roughly the q1 region." Because band 13q14 is near the distal end of the q1 region and because their exclusion is not precise (*11*), our assignment of the esterase D locus to 13q14 may not be inconsistent with their findings.

Gray *et al.* (*12*) described heterozygous expression of esterase D in an individual with deletion of 13q21 → 13q31, thus excluding the locus from this region. Based upon the earlier findings of Robson *et al.* (*11*), Gray and colleagues (*12*) concluded that assignment to 13q32

or 13q33 seemed most likely. Turleau *et al.* (*13*) found heterozygous expression of esterase D in a patient with deletion of 13q33 and 13q34, which excludes the locus from this region. The results of these three earlier studies are not inconsistent with our assignment of the esterase D locus to band 13q14.

Retinoblastoma has served as the prototype model for hereditary human tumors. Thus, our finding that the esterase D locus maps in the same band (q14) that is responsible for predisposition to retinoblastoma (*7*, *8*) is important. Because esterase D is a polymorphic genetic marker, we now have the opportunity to determine if there is a single retinoblastoma mutation in the familial form and if the point mutations differ from the chromosome deletion form of retinoblastoma in the locus involved. If close linkage is demonstrated in the dominantly inherited cases, esterase D determination in appropriate families could be used for genetic counseling, probably including prenatal diagnosis, as well as for identification of persons at risk of developing retinoblastoma. This could allow an early diagnosis to be made and permit early institution of treatment.

The close synteny of the esterase D locus and retinoblastoma may also permit the presumptive identification of a chromosome deletion too small to be detected by cytogenetic techniques in some patients with retinoblastoma. This would be an interesting application of human gene mapping as an aid to chromosome analysis.

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References and Notes

1. V. van Heyningen, M. Bobrow, W. F. Bodmer, S. E. Gardiner, S. Povey, D. A. Hopkinson, *Ann. Hum. Genet.* **38**, 295 (1975).
2. S. Chen, R. P. Creagan, E. A. Nichols, F. H. Ruddle, *Birth Defects Orig. Artic. Ser.* **11** (No. 3), 99 (1975).
3. J. J. Yunis, J. R. Sawyer, D. W. Ball, *Chromosoma* **67**, 297 (1978).
4. D. A. Hopkinson, M. A. Mestriner, J. Cortner, H. Harris, *Ann. Hum. Genet.* **37**, 119 (1973).
5. R. S. Sparkes, S. Targum, E. Gershon, G. F. Sensabaugh, M. C. Sparkes, M. Crist, *Hum. Genet.* **46**, 319 (1979).
6. R. S. Sparkes, H. Muller, I. Klisak, J. A. Abram, *Science* **203**, 1027 (1979).
7. M. G. Wilson, A. J. Ebbin, J. W. Towner, W. H. Spencer, *Clin. Genet.* **12**, 1 (1977).
8. J. J. Yunis and N. Ramsay, *Am. J. Dis. Child.* **132**, 161 (1978).
9. S. J. Moedjono and R. S. Sparkes, *Hum. Genet.*, **50**, 241 (1979).
10. R. S. Sparkes, M. C. Sparkes, M. Crist, *ibid.* **52**, 179 (1979).
11. E. B. Robson *et al.*, *Birth Defects Orig. Artic. Ser.* **12** (No. 7), 351 (1976).
12. J. E. Gray, M. Bobrow, P. J. L. Cook, E. B. Robson, *Cytogenet. Cell Genet.* **22**, 487 (1978).
13. C. Turleau, J Seger, J. de Grouchy, F. Dore, J. C. Job, *Ann. Genet.* **21**, 189 (1978).
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Pro-Adrenocorticotropin/Endorphin-Derived Peptides: Coordinate Action on Adrenal Steroidogenesis

Abstract. *A synthetic peptide, representing a portion of the 16K (16,000 dalton)-fragment sequence within the pro-adrenocorticotropin/endorphin precursor molecule, potentiates the steroidogenic action of the 1 to 24 portion of adrenocorticotropin [ACTH(1-24)] on the rat adrenal cortex. The peptide has 27 amino acid residues and consists of γ-melanotropin with a carboxyl terminal extension. It affects both the inner and outer adrenocortical zones of hypophysectomized animals, as evidenced by a synergistic augmentation of corticosterone and aldosterone production, respectively. The peptide can be distinguished from adrenocorticotropin by its activation of cholesterol ester hydrolase and its failure to stimulate cholesterol side-chain cleavage.*

Evidence suggests that the hormonal products of the pituitary corticotroph cell are initially synthesized as parts of a common precursor prohormone—pro-adrenocorticotropin (ACTH)/endorphin (*1*). The amino terminal region of this precursor has been designated the 16K fragment (apparent molecular weight, 16,000) (*2*, *3*) and it includes a melanotropin (MSH)-like sequence (γ-MSH) which is partially homologous with the α- and β-MSH segments of the same prohormone (*4*). No physiological activity for either the 16K fragment itself or any derivative of it has been described.

A peptide consisting of γ-MSH with a carboxyl terminal extension of 15 additional residues has been synthesized (*5*) based on the amino acid sequence of pro-ACTH/endorphin from the intermediate lobe of bovine pituitary (*4*). We report that this peptide, denoted γ_3-MSH, stimulates the activity of adrenocortical cholesterol ester hydrolase and is capable of synergistically potentiating ACTH-stimulated corticosterone and aldosterone biosynthesis in the hypophysectomized rat.

Female Sprague-Dawley rats (Holtzman) weighing 160 to 180 g were individ-

Table 1. Response of rat adrenocortical glands to γ_3-MSH injected intravenously 7 minutes before the animals were killed. The experiments and serum steroid determinations were performed as described in the text and the legend to Fig. 1. The adrenal glands were rapidly enucleated in situ to eliminate zona glomerulosa and tissue, and the glands from animals in each group were then pooled. The mitochondrial and 105,000*g* supernatant fractions were prepared by differential centrifugation (*16*) for determination of cholesterol side-chain cleavage (*16*) and cholesterol ester hydrolase (*8*) activities, respectively. Protein was measured by the method of Bradford (*17*). Comparison of serum steroid results between groups was performed by analysis of variance; $N = 5$ for all groups. Enzyme activities were measured in triplicate or quadruplicate.

Group	Peptide given (ng)		Concentration in serum of		Enzyme activity (nmole/min-mg protein)†	
	ACTH(1-24)	γ_3-MSH	Corticosterone (μg/dl)*	Aldosterone (ng/dl)*	Cholesterol ester hydrolase	Cholesterol side-chain cleavage
A			2.2 ± 0.3	0.7 ± 0.2	1.54 ± 0.06	0.34 ± 0.24
B	10 (1 mU)		26.9 ± 2.8	10.5 ± 2.2	1.62 ± 0.11	1.58 ± 0.32
C		10	3.7 ± 1.0	2.8 ± 0.4	2.01 ± 0.15	0.41 ± 0.14
D	10	10	63.0 ± 1.7‡	66.6 ± 4.8‡	2.19 ± 0.07	3.05 ± 0.34

*Mean ± standard error. †Mean ± 2 standard deviations. ‡$P < .01$ compared with groups B and C.

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