pole. The available evidence of phenacodontid phylogeny suggests that both the earliest perissodactyls and *Phenacodus* evolved from the middle Paleocene phenacodontid genus *Tetraclaenodon* (*6, 7*). If that is correct, the endocast of *Phenacodus* indicates the maximum degree of neocortical expansion one would expect in the phenacodontid condylarth ancestor of perissodactyls. Thus, expansion of the neocortex may reflect one of the adaptations responsible for the emergence and early success of the order Perissodactyla.

The new *Hyracotherium* endocasts provide a baseline against which to compare later horse brain evolution. After *Hyracotherium*, the next good record is from endocasts of *Mesohippus*, a 30-million-year-old ancestor of modern horses (*8*). The brain of *Mesohippus* (Fig. 1D) had a more expanded neocortex than is seen in *Hyracotherium*, with the occipital and frontal lobes in more extensive contact with cerebellum and olfactory bulbs, respectively. The ectolateral and lateral sulci were longer in *Mesohippus*, but the greatest change appears to have been in the frontal lobe, which was considerably larger and more convoluted in *Mesohippus* than in *Hyracotherium*. Thus, in the first 20 to 30 million years of horse brain evolution, there was continued expansion of the neocortex, with the most marked increase in the area of the frontal lobe.

The *Hyracotherium* endocasts suggest a brain volume of about 25 cm^3, and associated skeletal materials suggest a body weight of about 9 kg (*9*). A useful index of relative brain size is the encephalization quotient, or E.Q., proposed by Jerison (*10*), which is the ratio of a given species' brain size compared to the brain size one would expect in an average living mammal of that species' body weight (*11*). The E.Q. of *Hyracotherium* was about 0.47, meaning that it had a brain about half the size one would expect in an average living mammal of the same body weight as *Hyracotherium*. For the condylarth *Phenacodus*, I estimate an E.Q. of 0.22; for *Mesohippus*, an E.Q. of 0.77; and for modern horses and zebras, E.Q.'s of 0.95 to 1.09 (*12*). In other words, the relative brain size of *Hyracotherium* was about twice that of the contemporaneous condylarth *Phenacodus*, but about half that of modern horses; and during the 20 to 30 million years between *Hyracotherium* and *Mesohippus*, relative brain size increased by about 65 percent.

The evolutionary trends toward increased amount of neocortex and increase in relative brain size are probably correlated (*13*), and are seen in many other groups of mammals besides horses (*10, 14*). The functional significance of those trends is unclear, and their elucidation remains one of the important unsolved problems in studies of mammalian brain evolution. Expansion of the frontal lobe, seen in evolution from *Hyracotherium* to *Mesohippus*, is a common trend in ungulate brain evolution (*15*). Extrapolation from cortical maps of living ungulates suggests that increased tactile sensitivity of the lips, reflected in expansion of lip region representation in somatic sensory cortex, was at least in part responsible for frontal lobe expansion in ungulates. Increased lip sensitivity, necessary for manipulation of vegetation prior to ingestion, would be expected in mammals specializing in browsing or grazing.

LEONARD RADINSKY

Anatomy Department,
University of Chicago,
Chicago, Illinois 60637

References and Notes

1. T. Edinger, *Mem. Geol. Soc. Am.* **25**, 19 (1948).
2. G. Simpson, *J. Linnean Soc.* **42**, 198 (1952). Simpson suggested that the most anterior of the orbital foramina preserved in YPM 11694, identified by Edinger as the optic foramen, was actually the ethmoid foramen. However, since the foramen in question lies caudal to the olfactory bulb, I believe Edinger was correct in identifying it as the optic foramen. In preserved morphology, including endocast gross morphology, YPM 11694 matches the condylarth *Meniscotherium* [described by C. L. Gazin, *Smithsonian Misc. Coll.* **149** (2), 36 (1965)]. Edinger (*1*) writes that YPM 11694 came from the Almagre facies of the San Juan Basin, N.M., but the stratigraphic datum on the original specimen label reads, "Almagre-Largo." *Meniscotherium* is rare in the Almagre facies, but common in the Largo facies.
3. American Museum of Natural History Nos. 55266, 55267, and 55268, from the Huerfano Formation early Eocene, Huerfano Basin, Colo.
4. L. Radinsky, in preparation.
5. W. Morris, *Los Angeles Co. Mus. Contrib. Sci.* **151**, 1 (1968); G. Jepsen and M. Woodburne, *Science* **164**, 543 (1969).
6. L. Radinsky, *Evolution* **20**, 408 (1966).
7. R. M. West, *Milwaukee Public Mus. Contrib. Biol. Geol.* **6**, 60 (1976).
8. T. Edinger, *Mem. Geol. Soc. Am.* **25**, 42 (1948).
9. Skull length averages 16.5 cm. Body length was about 4.7 times skull length [see skeleton figured in A. S. Romer, *Vertebrate Paleontology* (Univ. of Chicago Press, Chicago, 1966), p. 266], or about 77.6 cm. For 59 modern ungulate species, body weight (P, grams) relates to body length (L, centimeters), as: $P = 0.0027\,L^{3.45}$ [data from E. Walker, *Mammals of the World* (Johns Hopkins Press, Baltimore, 1964)].
10. H. Jerison, *Evolution of the Brain and Intelligence* (Academic Press, New York, 1973), p. 61.
11. The relationship of brain weight (E, grams) to body weight (P, grams) in a large sample of living mammals is described by the equation: $E = 0.12P^{0.67}$ (*10*).
12. Brain weight and body weight data for living perissodactyls is summarized by D. Kruska [*Z. Zool. Systematik Evolutionsforsch.* **11**, 81 (1973)].
13. K. Wirz, *Acta Anat.* **63**, 449 (1966); G. Sacher, in *The Primate Brain*, C. Noback and W. Montagna, Eds. (Appleton-Century-Crofts, New York, 1970), p. 278.
14. C. Dechaseaux, *Ann. Paléont.* **55**, 195 (1969); L. Radinsky, *Brain Behav. Evol.* **7**, 169 (1973); *ibid.* **11**, 214 (1975).
15. L. Radinsky, *Am. Zool.* **16**, 207 (1976); in preparation.
16. Supported by NSF grant BMS 74-17937. I thank Dr. M. McKenna for permission to work on AMNH specimens. A more detailed description and analysis of the endocasts of *Hyracotherium* and other early perissodactyls is in preparation.

15 July 1976

3,4,3′,4′-Tetrachloro Azoxybenzene and Azobenzene: Potent Inducers of Aryl Hydrocarbon Hydroxylase

Abstract. *Two unwanted contaminants, 3,4,3′,4′-tetrachloroazoxybenzene (TCAOB) and 3,4,3′,4′-tetrachloroazobenzene (TCAB), formed in the commercial synthesis of 3,4-dichloroaniline or of herbicides made from 3,4-dichloroaniline, were responsible for three outbreaks of acne among chemical workers. TCAOB and TCAB are approximately isosteric to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 2,3,7,8-tetrachlorodibenzofuran, two well-known contaminants that cause acne. All four of these agents are potent inducers of hepatic aryl hydrocarbon hydroxylase activity and compete for stereospecific binding sites in the hepatic cytosol, which are thought to be the receptor sites for the induction of this enzyme. Among the chlorinated azoxy and azobenzenes, the potency of a congener to induce aryl hydrocarbon hydroxylase activity correlates with its binding affinity for the hepatic cytosol specific binding sites and its capacity to induce acne; this relation between structure and activity parallels that observed for the chlorinated dibenzo-*p*-dioxins and dibenzofurans.*

3,4-Dichloroaniline is the starting material for the synthesis of a number of commercially important herbicides (acylphenylamides, phenylcarbamates, and phenylureas). During the synthesis of 3,4-dichloroaniline or its further conversion to herbicides, the conditions employed (heat and mild oxidation) promote the condensation of two molecules, so that 3,4,3′,4′-tetrachloroazoxybenzene (TCAOB), or 3,4,3′,4′-tetrachloroazobenzene (TCAB) are formed as unwanted contaminants. Several outbreaks of acne (chloracne) have occurred among workers in chemical plants manufacturing 3,4-dichloroaniline or its deriva-

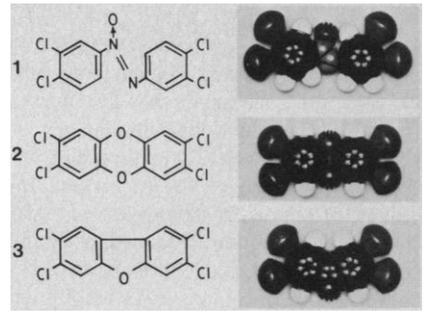

Fig. 1. The chemical structures and molecular models of (1) 3,4,3′,4′-tetrachloroazoxybenzene, (2) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, and (3) 2,3,7,8-tetrachlorodibenzofuran.

tives. The initial incident affected 41 workers in a small factory using *N*-(3,4-dichlorophenyl)-hydroxylamine, and the agent responsible for the acne was found to be TCAOB (*1*). Since that time, two additional outbreaks of acne, attributed to TCAB, have been recorded (*2*). The severe and persistent chloracne observed in these workers was characterized by epidermal cysts and often comedones, papules, and scarring on exposed and covered areas of the body (*1*), and was typical of the acne evoked by a number of chlorinated compounds (*3*).

Previous investigations have shown that several 3,4-dichloroacylanilide herbicides can be degraded in soil with the release of 3,4-dichloroaniline which can then be further transformed by soil fungi to TCAB and TCAOB (*4*). Despite awareness of the formation of these contaminants both in the manufacturing process and in the degradation of herbicides in soil, we are unaware of any previous reports on the toxicity of these compounds.

The chemical structure of TCAOB is shown in Fig. 1, together with a photograph of the space-filling molecular model. In the *trans* configuration, TCAOB and TCAB (not shown) can assume a planar conformation, and have a molecular shape similar to both 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDBF), two well-known acnegens encountered in the production of the herbicide 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) (*3*, *5*) and the PCB's (polychlorobiphenyls), respectively (*6*). We have shown (*7*) that TCDD and other halogenated dibenzo-*p*-dioxins and dibenzofurans are potent inducers of aryl hydrocarbon hydroxylase (AHH) activity, a microsomal monooxygenase activity which is mediated by cytochrome P-450 and which metabolizes foreign compounds, especially polycyclic aromatic hydrocarbons. The potency of various halogenated dibenzo-*p*-dioxins and dibenzofurans in inducing AHH activity closely parallels their toxic potency (including their potency to elicit acne) (*7*, *8*). The similarity of TCAOB and TCAB to TCDD and TCDBF prompted the present investigation.

Fertilized chicken eggs of 17 days gestation were injected with 25 μl of *p*-dioxane (control) or the same volume of solvent containing TCDD, TCDBF, TCAOB, or a combination of any two of these compounds. Twenty-four hours later the embryos were killed and their livers assayed for AHH activity as previously described (*7*). As shown in Table 1, all three compounds induce AHH activity to the same maximum response (approximately a tenfold increase), and a combination of maximally inducing doses of any two of these drugs produces no greater response than that elicited by any of the compounds administered alone. This suggests that the compounds act through a common mechanism.

Each compound was administered to chicken embryos and C57BL/6J mice at several dosage levels. The hepatic AHH activity was then assayed, and from the log-dose response curves generated, the dose which evoked half the maximum response (ED_{50}) was estimated. As shown in Table 2, all three compounds are nearly equipotent in the chicken embryo, having ED_{50}'s from 0.3 to 0.5 nmole/kg. While TCDD is only slightly less potent in the mouse than the chicken embryo, TCAOB is 18,000 times less potent, probably because it undergoes more rapid metabolic inactivation (*9*).

We have recently identified a macro-

Table 1. Comparison of the induction of hepatic AHH activity in the chick embryo by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, 2,3,7,8-tetrachlorodibenzofuran, and 3,4,3′,4′-tetrachloroazoxybenzene. Chicken embryos of 17 days gestation were injected with 25 μl of *p*-dioxane (control) or 25 μl of solvent containing TCDD (1.55×10^{-10} mole per egg), TCDBF (4.66×10^{-8} mole per egg), TCAOB (4.66×10^{-8} mole per egg), or a combination of two of these drugs. Twenty-four hours later the embryos were killed, and the livers from four identically treated embryos were pooled and assayed for AHH activity (*7*). The AHH activity is expressed as units of activity per milligram of liver weight; each value represents the mean ± standard error of four groups of pooled liver.

Treatment	AHH activity (unit/mg)	Percentage of maximum response*
Control (*p*-dioxane)	1.67 ± 0.11	0
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	16.40 ± 0.61	100.0
2,3,7,8-Tetrachlorodibenzofuran	16.50 ± 0.56	100.7
3,4,3′,4′-Tetrachloroazoxybenzene	16.22 ± 1.34	99.8
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin plus 2,3,7,8-tetrachlorodibenzofuran	17.27 ± 0.97	105.9
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin plus 3,4,3′,4′-tetrachloroazoxybenzene	15.90 ± 1.21	96.6
2,3,7,8-Tetrachlorodibenzofuran plus 3,4,3′,4′-tetrachloroazoxybenzene	14.09 ± 1.23	84.3

*The percentage of maximum response was calculated by subtracting the AHH activity of the control group from each of the experimental groups, equating the response of the TCDD-treated group to 100 percent, and expressing each of the other treatment groups as the percentage of the response produced by TCDD.

Table 2. Comparison of the potency of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, 2,3,7,8-tetrachlorodibenzofuran, and 3,4,3′,4′-tetrachloroazoxybenzene in inducing hepatic AHH activity in the chicken embryo and C57BL/6J mouse. Each compound was administered to 17-day chicken embryos at five or more dosage levels and hepatic AHH activity was assayed 24 hours later (see legend of Table 1). At each dosage level four groups of four pooled livers were assayed. At least two log-dose response curves were generated for each compound, the ED_{50} was estimated graphically for each curve, and then the mean of these estimated values was reported. To convert the dose to nanomoles per kilogram, it was assumed that the average weight of a chicken egg was 50 g. Each compound was also administered intraperitoneally to 6-week-old female C57BL/6J mice at five different dosage levels. The compounds were dissolved in *p*-dioxane, and control animals received only the solvent (0.4 ml/kg). Five mice were injected at each dose. Forty-eight hours later the animals were killed and their livers assayed for AHH activity (*13*). The ED_{50} for each compound was estimated graphically from the log-dose response curve.

Compound	ED_{50} for hepatic AHH induction (nmole/kg)	
	Chicken embryo	C57BL/6J mouse
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	0.31	0.9
2,3,7,8-Tetrachlorodibenzofuran	0.46	23
3,4,3′,4′-Tetrachloroazoxybenzene	0.45	8200

molecular species in the liver cytosol from C57BL/6J mice which appears to be the receptor for the induction of AHH activity (*10*). This hepatic cytosol species reversibly binds ^{3}H-labeled TCDD with a high affinity and, in vitro, the binding affinity of TCDD and its congeners correlates very well with their potency in vivo to induce AHH activity in the chicken embryo. If TCAOB and TCAB induce AHH activity by the same mechanism as TCDD, then these compounds should compete with TCDD for the stereospecific binding sites in the liver cytosol.

Hepatic cytosol (105,000*g* supernatant fraction) from C57BL/6J mice was incubated with [1,6-^{3}H]TCDD and varying concentrations of TCAOB, TCAB, and related compounds for 2 hours at 0°C. The unbound [^{3}H]TCDD was removed, the total amount of bound, labeled ligand was measured, and the results were corrected for nonspecific binding (see Fig. 2 for details). The specific binding of [^{3}H]TCDD as a percentage of control specific binding was plotted against the log of the concentration of the unlabeled ligand which was competing for binding sites. As shown in Fig. 2A, TCAOB, TCAB, and 3,4,3′,4′-tetrachlorohydrazobenzene all compete with [^{3}H]TCDD for specific binding sites, while azobenzene even at 1000 times the molar ratio of [^{3}H]TCDD fails to diminish the binding of the labeled ligand.

From such graphs of competitive binding, the equilibrium dissociation constants (K_d) for a series of these compounds was calculated (Fig. 3). The biological potency (ED_{50}) for each of these compounds was estimated from the log-dose response curves for the induction of hepatic AHH activity in the chicken embryo. As shown in Fig. 3, there is very good agreement between the binding affinity of these compounds in vitro and their biological potency in vivo. TCAOB is slightly more potent that both TCAB and 3,4,3′,4′-tetrachlorohydrazobenzene. 4,4′-Dichloroazoxybenzene is 20-fold less avid that TCAOB for hepatic cytosol specific binding, but four orders of magnitude less potent as an inducer of AHH activity. This discrepancy is at present unexplained but may arise from metabolic inactivation of the compound in vivo. Among the five congeners which fail to induce AHH activity and fail to compete for hepatic cytosol specific binding, the 3,5,3′,5′-tetrachloro compounds are of special interest because they differ from the biologically active 3,4,3′,4′-tetrachloro congeners only by the position of one chlorine atom in each ring. The compounds TCAOB, TCAB, TCDD, and TCDBF are approximately isosteric (Fig. 1), and all of them induce AHH activity and produce acne (*11, 12*). The compounds 3,5,3′,5′-tetrachloroazoxybenzene and 1,3,6,8-tetrachlorodibenzo-*p*-dioxin are also approximately isosteric, but neither of them induces AHH activity or produces acne. The evidence indicates that among the chlorinated azoxybenzenes, dibenzo-*p*-dioxins, and dibenzofurans, the capacity of any one of these compounds to induce AHH activity corresponds with its ability to elicit acne.

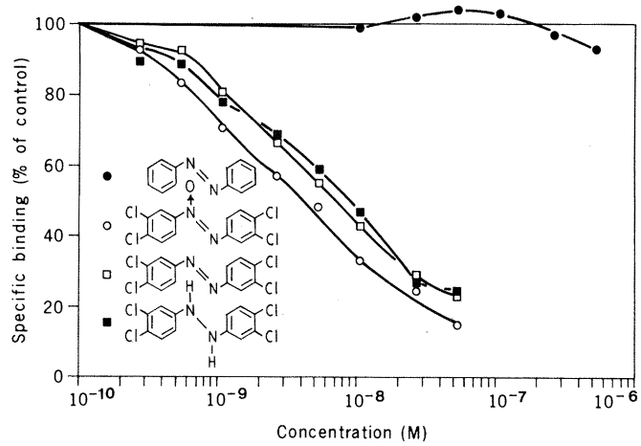

Fig. 2. The specific hepatic cytosol binding of TCAOB and congeners. The binding of unlabeled azoxy, azo, and hydrazo compounds was determined by their ability to compete with [^{3}H]TCDD for specific binding sites (*10*). The 105,000*g* supernatant fraction of liver from C57BL/6J mice was diluted to 2 mg of protein per milliliter with KTMD buffer, *p*H 7.5 (KCl, 150 m*M*; tris, 25 m*M*; $MgCl_2$, 5 m*M*; and dithiothreitol, 1 m*M*) and incubated with 0.54 n*M* [^{3}H]TCDD plus a 200-fold excess of unlabeled TCDBF, or with 0.54 n*M* [^{3}H]TCDD plus various concentrations of azoxy, azo, and hydrazo compounds for 2 hours at 0°C in a shaking water bath. One-half volume of a suspension of 3 percent charcoal and 0.03 percent dextran in KTMD buffer was added, the flasks were incubated for 5 minutes at 0°C, the mixture was centrifuged to sediment the charcoal, and a sample of the supernatant fraction was removed and counted by liquid scintillation spectrometry. The assays of total binding ([^{3}H]TCDD alone) and nonspecific binding ([^{3}H]TCDD plus a 200-fold excess of TCDBF) were performed in triplicate; all other assays were performed in duplicate. Each value was corrected for nonspecific binding and the data were plotted as the percentage of the control specific binding against the log of the competitor concentration.

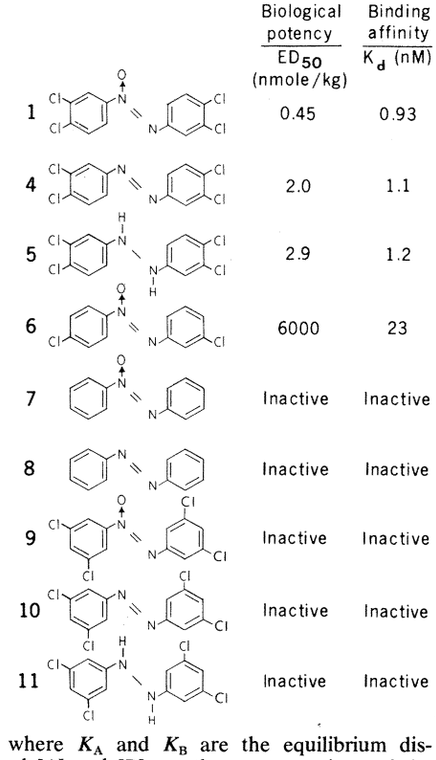

Fig. 3. A comparison of the biological potency (ED_{50}) and the binding affinity (K_d) of various azoxy, azo, and hydrazo benzenes. The compounds are as follows: (**1**) 3,4,3′,4′-tetrachloroazoxybenzene; (**4**) 3,4,3′,4′-tetrachloroazobenzene; (**5**) 3,4,3′,4′-tetrachlorohydrazobenzene; (**6**) 4,4-dichloroazoxybenzene; (**7**) azoxybenzene; (**8**) azobenzene; (**9**) 3,5,3,5-tetrachloroazoxybenzene; (**10**) 3,5,3,5-tetrachloroazobenzene; and (**11**) 3,5,3,5-tetrachlorohydrazobenzene. The ED_{50} of each compound was estimated from a log-dose response curve for the induction of hepatic AHH activity in the chicken embryo as described in Table 2. Compounds were considered inactive when they elicited less than 10 percent of the maximum response at a dose of 46.6 nmole/egg (0.93 μmole/kg). 4,4′-Dichloroazoxybenzene had extremely low potency; at the highest dose tested (9.3 μmole/kg) it evoked slightly more than half the maximum response. The affinity of these compounds for the TCDD-binding species in hepatic cytosol of C57BL/6J mice was determined by their capacity to compete with [^{3}H]TCDD specific binding as described in Fig. 2. Each active analog was tested in three separate experiments. The concentration of the active compound, which reduced the specific binding of [^{3}H]TCDD by one-half was determined graphically, and the mean of the log of these concentrations was calculated. The relative binding affinity of various compounds is related by the equation $K_A/K_B = [A]/[B]$ where K_A and K_B are the equilibrium dissociation constants of compounds A and B and [*A*] and [*B*] are the concentrations of the free drug which reduce the specific binding of [^{3}H]TCDD by the same amount (50 percent) (*10, 14–17*). The equilibrium dissociation constant of TCDD is 0.27 n*M*.

Note added in proof: A fourth chemical company, using *N*-(3,4-dichlorophenyl)-hydroxylamine, has reported an outbreak of chloracne involving more than 40 workers and is attributing the disease to TCAB.

ALAN POLAND
EDWARD GLOVER
Department of Pharmacology and Toxicology, University of Rochester School of Medicine, Rochester, New York 14642
ANDREW S. KENDE
MARK DECAMP
CHRISTEN M. GIANDOMENICO
Department of Chemistry, University of Rochester

References and Notes

1. J. S. Taylor, R. C. Wuthrich, K. M. Lloyd, A. Poland, *Arch. Dermatol.*, in press.
2. We learned of these industrial outbreaks of acne from Dr. James Taylor, Department of Dermatology, Cleveland Clinic, Cleveland, Ohio.
3. G. May, *Br. J. Ind. Med.* **30**, 276 (1973); N. E. Jensen, I. B. Sneddon, A. E. Walker, *Trans. St. John's Hosp. Dermatol. Soc.* **58**, 172 (1972); K. D. Crow, *ibid.* **56**, 77 (1970); R. D. Kimbrough, *Chem. Rubber Co. Crit. Rev. Toxicol.* **2**, 445 (1974).
4. R. Bartha and D. Pramer, *Science* **156**, 1617 (19 → R. Bartha, H. A. B. Linke, D. Pramer, *ibid.* **161**, 582 (1968); R. Bartha and D. Pramer, *Adv. Appl. Microbiol.* **13**, 317 (1970); H. Chisaka and D. C. Kearney, *J. Agric. Food Chem.* **18**, 854 (1970); L. M. Bordeleau, J. D. Rosen, R. Bartha, *ibid.* **20**, 573 (1972), D. D. Kaufman, J. R. Plimmer, J. Iwan, V. I. Kingebiel, *ibid.*, p. 916.
5. J. Kimmig and K. H. Schulz, *Dermatologica* **115**, 540 (1957); *Naturwissenschaften* **44**, 337 (1957); K. H. Schulz, *Arch. Klin. Exp. Dermatol.* **206**, 589 (1957).
→ J. G. Vos, J. H. Koeman, H. L. van der Maas, M. C. ten Noever de Brauw, R. H. de Vos, *Food Cosmet. Toxicol.* **8**, 625 (1970); A. Curley, V. W. Burse, R. W. Jennings, E. C. Villanueva, R. D. Kimbrough, *Bull. Environ. Contam. Toxicol.* **14**, 153 (1975); G. W. Bowes, M. J. Mulvihill, B. R. T. Simoneit, A. L. Burlingame, R. W. Risebrough, *Nature (London)* **256**, 305 (1975); G. W. Bowes, M. J. Mulvihill, M. R. DeCamp, A. S. Kende, *J. Agric. Food Chem.* **23**, 1222 (1975).
7. A. Poland and E. Glover, *Mol. Pharmacol.* **9**, 736 (1973).
8. B. A. Schwetz, J. M. Morris, G. L. Sparschu, V. K. Rowe, P. J. Gehring, J. L. Emerson, C. G. Gerbig, *Environ. Health Perspect. Exp. Issue No. 5* (1973), p. 87.
9. J. R. Gillette, in *Handbook of Experimental Pharmacology*, B. B. Brodie and J. R. Gillette, Eds. (Springer-Verlag, New York, 1971), vol. 28, part 2, pp. 349–361.
10. A. Poland, E. Glover, A. S. Kende, *J. Biol. Chem.* **251**, 4936 (1976).
11. The potency of TCAOB to elicit acne when applied to rabbits' ears is reported by Taylor *et al.* (*1*). The acnegenic potency and oral median lethal dose (LD_{50}) of TCAB were reported in a confidential communication from one of the chemical companies that manufactured 3,4-dichloroaniline. We have tested TCAOB, TCAB, 3,5,3′,5′-tetrachloroazoxybenzene and several chlorinated dibenzo-*p*-dioxins and dibenzofurans for their capacity to elicit experimental acne (*12*).
12. A. Poland and E. Glover, unpublished results.
13. ———, *Mol. Pharmacol.* **10**, 349 (1974).
14. Reduction of 3,4-dichloronitrobenzene with $LiAlH_4$ in ether (*15*) at 0°C led to the formation of 3,4,3′,4′-tetrachloroazoxybenzene (m.p. 139.5° to 140.5°C; known m.p. 139°C) (*16*). At 40°C this reduction yielded mainly 3,4,3′,4′-tetrachlorohydrazobenzene which on air oxidation (in ethanol, 70°C) was converted to the azo compound (m.p. 156° to 158.5°C; known m.p. 158°C) (*15*). Similar reductions of 3,5-dichloronitrobenzene yielded 3,5,3′,5′-tetrachloroazoxybenzene (m.p. 171° to 172°C) and 3,5,3′,5′-tetrachlorohydrazobenzene (m.p. 129° to 130°C), respectively. Zinc reduction of 3,5-dichloronitrobenzene produced 3,5,3′,5′-tetrachloroazobenzene (m.p. 193° to 194.5°C; known m.p. 194° to 194.5°C) (*17*). All the above compounds for which melting points are given had a purity of 95 percent or greater by thin-layer chromatography and mass spectroscopy and gave satisfactory mass spectra.
15. J. F. Corbett and P. F. Holt, *J. Chem. Soc.* **1963**, 2385 (1963).
16. R. Gaudy, K. F. Kirstead, *Can. J. Res.* **27B**, 890 (1949).
17. R. B. Carlin and W. O. Forshey, Jr., *J. Am. Chem. Soc.* **72**, 793 (1950).

18. Azobenzene, azoxybenzene, and 4,4′-dichloro azoxybenzene were purchased from Aldrich Chemical; 3,5,3′,5′-tetrachlorohydrazobenzene was purchased from ICN Pharmaceuticals. This study was supported in part by National Institute of Environmental Health Sciences grant 1-R01-ES-000965-03. A.P. is a recipient of a research career development award KO 4-ES-00017-01. M.D. was recipient of NIH research fellowship 1-FO-2-NS-55171.

10 May 1976; revised 12 July 1976

Endorphins: Profound Behavioral Effects in Rats Suggest New Etiological Factors in Mental Illness

Abstract. *The endogenous morphinomimetic brain peptides Met⁵-enkephalin and α-, β-, and γ-endorphins have been evaluated in rats after intracerebrospinal fluid injection. β-Endorphin produces marked, prolonged muscular rigidity and immobility similar to a catatonic state, counteracted by the opiate antagonist naloxone; this effect occurs at molar doses 1/100 to 1/400 that at which the other peptides or morphine block the response to painful stimuli. All peptides evoked dose-related, naloxone-reversible, wet-dog shakes in rats that had not been exposed to drugs. β-Endorphin produced hypothermia, whereas γ-endorphin produced hyperthermia. Such potent and divergent responses to naturally occurring substances suggest that alterations in their homeostatic regulation could have etiological significance in mental illness.*

Five endogenous peptides with morphine-like biological properties have now been isolated from brain and characterized chemically: Met⁵-enkephalin and Leu⁵-enkephalin by Hughes *et al.* (*1*, *2*) from whole brain extracts; and α-, β-, and γ-endorphins by Guillemin *et al.* (*3–5*) from extracts of hypothalamus-neurohypophysis. Met⁵-enkephalin is structurally identical (*2*) to the fragment of residues 61 to 65 of the pituitary hormone β-lipotropin (β-LPH) (*6*). α-Endorphin (*3*) is structurally identical to β-LPH-(61-76), γ-endorphin (*4*, *5*) is structurally identical to β-LPH-(61-77), and β-endorphin [see (*7*)] to β-LPH-(61-91) (*5*, *6*). The pharmacological properties of endorphins have so far been screened through application of tests in vitro or in vivo previously used to characterize opiate agonists and antagonists (*1–5*, *8–15*). Most (*1–5*, *8–11*, *13*, *15*), but not all (*12*, *14*) of these effects are counteracted by specific opiate antagonists.

Although early reports of the effects of the endorphins suggested their relative equipotency to enkephalin pentapeptides in classical opiate assays (*8*, *9*), much recent work indicates that β-endorphin is from 2 to 40 times more potent than Met⁵-enkephalin in opiate displacement assays (*4*, *15*) and in analgesia assays (*10*), and 4 to 5 times more potent than Met⁵-enkephalin in the guinea pig ileum assay (*5*). We now report that endorphins affect several behavioral and physiological measures in addition to responses to noxious agents and that each of the peptides exhibits different dose-effect profiles on these measures: β-endorphin induces a marked catatonic state lasting for hours (Fig. 1) (*16*) at molar doses 1/100 that at which Met⁵-enkephalin transiently inhibits responses to

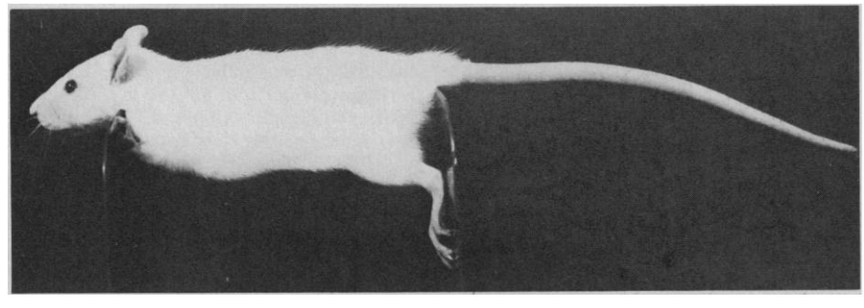

Fig. 1. Thirty minutes after the intracisternal injection of β-endorphin (14.9×10^{-9} mole) this rat exhibited sufficient rigid immobility to remain totally self-supporting when placed across metal bookends which are in contact only at the upper neck and base of the tail. Such postures were maintained for prolonged periods. Note the erect ears and tail, widely opened eyelids and extended lower limbs.