deficient enzyme activity with values of 0.4 as compared to 175 and 200 nmoles/mg per hour for two normal extracts. These extracts exhibited an increased apparent K_m for inosine of 770 μM as compared to the normal value of 22 μM. Therefore, the altered kinetic properties observed with the mutant enzyme were found in more than one cell type.

The discovery of a purine nucleoside phosphorylase with altered kinetic and physical properties in two patients with a disorder of cellular immunity is important for at least two reasons. First, an increase of the apparent K_m for inosine indicates that the enzyme will become more active if inosine concentrations increase. Such an elevation of inosine concentrations, by activating the mutant enzyme, could decrease the apparent block of nucleoside degradation and could account for the less severe clinical features in the two brothers studied (*2*). Elevated inosine in the concentration of plasma, presumably a reflection of intracellular inosine concentrations, has been observed in this disorder (*1, 2*). Second, this variant provides evidence for a structural alteration of the enzyme protein and implies that a structural gene mutation has resulted in purine nucleoside phosphorylase deficiency. These observations give the first direct evidence for genetic heterogeneity in this disease. Finally, this structural gene mutation supports the hypothesis that a block of purine nucleoside degradation is the cause of the immune disorder observed, rather than a consequence of a deletion of two closely linked genes. Such cause and effect relationship was initially suggested by the structural alterations of adenosine deaminase in severe, combined immunodeficiency (*6*). The two diseases are genetically distinct since adenosine deaminase and purine nucleoside phosphorylase are coded by different chromosomes (*7*). Thus the discovery of two inherited disorders of the immune system associated with enzyme deficiencies of purine catabolism provide important clues for a role of purine nucleoside degradation in the regulation of immune function.

IRVING H. FOX
CATHERINE M. ANDRES
Human Purine Research Center, Department of Internal Medicine and Biological Chemistry, University of Michigan Medical Center, Ann Arbor 48109
ERWIN W. GELFAND
DOUGLAS BIGGAR
Department of Immunology, Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X9

References and Notes

1. E. R. Giblett, A. J. Amman, R. Sandman, D. W. Wara, L. K. Diamond, *Lancet* **1975-I**, 1010 (1975); A. Cohen, D. Doyle, D. W. Martin, Jr., A. J. Amman, *N. Engl. J. Med.* **295**, 1449 (1976); S. K. Wadman, P. K. de Bree, A. H. Van Gennip, J. W. Stoop, B. J. M. Zegers, G. E. J. Staal, L. H. Siegenbeck, *J. Clin. Chem. Clin. Biochem.* **14**, 326 (1976).
2. N. L. Edwards, E. W. Gelfand, D. Biggar, I. H. Fox, in preparation; E. W. Gelfand, H. M. Dosch, I. H. Fox, in preparation.
3. K. C. Agarwal, R. P. Agarwal, J. D. Stoeckler, R. E. Parks Jr., *Biochemistry* **14**, 79 (1975).
4. B. K. Kim, S. Cha, R. E. Parks, Jr., *J. Biol. Chem.* **243**, 1771 (1968).
5. Isoelectric focusing is a technique that measures the charge on a molecule. Ampholytes are used to set up a *p*H gradient when an electrical current is applied. Proteins migrate in the *p*H gradient until they have no charge. The latter is the isoelectric *p*H. We used a previously described technique [I. H. Fox, I. L. Dwosh, P. J. Marchant, S. Lacroix, M. R. Moore, S. Omura, V. Wyhofsky, *J. Clin. Invest.* **56**, 1239 (1975)].
6. M. B. Van der Weyden, R. H. Buckley, W. N. Kelley, *Biochem. Biophys. Res. Commun.* **57**, 590 (1974); M. B. Van der Weyden and W. N. Kelley, *J. Clin. Chem. Clin. Biochem.* **14**, 326 (1976); P. P. Trotta, E. M. Smithwick, M. E. Balis, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 104 (1976); S. Chen, R. C. Scott, K. R. Swedberg, *Am. J. Hum. Genet.* **27**, 46 (1975); R. Hirschhorn, N. Beratis, F. S. Rosen, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 213 (1976); D. A. Carson, R. Goldblum, R. Keightley, J. E. Seegmiller, *J. Clin. Chem. Clin. Biochem.* **14**, 283 (1976); P. E. Daddona, M. B. Van der Weyden, W. N. Kelley, *Clin. Res.* **24**, 575A (1976).
7. Adenosine deaminase coding has been assigned to chromosome 20 [R. P. Creagan, J. A. Tischfield, E. A. Nichols, F. H. Ruddle, *Lancet* **1973-II**, 1449 (1973)], and coding for purine nucleoside phosphorylase has been assigned to chromosome 14 [F. Ricciuti and F. H. Ruddle, *Nature (London) New Biol.* **241**, 180 (1973); J. L. Hamerton *et al.*, *Cytogenet. Cell Genet.* **12**, 128 (1973); D. L. George and U. Francke, *Science* **194**, 851 (1976)].
8. We thank Dr. M. Buchwald for providing fibroblast cells from the deficient children, and J. Berry for performing the isoelectric focusing. Supported by PHS grants AM 19674 and 5M01 RR-42-14 and by the Medical Research Council of Canada grant MT-4875.

23 February 1977; revised 3 May 1977

A Specific High-Affinity Binding Macromolecule for 1,25-Dihydroxyvitamin D_3 in Fetal Bone

Abstract. *Cytosol fractions were prepared from fetal rat or embryonic chick calvaria and analyzed for binding of vitamin D_3 metabolites on sucrose density gradients. Both cytosol fractions contain a 3.5*S *macromolecule which specifically binds 1,25-dihydroxy-[^3H]vitamin D_3 and in addition, a 5 to 6*S *macromolecule which binds 25-hydroxy-[^3H]vitamin D_3. In rat calvaria cytosol, 1,25-dihydroxy-[^3H]vitamin D_3 also binds to the 5 to 6*S *macromolecule but appears to have greater affinity for the 3.5*S *component.*

Several observations have suggested that 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$], the metabolically active form of vitamin D_3, can be considered a hormone. 1,25-$(OH)_2D_3$ is produced in the kidney but acts in intestine and bone, and its synthesis is regulated by the need for calcium (*1*). Accordingly, evidence has been collected to demonstrate that 1,25-$(OH)_2D_3$ may stimulate calcium absorption in the intestine by a receptor protein-mediated mechanism. A 3.0 to

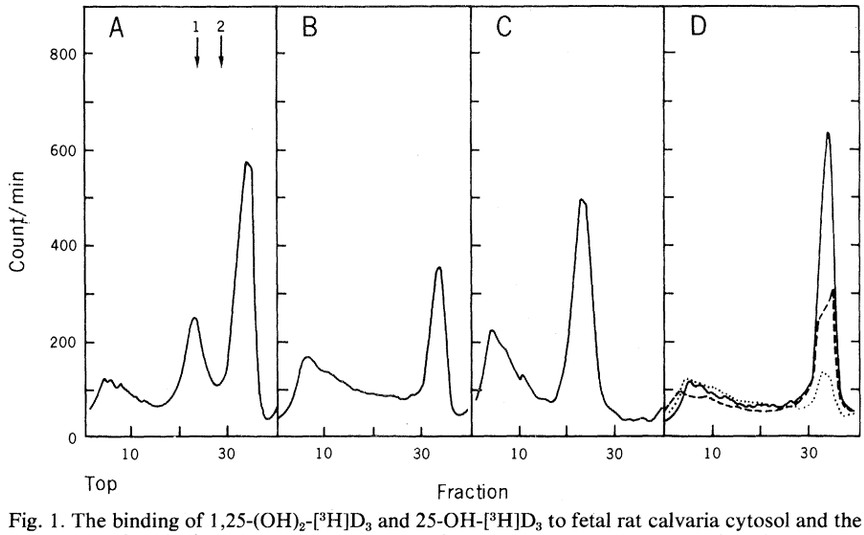

Fig. 1. The binding of 1,25-$(OH)_2$-[^{3}H]D_3 and 25-OH-[^{3}H]D_3 to fetal rat calvaria cytosol and the competition for binding. Cytosol was prepared from fetal rat calvaria as described in the text. Cytosol (2.7 mg protein) was incubated with vitamin D_3 metabolites and binding was analyzed on 4 to 20 percent sucrose gradients. (A) Binding of 0.25 n*M* 1,25-$(OH)_2$-[^{3}H]D_3 and competition by (B) 3 n*M* 1,25-$(OH)_2D_3$ and (C) 3 n*M* 25-OH-D_3. (D) Binding of 0.25 n*M* 25-OH-[^{3}H]D_3 (—) and competition by 3 n*M* 1,25-$(OH)_2D_3$ (---) or 3 n*M* 25-OH-D_3 (···). The arrows indicate the sedimentation of ovalbumin (1) and bovine serum albumin (2).

3.7*S* cytoplasmic protein having high affinity and low capacity for 1,25-$(OH)_2D_3$ has been demonstrated in chick intestine (*2, 3*). In vitro, the protein-hormone complex migrates from cytoplasm to nucleus (*4*) wherein it stimulates the synthesis of messenger RNA (mRNA) (*5*).

However, little is known about the process of bone mineral mobilization in which 1,25-$(OH)_2D_3$ has a direct role. It is clear that physiologic doses of 1,25-$(OH)_2D_3$ bring about the mobilization of mineral from bone (*6*). In vivo, this process requires the presence of parathyroid hormone (*7*), whereas in organ culture, 1,25-$(OH)_2D_3$ stimulates bone resorption in the absence of added parathyroid hormone (*8*). This mobilization of calcium from bone is blocked by prior administration of actinomycin D (*9*), suggesting that transcription is involved. Furthermore, in vitro, 1,25-$(OH)_2D_3$ accumulates in the nuclear fraction derived from bone homogenates (*10*), substantiating the idea that nuclear events are involved in 1,25-$(OH)_2D_3$–induced bone resorption. The present study demonstrates that cytosols prepared from fetal chick and rat bone homogenates contain a high-affinity binding protein for 1,25-$(OH)_2D_3$ similar to that observed in chick intestinal cytosol, suggesting that the action of 1,25-$(OH)_2D_3$ in bone may be mediated by an analogous receptor protein mechanism.

Pregnant rats (Holtzman) were obtained on days 19 or 20 of gestation. Chick embryos were obtained from Sunnyside Hatcheries (Oregon, Wis.) on day 18 of incubation. 25-OH-[23,24-^{3}H]D_3 (78 c/mmole) was synthesized by a modification of the method of Partridge *et al.* (*11*), and 1,25-$(OH)_2$-[23,24-^{3}H]D_3 (78 c/mmole) was synthesized enzymatically from 25-OH-[23,24-^{3}H]D_3 as described (*12*).

Fetal rat or chick calvaria were cleaned of blood and adherent connective tissue and rinsed in buffer (at 4°C) containing 0.3*M* potassium chloride, 0.5 m*M* dithiothreitol, 1.5 m*M* EDTA, and 10 m*M* tris-hydrochloride, *p*H 7.4. The calvaria were blotted and minced and then homogenized in three volumes (weight to volume) of the same buffer in a Polytron model PT-20 (Brinkmann Instruments). The homogenate was passed through cheesecloth and the filtrate was centrifuged at 106,500*g* for 1 hour to obtain a clear cytosol. Protein was determined by the method of Lowry *et al.* (*13*). Bone cytosol (0.3 to 1.0 ml) was incubated with 0.1 to 0.3 pmole of radioactive metabolite dissolved in 95 percent ethanol in the presence or absence of competing nonradioactive metabolite. After 1 hour at 4°C, 0.2 ml of incubation mixture was layered onto a linear 4 to 20 percent sucrose density gradient prepared by layering 0.71 ml each of 20, 16, 12, 8, and 4 percent sucrose in 10 m*M* tris-hydrochloride, 1.5 m*M* EDTA, and 0.3*M* potassium chloride into a Polyallomer tube and allowing the layers to diffuse at 4°C for 6 hours. The gradients were centrifuged for 18 hours at 257,000*g* in an SW60 rotor contained in a Beckman L5-50 ultracentrifuge. The gradients were fractionated from the bottom by displacement with mineral oil. Thirty-nine 0.1-ml fractions were collected in 4-dram vials and the bottom of each tube was cut off and counted as the 40th fraction. Radioactivity was determined in 4 ml of scintillation fluid containing 1:3 (by volume) Triton X-100 and toluene, 2,5-diphenyloxazole (PPO; 4 g/liter), 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]-benzene (POPOP; 50 mg/liter), and sufficient water to produce clarification.

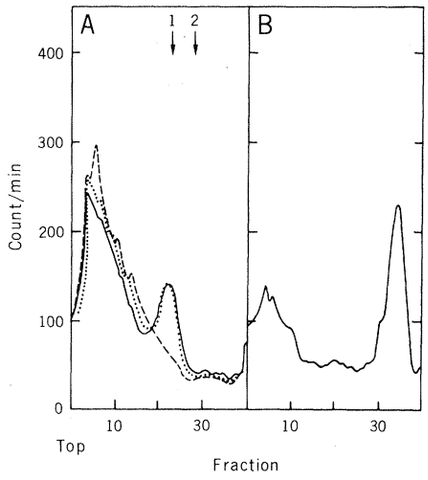

Fig. 2. The binding of 1,25-$(OH)_2$-[^{3}H]D_3 and 25-OH-[^{3}H]D_3 to fetal chick calvaria cytosol. Cytosol was prepared from fetal chick calvaria as described in the text. Cytosol (0.25 mg protein) was incubated with vitamin D_3 metabolites and binding was analyzed on 4 to 20 percent sucrose density gradients. (A) Binding of 0.48 n*M* 1,25-$(OH)_2$-[^{3}H]D_3 (—) and competition for binding by 5 n*M* 1,25-$(OH)_2D_3$ (---) or 5 n*M* 25-OH-D_3 (···). (B) Binding of 0.5 n*M* 25-OH-[^{3}H]D_3. The arrows indicate the position of sedimentation of ovalbumin (1) and bovine serum albumin (2).

The binding in vitro of 1,25-$(OH)_2$-[^{3}H]D_3 and 25-OH-[^{3}H]D_3 to cytosol prepared from fetal bone was examined by means of sucrose density gradient centrifugation. Experimental conditions which have been used successfully to detect and stabilize the 3.7*S* chick intestinal binding protein for 1,25-$(OH)_2D_3$ (*3*) were applied to the analysis of chick and rat bone cytosol. In addition, by using 1,25-$(OH)_2$-[^{3}H]D_3 and 25-OH-[^{3}H]D_3 with high specific activities (78 c/mmole), binding experiments could be performed with as little as 0.2 n*M* radioactive metabolite.

Cytosol prepared from fetal rat calvaria contained two binding macromolecules for 1,25-$(OH)_2$-[^{3}H]D_3 having sedimentation coefficients of 3.5 to 3.7*S* and 5 to 6*S* as determined by comparison with ovalbumin (3.7*S*) and bovine serum albumin (4.4*S*) (Fig. 1A). An excess of nonradioactive 1,25-$(OH)_2D_3$ totally displaced 1,25-$(OH)_2$-[^{3}H]D_3 bound in the 3.5*S* region and partially displaced 1,25-$(OH)_2$-[^{3}H]D_3 bound in the 7*S* region (Fig. 1B). In this experiment, unbound 1,25-$(OH)_2$-[^{3}H]D_3 was recovered in the mineral oil used to fractionate the gradient. Other experiments indicated that with increasing concentrations of 1,25-$(OH)_2D_3$, 3.5*S*-bound 1,25-$(OH)_2$-[^{3}H]D_3 was displaced onto the 6*S* macromolecule, and if sufficient nonradioactive 1,25-$(OH)_2D_3$ was added it was displaced into the "free" region. Nonradioactive 25-OH-D_3 was effective in displacing 6*S*-bound 1,25-$(OH)_2$-[^{3}H]D_3 onto the 3.5*S* macromolecule (Fig. 1C) indicating that the 3.5*S* macromolecule was not initially saturated with 1,25-$(OH)_2D_3$. Since 25-OH-[^{3}H]D_3 was bound exclusively to the 6*S* macromolecule and was not displaced to the 3.5*S* region by either 1,25-$(OH)_2D_3$ or 25-OH-D_3 (Fig. 1D), it appears that the 3.5*S* and 6*S* macromolecules are specific for 1,25-$(OH)_2D_3$, respectively.

The binding of 1,25-$(OH)_2$-[^{3}H]D_3 and 25-OH-[^{3}H]D_3 to cytosol prepared from embryonic chick calvaria was assessed by the same method. In contrast to rat calvaria cytosol, 1,25-$(OH)_2$-[^{3}H]D_3 bound to a single macromolecule having a sedimentation coefficient of about 3.5*S* (Fig. 2A). Nonradioactive 1,25-$(OH)_2D_3$ totally displaced 3.5*S*-bound 1,25-$(OH)_2$-[^{3}H]D_3 whereas the same molar excess of nonradioactive 25-OH-D_3 was ineffective (Fig. 2A), indicating specificity of this macromolecule for 1,25-$(OH)_2D_3$. As in rat calvaria cytosol, 25-OH-[^{3}H]D_3 bound exclusively to a 6*S* macromolecule. In both bone cytosol preparations, it was unlikely that the 3.5*S* macromolecules were a result of serum contamination since the serum transport proteins which bind 1,25-$(OH)_2D_3$ in rat and chick migrated differently from the 3.5*S* species, namely in the 4.0 to 4.1 regions on the sucrose gradients. It is, therefore, reasonable to suspect that the 3.5*S* protein is of intracellular origin and could represent receptor protein. It is known that 25-OH-D_3 will induce resorption of bone in culture (*9, 14, 15*), yet it did not bind to the 3.5*S* protein at the concentration of 25-OH-[^{3}H]D_3 used in

these experiments. Since 1000 to 5000 times more 25-OH-D_3 than 1,25-$(OH)_2D_3$ is required for these biological responses (*15*), it is not surprising that binding or competition were not observed with the concentration of 25-OH-D_3 used here. It is likely that displacement of 1,25-(OH)-[^{3}H]D_3 from 3.5*S* protein will be observed at concentrations of 30 to 300 n*M* 25-OH-D_3 in agreement with its relative activity in the bone system.

Both bone cytosols examined contained two high-affinity binding proteins for vitamin D_3 metabolities: a 3.5*S* protein specific for 1,25-$(OH)_2D_3$ and a 5 to 6*S* protein specific for 25-OH-D_3. Although 1,25-$(OH)_2$-[^{3}H]D_3 is distributed between 3.5*S* and 6*S* macromolecules in rat calvaria cytosol, it binds preferentially to the 3.5*S* component. A 5 to 6*S* protein which binds 25-OH-D_3 in cytosol prepared from rat bone has been demonstrated by other investigators using ion exchange chromatography (*16*). Furthermore, a similar 5 to 6*S* binding protein for 25-OH-D_3 has been demonstrated in all rat and chick tissues examined (*3, 17*). In experiments not reported here, the 5 to 6*S* 25-OH-D_3 cytosol binding protein found in rat tissues reacts with an antibody directed to the 4*S* plasma transport protein for 25-OH-D_3, suggesting that these binding proteins are similar or closely related and may not be related to receptor activity.

In chick calvaria cytosol, 1,25-$(OH)_2$-[^{3}H]D_3 bound only to the 3.5*S* protein in a manner analogous to its association with the 3.7*S* protein present in chick intestinal cytosol. Although further investigation is necessary to establish these 3.5*S* proteins as physiologic receptors for the action of 1,25-$(OH)_2D_3$ in bone, at least these proteins have high affinity and low capacity for 1,25-$(OH)_2D_3$, which are important criteria expected of a steriod hormone receptor.

B. E. KREAM, M. JOSE
S. YAMADA, H. F. DELUCA
Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin–Madison, Madison 53706

References and Notes

1. H. F. DeLuca, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 2211 (1974); E. Kodicek, *Lancet* **1974-I**, 325 (1974); A. W. Norman, *Vitam. Horm. (N.Y.)* **32**, 325 (1974).
2. P. F. Brumbaugh and M. R. Haussler, *J. Biol. Chem.* **249**, 1258 (1974); D. E. M. Lawson and P. W. Wilson, *Biochem. J.* **144**, 573 (1974).
3. B. E. Kream, R. D. Reynolds, J. C. Knutson, J. A. Eisman, H. F. DeLuca *Arch. Biochem. Biophys.* **176**, 779 (1976).
4. P. F. Brumbaugh and M. R. Haussler, *J. Biol. Chem.* **249**, 1251 (1974).
5. J. E. Zerwekh, T. J. Lindell, M. R. Haussler, *ibid.* **251**, 2388 (1976).
6. H. F. DeLuca and H. K. Schnoes, *Annu. Rev. Biochem.* **45**, 631 (1976).
7. M. Garabedian, Y. Tanaka, M. F. Holick, H. F. DeLuca, *Endocrinology* **94**, 1022 (1974).
8. L. G. Raisz, C. L. Trummel, M. F. Holick, H. F. DeLuca, *Science* **175**, 768 (1972).
9. Y. Tanaka and H. F. DeLuca, *Arch. Biochem. Biophys.* **146**, 574 (1971).
10. J. C. Weber, V. Pons, E. Kodicek, *Biochem. J.* **125**, 147 (1971).
11. J. J. Partridge, S. Faber, M. R. Uskokovic, *Helv. Chim. Acta* **57**, 764 (1974).
12. C. A. Frolik and H. F. DeLuca, *J. Clin. Invest.* **52**, 543 (1973).
13. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
14. J. J. Reynolds, M. F. Holick, H. F. DeLuca, *Calcif. Tissue Res.* **12**, 295 (1973).
15. P. H. Stern, C. L. Trummel, H. K. Schnoes, H. F. DeLuca, *Endocrinology* **97**, 1552 (1975).
16. D. E. M. Lawson, M. Charman, P. W. Wilson, S. Edelstein, *Biochim. Biophys. Acta* **437**, 403 (1976).
17. J. G. Haddad and S. J. Birge, *J. Biol. Chem.* **250**, 299 (1975).
18. The 25-OH-D_3 was a gift of J. Babcock (Upjohn) and the 1,25-$(OH)_2D_3$ was generously supplied by M. Uskokovic (Hoffmann–LaRoche). Supported by PHS grant AM-14881, U.S. Energy Research and Development Administration EY-76-S-02-1668, and the Harry Steenbock Research Fund.

10 March 1977; revised 29 April 1977

Temporal Lobe Aggression in Rats

Abstract. *Although reports of aggressive behavior in temporal lobe epileptics are common, it has proven difficult in clinical settings to gain the experimental control necessary to systematically investigate temporal lobe aggression or even to provide unequivocal evidence of its existence. Increases in aggressive behavior were observed in rats with experimentally induced epileptic foci in temporal lobe structures but not in control rats or those with foci in the caudate.*

There have been reports of aggressive behavior in temporal lobe epileptics (*1*) and of epileptic electrographic anomalies, apparently of temporal lobe origin, in subjects hospitalized or imprisoned for their violent behavior (*2*). Nevertheless, it has proven difficult in human clinical studies to gain the degree of quantification and experimental control necessary to provide unequivocal support for the view that the neural changes associated with temporal lobe epilepsy can predispose an individual to aggression (*3*). We describe here the controlled induction of temporal lobe aggression in experimental animals "kindled" with periodic electrical stimulation of the brain. Rats were periodically stimulated at low levels at one of three brain loci until motor seizures, which developed and increased in severity with each successive stimulation, were reliably elicited. Rats with an epileptic focus kindled in a temporal lobe structure (amygdala or hippocampus) were found to be more aggressive than control subjects or those with a kindled focus in the caudate nucleus.

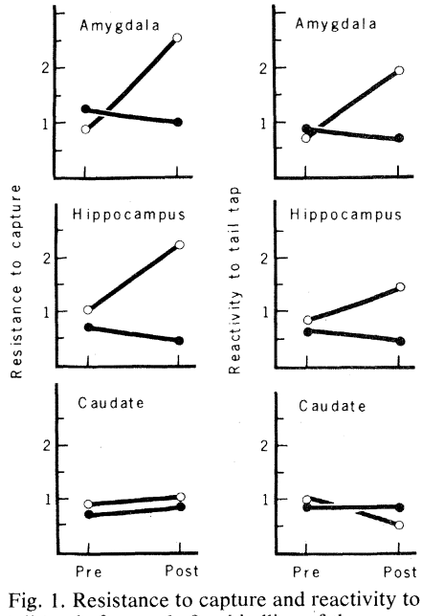

Fig. 1. Resistance to capture and reactivity to tail tap before and after kindling of the amygdala, hippocampus, or caudate. The means of the kindled and control animals are illustrated by open and closed circles, respectively.

A single bipolar electrode was implanted in the amygdala (N = 25), the hippocampus (N = 25), or the caudate nucleus (N = 25) of adult male hooded rats (*4*). After at least 2 weeks of recovery from surgery during which all 75 subjects were handled each day, 15 subjects from each of the three groups were randomly selected to be kindled; the remaining 10 served as handled but unstimulated controls. Each subject in the kindling groups was stimulated (1 second, 60 hertz, 400 μa root mean square) 6 days per week for the 8 weeks of the experiment. No more than three stimulations were administered on any one day, and the interval between consecutive stimulations was always greater than 2 hours (*5*). Initial stimulations rarely produced behavioral responses, but after a few stimulations, mild facial clonus was typically elicited. Then, with each successive stimulation, the convulsive reaction gradually increased in severity. In the last 6 weeks of the experiment, bilateral seizures characterized by clonus of the jaw, head, and forelimbs were reliably elicited in all three groups of kindled subjects (*6*). Behavioral tests of aggression were conducted according to the procedure described by Seggie (*7*). The aggressiveness of each subject was