bonds whose rotational angles have opposite signs. The $t_m g t g^* t_{m'}$ conformation connects parallel but laterally displaced planar segments; it has been postulated to exist in biomembranes (*23*) and crystalline polyethylene (*24*) and has been invoked to explain certain dielectric relaxation phenomena in polyethylene (*25*). Although the presence of the 1305 cm^{-1} band does not unambiguously prove the existence of either of these conformational sequences, it does indicate *gauche* disruptions in the interior regions of some chains.

All the solid-solid phase transitions lead to similar changes in the infrared spectrum. The change in band intensities at each transition is approximately proportional to the change in enthalpy. Of the bands associated with nonplanar forms, those most easily detected belong to the end-*gauche* conformation. Consequently, only these bands could be clearly observed to increase in intensity at the weak δ transition.

It is significant that a band near 1352 cm^{-1}, known to be characteristic of pairs of adjacent *gauche* bonds (Table 1) (*11*), was not observed except at temperatures near the melting point. This band is prominent in the infrared spectra of liquid *n*-alkanes and is found in the spectrum of highly crystalline polyethylene (*11*). Its absence in the high-temperature phases of the crystalline *n*-alkanes, where nonplanar chains are known to be present, indicates that only very limited kinds of nonplanar conformations are allowed in the crystal. The appearance of a band at 1352 cm^{-1} when the temperature of the *n*-alkane approaches the melting point may signal the onset of a new conformational regime, although more measurements are needed to settle this point.

Plots of integrated band intensities against temperature (not shown) led to the following observations. (i) As the *n*-alkane is warmed, abrupt changes in the concentration of nonplanar conformers occur at temperatures that correspond, within the estimated experimental error (< 1 K), to the transition temperatures determined from the DSC measurements. (ii) Nonplanar conformers are observed in phase I at temperatures at least 20 K below the first (lowest temperature) transition. As the sample is warmed, their concentration gradually increases; a discontinuous jump occurs at each transition. (iii) The concentration of nonplanar conformers in the highest temperature phase is significantly greater for longer *n*-alkanes. In C_{29} in phase II, roughly half the molecules are nonplanar. (iv) The temperature dependence of nonplanar forms varies in the temperature regions between transitions depending on the *n*-alkane. (v) The T_α transitions occur over a temperature interval of about 0.5 K or less. The other solid-solid transitions are somewhat broader. (vi) In cases where there are multiple transitions, if two transition temperatures are sufficiently close the transitions, especially the lower, are broadened. (vii) Where there are multiple transitions, there is a qualitative correspondence between the relative values of ΔH determined from the DSC measurements and the change in concentration of nonplanar conformation at the transition. Thus for C_{27}, $\Delta H_\delta \ll \Delta H_\gamma < \Delta H_\alpha$, and these inequalities are reflected in the relative magnitude of changes in band intensities at the transitions. The sensitivity of the infrared method is indicated by the fact that the entropy change associated with the δ transition is about 0.1 entropy unit.

We have demonstrated the existence of a variety of nonplanar conformers in the solid phases of the *n*-alkanes and have found a new phase transition. The concentrations of these conformers increase with temperature. The existence of these nonplanar molecules must be linked with the existence of the many different solid phases of hydrocarbon chain systems.

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References and Notes

1. D. C. Bassett and B. Turner, *Nature (London) Phys. Sci.* **240**, 146 (1972).
2. M. Yasuniwa, C. Nakafuku, T. Takemura, *Polym. J.* **4**, 526 (1973).
3. J. F. Nagle, *Annu. Rev. Phys. Chem.* **31**, 157 (1980).
4. D. Chapman, R. M. Williams, B. D. Ladbrooke, *Chem. Phys. Lipids* **1**, 445 (1967); H.-J. Hinz and J. M. Sturtevant, *J. Biol. Chem.* **247**, 6071 (1972).
5. S. C. Chen, J. M. Sturtevant, B. J. Gaffney, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5060 (1980).
6. D. L. Melchior and J. M. Stein, *Prog. Surf. Membr. Sci.* **13**, 211 (1979); J. F. Nagle and H. L. Scott, *Phys. Today* **31**, 38 (1978).
7. A. Müller, *Proc. R. Soc. London Ser. A* **138**, 514 (1932).
8. G. R. Strobl, *J. Polym. Sci. Polym. Symp.* **59**, 121 (1977); B. Ewen, G. R. Strobl, D. Richter, *Faraday Spec. Discuss, Chem. Soc.* **69**, 19 (1980).
9. R. G. Snyder and J. H. Schachtschneider, *Spectrochim. Acta* **19**, 85 (1963).
10. J. H. Schachtschneider and R. G. Snyder, *ibid.*, p. 117.
11. R. G. Snyder, *J. Chem. Phys.* **47**, 1316 (1967).
12. This study is confined to the odd *n*-alkanes, whose members form a continuous series with respect to phase behavior. This is not true for the even *n*-alkanes (*15*).
13. The *n*-alkanes used were described by A. A. Schaerer *et al.*, *J. Am. Chem. Soc.* **77**, 2017 (1955)]. Our infrared spectra of these samples showed no evidence of impurities.
14. Y. Kakiuchi, *J. Phys. Soc. Jpn.* **6**, 313 (1951); J. D. Hoffman, *J. Chem. Phys.* **20**, 541 (1952); Y. V. Mnyukh, *J. Phys. Chem. Solids* **24**, 631 (1963); D. W. McClure, *J. Chem. Phys.* **49**, 1830 (1968); T. Ishinabe, *ibid.* **72**, 353 (1980).
15. M. G. Broadhurst, *J. Res. Natl. Bur. Stand. Sect. A* **66**, 241 (1962).
16. T. Oyama, K. Takamizawa, Y. Urabe, Y. Ogawa, *Kyushu Daigaku Kogaku Shuho* **52**, 129 (1979); T. Oyama, K. Takamizawa, Y. Ogawa, *Kobunshi Ronbunshu* **37**, 711 (1980).
17. These measurements were made with a Perkin-Elmer model DSC-2 differential scanning calorimeter. Our measured values for T_α and T_{mp} (melting point temperature) of C_{25}, C_{27}, and C_{29} agree with the values tabulated by Broadhurst (*15*) with one exception. For T_α of C_{27} Broadhurst used the average of the two solid-solid transition temperatures reported by Schaerer *et al.* (*13*). These two transitions are actually α and γ, and the temperatures reported (*13*) for them are also what we found.
18. A. E. Smith, *J. Chem. Phys.* **21**, 2229 (1953).
19. The infrared spectra were measured with a model 8000 Nicolet Fourier transform infrared spectrometer with a mercury-cadmium-telluride/InSb sandwich detector. The resolution was 1.0 cm^{-1}. The temperature of the sample was controlled to better than ± 0.05 K.
20. R. G. Snyder, *J. Mol. Spectrosc.* **7**, 116 (1961).
21. ———, *J. Chem. Phys.* **42**, 1744 (1965).
22. A more complete account of these calculations is being prepared by M. Maroncelli, R. G. Snyder, and H. L. Strauss.
23. D. A. Pink, T. J. Green, D. Chapman, *Biochemistry* **19**, 349 (1980).
24. W. Pechhold, S. Blasenbrey, S. Woerner, *Colloid Polym. Sci.* **189**, 14 (1963).
25. R. H. Boyd and J. A. Sayre, *J Polym. Sci. Polym. Phys. Ed.* **17**, 1627 (1979).
26. We gratefully acknowledge support by the National Institutes of Health and the National Science Foundation. We thank Y. Oono for making us aware of the Japanese literature and F. Okino, T. Nagata, and again Y. Oono for help with the translations.

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Steady-State Relationship of Calcium-45 Between Bone and Blood: Differences in Growing Dogs, Chicks, and Rats

Abstract. *In young animals that had received multiple doses of calcium-45, a constant ratio of calcium-45 specific activity in blood to that in bone was found in growing dogs and chicks but not in rats. This steady-state relationship of calcium-45 between bone and blood suggests that during growth in dogs and chicks most of the skeletal calcium is in an active state of turnover. In growing rats, after the first 2 weeks of life, the blood/bone ratio of calcium-45 decreases due to a decrease in bone resorption.*

The stability of the serum calcium concentration under various clinical and experimental conditions has led to the concept of a strong homeostatic mechanism for the regulation of calcium (*1*). The rapidity with which the skeleton responds and maintains normal levels of serum calcium after multiple calcium-

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depleting blood transfusions in dogs (*2, 3*) suggested that the skeleton is in equilibrium with extracellular fluids (*1*). Early experiments in rats (*4*) did not provide isotopic evidence for this relationship. Later, comparative studies of the specific radioactivity of serum ^{45}Ca and bone ^{45}Ca from young rats (*5*) and from isolated perfused dog limbs (*6*) led to the conclusion that the skeleton's calcium is not in equilibrium with blood. However, Hevesy's demonstration (*7*) of highly efficient conservation of total body ^{45}Ca in young mice undergoing rapid growth modeling of bone supports the idea that there is a dynamic relationship between bone calcium and blood calcium.

Some of the problems inherent in measuring skeletal dynamics for the study of metabolically stable constituents of the body such as calcium (*8*) and collagen (*9*) are related to the slowness with which isotopic equilibria are reached after single-dose labeling of mature animals. The experimental design can be improved by using multiple-dose techniques (*10*) with rapidly growing animals and waiting long enough after labeling to minimize the heterogeneity in the different metabolic compartments (*10*). Unlike pulse-labeling of mature animals and humans when most of the skeleton has already formed, which results in an irregular distribution of ^{45}Ca, multiple labeling of young growing animals results in a widespread and uniform distribution of ^{45}Ca and [^{3}H]-tetracycline in whole bones (*11*).

The experiments reported here illustrate the use of multiple-dose labeling to achieve rapid isotopic equilibrium between bone and blood. Young rats, dogs, and chicks were labeled repeatedly with ^{45}Ca (*11*) during their period of rapid growth in utero or during the early weeks of life. Soon after birth the labeled dog and rat pups were placed with unlabeled foster mothers. One or two weeks after the end of labeling, groups of chicks and rats were bled and then killed at intervals during the first 14 to 16 weeks of life and each of nine dogs during the first 14 months. All animals were maintained on a normal diet. After isolation and demineralization (*11*) of whole femurs and humeri, serum and bone calcium were analyzed chemically and isotopically (*11*) to determine the specific activity of ^{45}Ca (disintegrations per minute per milligram) in blood and whole bones. The ratio of the ^{45}Ca specific activity of serum to that of bone was calculated for each animal and correlated with age.

With growth and accretion of dietary calcium, the specific activity of ^{45}Ca decreased markedly (10- to 60-fold) in bone and serum in all three species studied (Fig. 1, A, C, and E) and was always lower in serum than in bone. During the early weeks after birth the specific activity of bone and serum ^{45}Ca decreased rapidly in dogs (*11*), chicks, and rats due to the rapid growth and influx of nonradioactive dietary calcium into bone. In dogs the specific activity decreased from 720 to 24.5 dpm/mg in bone and from 470 to 14.9 dpm/mg in serum during 14 months (Fig. 1A). Similarly, the specific activity in chick bone decreased from 10,500 to 1620 dpm/mg and that in serum from 7200 to 1060 dpm/mg during 16 weeks (Fig. 1C), while the activity in rat bone decreased from 270,000 to 4000 dpm/mg and that in serum from 180,000 to 1010 dpm/mg during 14 weeks (Fig. 1E). The ratio of the specific activity in serum relative to that in bone remained constant (0.56 to 0.64) for dogs during the first 14 months of life (Fig. 1B) and for chicks (0.65 to 0.69) during the first 16 weeks (Fig. 1D). For rats the ratio was relatively constant (0.62 to 0.75) for the first 2 weeks, decreased to 0.42 at 5 weeks, and was 0.25 by 14 weeks of age (Fig. 1F).

The constancy of the blood/bone specific activity ratio suggested that bone calcium is in a dynamic steady-state relationship with blood calcium for the peri-

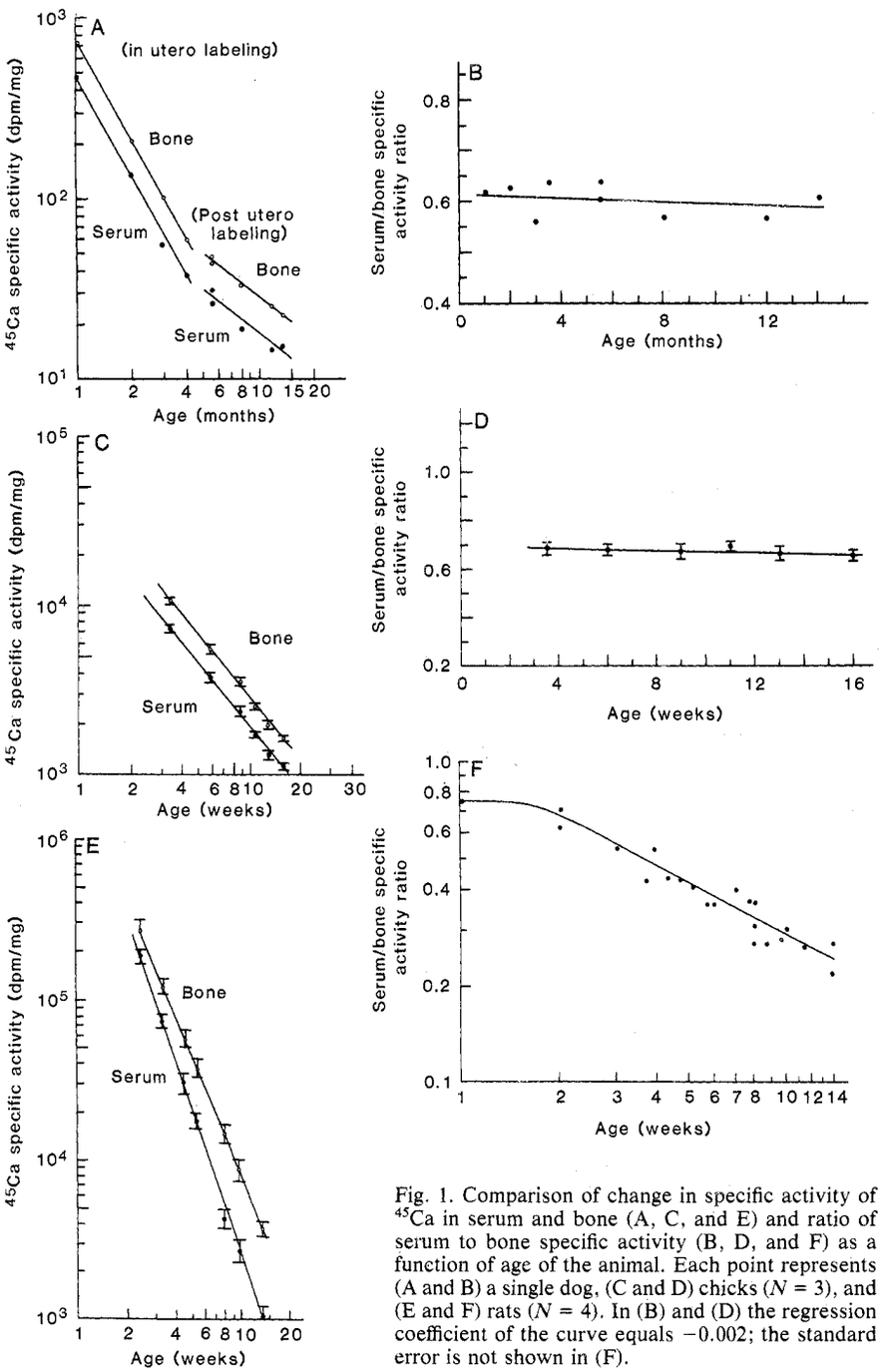

Fig. 1. Comparison of change in specific activity of ^{45}Ca in serum and bone (A, C, and E) and ratio of serum to bone specific activity (B, D, and F) as a function of age of the animal. Each point represents (A and B) a single dog, (C and D) chicks ($N = 3$), and (E and F) rats ($N = 4$). In (B) and (D) the regression coefficient of the curve equals -0.002; the standard error is not shown in (F).

od studied in dogs (14 months) and for the first 16 weeks of life in chicks. Except for the first 2 weeks of life, rats did not demonstrate a constant relationship between bone calcium and blood calcium. The constancy of the ratio is consistent with active bone resorption in dogs (*12*) and chicks (*13*) due to growth modeling and haversian remodeling of bone. In contrast, normal rats quickly cease most growth modeling of bone and have few haversian osteons (*14, 15*). In addition, the type and vascularity of bone vary in these three species. Laminar bone, which is the predominant type in chicks, is more vascularized than the predominant haversian bone in dogs, which is much more vascularized than the nonhaversian bone in rats (*16*). In rats the loss of bone vascularity with age could result in a decrease in the blood/bone ratio of ^{45}Ca.

In growing dogs and chicks the numerical value of the ratio suggests that under steady-state conditions 60 to 70 percent of the serum calcium is derived from bone calcium and 30 to 40 percent is derived from the diet. The numerical value of the ratio in dogs (0.56 to 0.64) is similar to that reported for the ^{48}Ca abundance in serum from young adult human subjects (*17*) who naturally have a uniformly distributed tracer (^{48}Ca) and then are given food containing ^{40}Ca that is free of ^{48}Ca. During 3 to 8 weeks of prolonged vitamin D deficiency in chicks (*18*) and dogs (*19*) the blood/bone ratio approaches 1, suggesting that when the dietary source of calcium is markedly inhibited, an equilibrium distribution of ^{45}Ca is reached between bone and blood and almost all the serum calcium is derived from bone.

To elucidate the mechanism for the decrease in the blood/bone ratio in rats, 3-week-old rats were given ^{45}Ca and [^{3}H]tetracycline for 2 weeks (*11*). One week later, one-half of the rats were given 20 daily subcutaneous injections of the diphosphonate, ethane-1-hydroxy-1,1-diphosphonic acid (EHDP, 20 mg per kilogram of body weight per day), which is thought to inhibit bone resorption (*20*). Since loss of [^{3}H]tetracycline directly reflects cortical bone resorption (*18*), changes in blood ^{45}Ca were compared to losses of [^{3}H]tetracycline from whole bone.

During the 20 days, the blood/bone ratio of ^{45}Ca in the untreated rats decreased from 0.45 to 0.32 (Fig. 2), while that in the EHDP-treated rats decreased to 0.14. The control rats lost 30 percent of their incorporated [^{3}H]tetracycline ($P < .001$; Fig. 2); the EHDP-treated rats lost 10 percent, which was not significantly different from the baseline (initial) value. A similar inhibition of [^{3}H]tetracycline loss and decrease of ^{45}Ca in blood were observed with EHDP-treated chicks (*21*) and parathyroidectomized dogs (*22*). In contrast, there was rapid loss of [^{3}H]tetracycline from whole bones in rapidly growing untreated dogs (*11, 23*) and chicks (*18*).

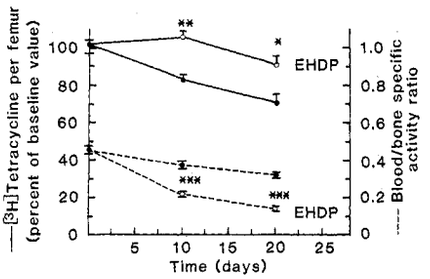

Fig. 2. Comparison of loss of incorporated [^{3}H]tetracycline from whole femurs of (●) control and (○) EHDP-treated rats with decrease in the serum/bone ^{45}Ca specific activity ratio for (●) control and (○) EHDP-treated rats. Data are presented as means ± standard errors for $N = 4$; for differences from the control group (*) $P < .05$, (**) $P < .01$, and (***) $P < .001$ (Student's t-test).

The correlation between the inhibition of [^{3}H]tetracycline loss and decrease in the blood/bone ratio of ^{45}Ca in EHDP-treated rats suggests that the decrease in the blood/bone ratio with age in normal rats is due to a decrease in bone resorption, resulting in a smaller release of bone ^{45}Ca into blood. The decrease in the blood/bone ratio in young growing rats would be consistent with (i) the small areas of resorbing surface and small rates of bone resorption, which are one-third the areas and rates of bone formation observed by quantitative histological methods in young rats (*24*), and (ii) the zero rate of bone resorption observed by ^{45}Ca kinetic analysis in 9- to 10-week-old rats (*25*). The assembled isotopic and morphological data suggest that bone resorption is dissociated early from bone formation in rats, whereas resorption and formation are coupled temporally in dogs and chicks through systemic reutilization of calcium. The assembled data suggest that the rat is not an appropriate animal model (*15*) in which to study active bone turnover.

The concept that most bone calcium is nonexchangeable biologically is based on ^{45}Ca data for maturing or adult rats (*26*), in which our data indicate that little bone resorption is occurring. In contrast, the consistently high blood/bone ratios observed for 16 weeks in chicks and 14 months in dogs suggest that in these species bone calcium is exchanged with blood calcium as a result of continuous bone resorption. Continuous bone resorption and exchangeability of ^{45}Ca in young dogs and chicks under normal conditions and in older dogs, chicks, and rats (*27*) after a calcium-deficient diet support the idea (*2, 28*) that the skeleton serves as a calcium buffer and regulator for blood calcium.

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References and Notes

1. J. E. Howard, in *Ciba Foundation Symposium on Bone Structure and Metabolism*, G. E. W. Wolstenholme and C. M. O'Connor, Eds. (Little, Brown, Boston, 1956), pp. 206–217; W. F. Neuman and M. W. Neuman, *The Chemical Dynamics of Bone Mineral* (Univ. of Chicago Press, Chicago, 1958), pp. 101–168; B. E. C. Nordin, in *Annual Reviews on the Scientific Basis of Medicine* (Athlone, London, 1964), pp. 308–316; J. MacGregor, in *Bone Dynamics*, H. M. Frost, Ed. (Little, Brown, Boston, 1964), pp. 409–421.
2. A. B. Hastings and C. B. Huggins, *Proc. Soc. Exp. Biol. Med.* **30**, 458 (1933).
3. H. M. Patt and A. B. Luckhardt, *Endocrinology* **31**, 384 (1942); A. B. Hastings, in *Transactions of the Third Conference on Metabolic Interrelationships* (Josiah Macy, Jr., Foundation, New York, 1951), p. 38.
4. W. F. Neuman and M. W. Neuman, *The Chemical Dynamics of Bone Mineral* (Univ. of Chicago Press, Chicago, 1958), p. 116; J. H. Weikel and W. F. Neuman, *Metabolism* **10**, 83 (1961).
5. H. Jeffay and H. B. Bayne, *Am. J. Physiol.* **206**, 415 (1964).
6. G. A. Rodan, U. H. Liberman, M. Paran, M. Anbar, *Isr. J. Med. Sci.* **3**, 702 (1967).
7. G. Hevesy, *K. Dan. Vidensk. Selsk. Biol. Skr.* **22**, 1 (1955).
8. W. F. Neuman, A. R. Terepka, F. Canas, J. T. Triffitt, *Calcif. Tissue Res.* **2**, 262 (1968).
9. L. Klein, *Adv. Tracer Methodol.* **4**, 215 (1968).
10. A. N. Davison and J. Dobbing, *Nature (London)* **191**, 844 (1961).
11. L. Klein and K. V. Jackman, *Calcif. Tissue Res.* **20**, 275 (1976).
12. W. S. S. Jee, M. H. Bartley, R. R. Cooper, N. L. Dockum, in *The Beagle as an Experimental Dog*, A. C. Andersen, Ed. (Iowa State Univ. Press, Ames, 1970), pp. 162–188; E. H. Weinberg, R. P. Heaney, W. H. Harris, *Trans. Orthop. Res. Soc.* **3**, 250 (1978); C. Anderson and K. D. Danylchuk, *Am. J. Vet. Res.* **40**, 869 (1979).
13. J. S. Foote, *Smithson. Contrib. Knowl.* **35** (No. 3) (1916); L. E. Church and L. C. Johnson, *Am. J. Anat.* **114**, 521 (1964).
14. D. H. Enlow and S. O. Brown, *Tex. J. Sci.* **10**, 187 (1958); J. Jowsey, *J. Anat.* **100**, 857 (1966); *Cornell Vet.* **58**, 74 (1968).
15. I. J. Singh and D. L. Gunberg, *J. Morphol.* **133**, 241 (1971).
16. J. S. Foote, *Smithson. Misc. Collect.* **72** (No. 10) (1921); *Bone as a Measure of Development* (Douglas, Omaha, Neb., 1928), pp. 1–94; J. D. Currey, *Q. J. Microsc. Sci.* **101**, 351 (1960).
17. J. W. Hansen, G. S. Gordan, S. G. Prussin, *J. Clin. Invest.* **52**, 304 (1973).
18. L. Klein, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1818 (1980).
19. _____ and K. M. Wong, in *Hormonal Control of Calcium Metabolism*, D. V. Cohn, R. V. Talmage, J. L. Matthews, Eds. (Excerpta Medica, Amsterdam, 1981), p. 422.
20. H. Fleisch, R. G. G. Russell, M. D. Francis, *Science* **165**, 1262 (1969); R. Schenk, W. A. Merz, E. Muhlbauer, R. G. G. Russell, H. Fleisch, *Calcif. Tissue Res.* **11**, 196 (1973).
21. L. Klein, *Calcif. Tissue Int.* **27** (Suppl.), A21 (1979); *Trans. Orthop. Res. Soc.* **4**, 127 (1979).
22. K. M. Wong, L. Klein, R. M. Zollinger, *Surg. Forum* **30**, 100 (1979); L. Klein, in *Hormonal Control of Calcium Metabolism*, D. V. Cohn, R. V. Talmage, J. L. Matthews, Eds. (Excerpta Medica, Amsterdam, 1981), p. 447.
23. L. Klein and K. V. Jackman, in *Calcified Tissue, 1972, Proceedings of the 9th European Symposium on Calcified Tissues*, H. Czitober

and J. Eschberger, Eds. (Facta, Vienna, 1973), pp. 327–331.
24. D. Baylink, M. Stauffer, J. Wergedal, C. Rich, *J. Clin. Invest.* **49**, 1122 (1970); R. A. Evans, D. J. Baylink, J. Wergedal, *Metab. Bone Dis. Rel. Res.* **2**, 39 (1979).
25. F. Bronner and J.-P. Aubert, *Am. J. Physiol.* **209**, 887 (1965); S. Hurwitz, R. E. Stacey, F. Bronner, *ibid.* **216**, 254 (1969).
26. L. Singer and W. D. Armstrong, *Proc. Soc. Exp. Biol. Med.* **76**, 229 (1951); L. Singer, W. D. Armstrong, M. L. Premer, *ibid.* **80**, 643 (1952).
27. L. Klein and K. M. Wong, unpublished observations.
28. D. H. Copp, E. D. Mensen, G. D. McPherson, *Clin. Orthop.* **17**, 288 (1960).
29. Supported by NIH grants AG-00258, AG-00361, and DE-05487.

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Hindbrain GABA Receptors Influence Parasympathetic Outflow to the Stomach

Abstract. *Blockade of γ-aminobutyric acid receptor function by direct microinjection of bicuculline into the nucleus ambiguus in cats produced a marked increase in gastric motility which was mediated by the vagus nerve. This effect was reversed by muscimol. These data indicate that the nucleus ambiguus may be an important brain site influencing gastric function and that the neurotransmitter controlling parasympathetic outflow from this nucleus to the stomach is γ-aminobutyric acid.*

There are large gaps in our knowledge of the central nervous system (CNS) pathways and neurotransmitters that control parasympathetic outflow to the gastrointestinal tract. Anatomical and physiological evidence indicates that the nucleus tractus solitarius, dorsal motor nucleus of the vagus, parabrachial nucleus, hypothalamus, and central nucleus of the amygdaloid complex comprise part of the pathways for gastrointestinal control (*1–3*). The neurotransmitters associated with these pathways are unknown. However, recent cardiovascular studies suggest that γ-aminobutyric acid (GABA) may function as a CNS neurotransmitter controlling parasympathetic outflow (*4*). In addition, these studies have revealed that the nucleus ambiguus is also important for control of parasympathetic outflow and that activation of GABA receptors on this nucleus exerts a profound effect on parasympathetic activity to the heart (*4*). To obtain new information on CNS control of gastrointestinal function, we studied the effect of augmentation and reduction of GABA receptor activity at the nucleus ambiguus on contractile activity of the stomach.

Cats were anesthetized with α-chloralose (70 to 80 mg/kg, intravenously) and artificially ventilated with room air through a tracheal cannula. The animals were then paralyzed with decamethonium bromide (0.25 mg/kg, intravenously), given every 45 minutes or as needed. The femoral artery was catheterized for recording blood pressure and limb leads (lead 2) were placed on the extremities for recording the electrocardiogram. Arterial blood pressure and the electrocardiogram were monitored on a Gould brush recorder. Rectal temperature was monitored and maintained between 36° and 38°C with an infrared lamp. A midline abdominal incision was made and extraluminal strain gage force transducers (*5*) were sutured to the antrum and pylorus in the transverse axis to record circular muscle activity. The force transducers were calibrated prior to use and had equal sensitivities. Smooth muscle activity was registered on a Grass polygraph and motility indices (*6*) were calculated for antral and pyloric responses. The splanchnic nerves were sectioned and the adrenal glands were ligated in each animal because adrenergic innervation of the stomach and circulating catecholamines from the adrenal glands oppose parasympathetic effects on gastric motility (*7*).

The cats were then mounted in a David Kopf stereotaxic instrument and the dorsal surface of the lower brainstem was exposed by limited occipital craniotomy. Coordinates from Berman (*8*) were used to locate the nucleus ambiguus. In a few experiments we studied the effect on gastric motility of reducing GABA receptor activity at the dorsal motor nucleus of the vagus. This nucleus was also located with Berman's coordinates. The shaft of a 26-gauge Quincke (Babcock) spinal needle that had been cut from its base and filed smooth was guided stereotaxically at a 36° caudal angle into the nuclear area. The needle was attached to a 10-μl Unimetrics syringe with PE-20 tubing. Pharmacologic agents that augment (muscimol) and reduce (bicuculline methiodide) GABAergic tone (*9*) were dissolved in artificial cerebrospinal fluid (*10*) and administered by infusion with a Sage infusion pump at the rate of 0.1 μl/min for 10 minutes. For control infusions artificial cerebrospinal fluid alone was used. At the termination of each experiment the animal was killed and the brain was removed and placed in 10 percent Formalin. Frozen 50-μm sections were cut and mounted on slides. From these sections the cannula track and the injection site were identified and verified.

Microinjections of the GABA receptor antagonist bicuculline (10 ng/min for 10 minutes) into the left nucleus ambiguus resulted in pronounced increases in antral and pyloric contractile activity (Fig. 1B). The GABA receptor agonist muscimol was then microinjected (10 ng/min for 10 minutes) into the same site, and a striking decrease in gastric motility was observed (Fig. 1C). The same procedure was carried out for the right nucleus ambiguus (Fig. 1, D and E).

From the gastric motility tracings, the

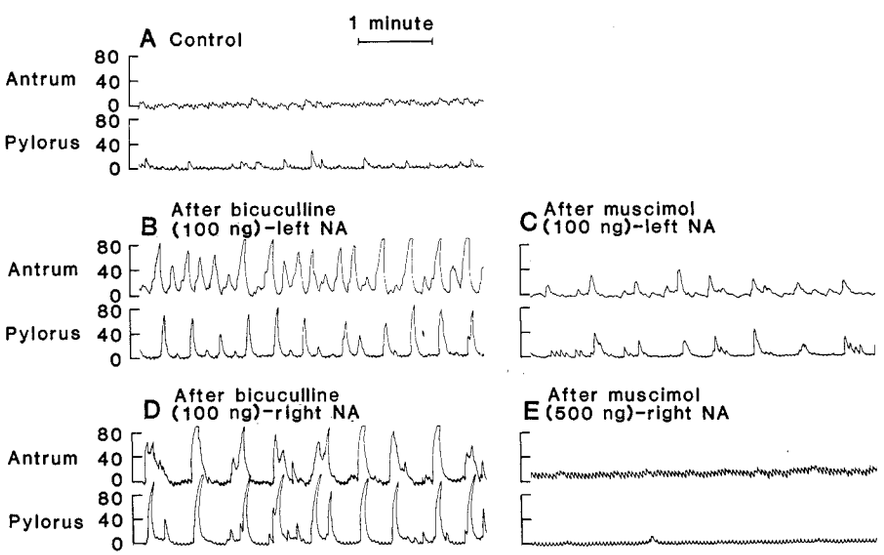

Fig. 1. (A to E) Effects of GABA receptor blockade (bicuculline) and stimulation (muscimol) at the nucleus ambiguus (*NA*) on antral and pyloric smooth muscle activity. Values on ordinate represent force in grams.

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