stimulation should not be attenuated by H-2 receptor antagonists. The conditions for testing this prediction appear to be met in the 13- to 14-day-old rat, and the results support the hypothesis.

These experiments also show that the study of developmental schedules in vivo can be a powerful tool for exposing the elements of complex physiological systems. In the mature subject one may not be able to isolate separate elements for examination because they are embedded in the interactions that characterize the complex system.

In summary, the results indirectly support the concept of multiple, independent acid-secretory response systems. In the developing rat, some of these systems appear to become functional before the histamine-mediated system is functional and may operate independently of the actions of histamine.

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7. The dose of pentobarbital was 7 mg/kg (intraperitoneally) for 13- to 16-day-old rats and 50 mg/kg for all older rats. All rats were deprived of food for 16 to 18 hours prior to surgery. Acid output was corrected for body weight. The rate of saline perfusion was 0.35 ml/min.
8. Acid output by ten rats infused with histamine diphosphate (8 mg/kg per hour) in 5 percent dextrose and water (D5W) was compared with output by five age- and weight-matched rats infused with D5W alone. After a baseline was established, acid output tended to rise slightly in both groups. However, there was no significant between-group difference in acid output, either between the basal and maximum outputs or between the maximum outputs. These data were analyzed both parametrically (by analysis of variance) and nonparametrically (by a Kruskal-Wallis analysis of variance for ranked data).
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10. The effects of cimetidine were compared with those of saline by multiple linear regression because the data at each sample period met criteria of homogeneity (F tests) and normal distribution ($D > .2$). The variables, in order of entry to maximize R^2, were pentagastrin ($R^2 = .3994$), time ($R^2 = .0327$), animal ($R^2 = .0072$), and cimetidine ($R^2 = .0001$). I also analyzed the same data by a more conservative Kruskal-Wallis analysis of variance for ranked data. In this analysis, as in the multiple linear regression, there was no significant cimetidine effect ($P > .05$), although there was a time effect and a highly significant animal effect ($P < .001$) accounting for 57 percent of the variance in rank order change over time.
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Purinergic Regulation of Food Intake

Abstract. *Inosine peripherally administered to rats markedly suppressed spontaneous food intake and food intake induced by diazepam, muscimol, insulin, and food deprivation. The purines 2-deoxyguanosine and 2-deoxyinosine also suppressed food deprivation–induced feeding, whereas 7-methylinosine, which does not bind to the benzodiazepine binding site in vitro, had no effect on food intake when compared with controls. These results suggest that purines may represent endogenous substances that regulate food intake through interactions with the benzodiazepine receptor.*

Purines occur in the brain in high concentrations (*1*) and have been reported to inhibit neuronal firing (*2*), regulate adenosine 3′,5′-monophosphate (cyclic AMP) formation in a variety of tissues (*3*), and act as competetive inhibitors of [^{3}H]diazepam binding in vitro (*4*). Although the purines have a relatively low affinity for the benzodiazepam receptor there is evidence that purines may represent the endogenous modulators of the benzodiazepine receptor (*4*). Isolates of endogenous ligands from brain fractions that inhibit [^{3}H]diazepam binding were identified by mass spectroscopy and radioimmunoassay as inosine and hypoxanthine (*5*). Central administration of inosine and 2-deoxyinosine increased the seizure latency induced by pentylenetetrazole (*6*), and peripheral administration of these purines reversed the exploratory activity elicited in mice by diazepam (*7*); however, neither the seizure latency (*6*) nor exploratory behavior (*7*) were influenced by purines that do not compete with [^{3}H]diazepam for receptor sites. Microiontophoretic application of inosine to primary cultures of mouse spinal

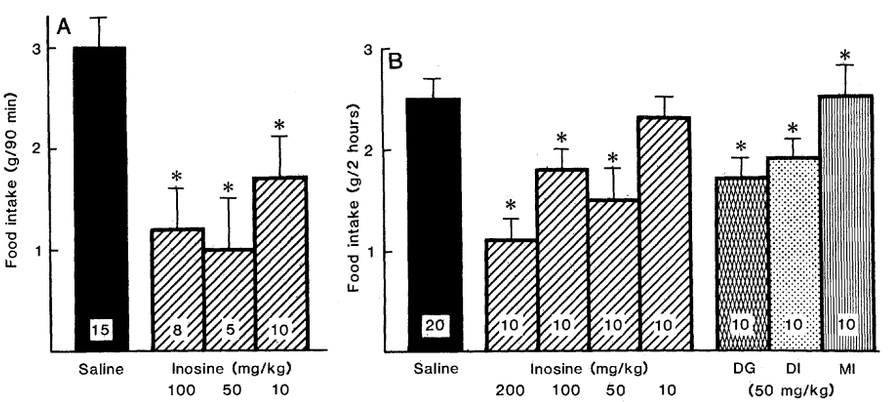

Fig. 1. (A) Effect of inosine on diazepam-enhanced feeding. Rats received diazepam (2.5 mg/kg) intraperitoneally at 2000 hours immediately followed by intraperitoneal administration of inosine or saline. Food intake was measured for the ensuing 90 minutes. The bars represent means ± standard error; the number of animals in each group is shown at the base of each bar. The protected least significance difference method was used to determine statistical significance in all studies. *$P < .05$ [$F(3, 34) = 5.65$, $P < .005$]. (B) Effect of purines on food deprivation–induced feeding. Purines [inosine, 2-deoxyguanosine (*DG*), 2-deoxyinosine (*DI*), and 7-methylinosine (*MI*)] or saline were administered intraperitoneally to rats that had been food-deprived for 30 hours, and food intake was measured for the ensuing 2 hours. The bars represent means ± standard error; the number of animals in each group is shown at the base of each bar. *$P < .05$ [$F(7, 82) = 5.48$, $P < .005$].

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cord neurons resulted in a nondesensitizing exciting response which was blocked by benzodiazepines and a desensitizing response which showed cross-desensitization with flurazepam (*8*).

Although many studies have focused on the anxiolytic, sedative, and muscle relaxant properties of benzodiazepines, there is also evidence that the benzodiazepines have an appetite-enhancing effect which appears to be independent of its anxiolytic properties (*9*). Benzodiazepines initiate feeding in sated animals (*10*), increase spontaneous food intake (*11, 12*), enhance food deprivation–induced feeding (*13, 14*) and tail-pinch–induced feeding (*15*), overcome the aversive effect of quinine adulteration (*16*), antagonize the anorectic effect of *d*-amphetamine (*14, 17*), and overcome food neophobia (*18*). The appetite-enhancing effect seems to be relatively selective at lower doses and is similar to the stimulus of food deprivation (*19*), whereas at higher doses the benzodiazepines may reduce food neophobia (perhaps because of the anxiolytic properties of the drug) (*18*). The neurochemical basis for appetite enhancement by benzodiazepines is not clear, although it has been suggested that serotonin (*11, 20*) and γ-aminobutyric acid (GABA) (*21*) may be involved. In the present study we report that purines suppress feeding induced by diazepam and the GABA agonist muscimol as well as food deprivation–induced eating, insulin-induced feeding, and nocturnal feeding. These findings suggest a role for purines in the central regulation of satiety.

Male Sprague-Dawley rats (125 to 175 g) were given free access to Purina Lab Chow and tap water and housed under conditions of controlled temperature and illumination (0600 to 1800 hours). The rats were tested in plastic boxes with which they were unfamiliar, and with Purina Lab Chow as the food source (7 to 10 g) (except when noted). Intraperitoneal administration of diazepam (Hoffmann–La Roche; 2.5 mg/kg in 2 percent ethyl alcohol and 4 percent propylene glycol in phosphate-buffered saline at *p*H 7.2) was used to enhance nocturnal feeding (2000 hours). Immediately after the diazepam injection, inosine (dissolved in saline, *p*H 9.0) at doses of 100, 50, and 10 mg/kg or saline (*p*H 9.0) was administered intraperitoneally and the rats were placed in the testing arena. All doses of inosine tested suppressed diazepam-enhanced feeding (Fig. 1A). These doses of inosine are significantly below the sedative range (500 to 1000 mg/kg), which reduces behavioral activity (*7*).

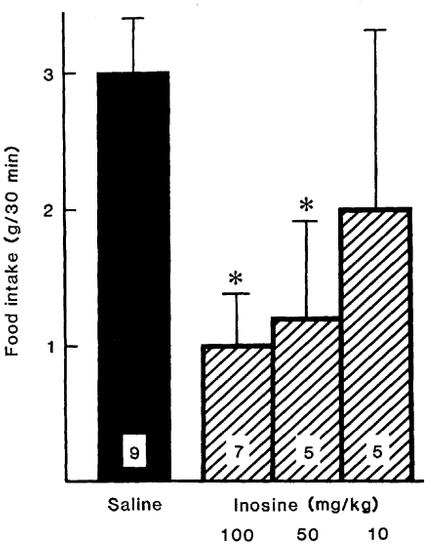

Fig. 2. Effect of inosine on muscimol-induced feeding. Eating was induced by intracerebroventricular administration of the GABA agonist muscimol (500 ng). Immediately after the muscimol injection, inosine or saline was administered intraperitoneally and food intake was measured for the ensuing 30 minutes. The bars represent means ± standard error; the number of animals in each group is shown at the base of each bar. $^*P < .05$ [log transformation: $F(3, 22) = 3.09$, $P < .05$].

Using a 30-hour food deprivation model we studied the ability of a number of purines to suppress food intake. Inosine, 2-deoxyinosine, 2-deoxyguanosine, 7-methylinosine (Sigma), or saline was administered intraperitoneally to food-deprived rats. The rats were then immediately placed into plastic boxes containing Purina Lab Chow and food intake was measured for 2 hours. Inosine suppressed food deprivation–induced feeding at doses of 200, 100, and 50 mg/kg (Fig. 1B). 2-Deoxyguanosine and 2-deoxyinosine also suppressed feeding at the 50 mg/kg dose, whereas 7-methylinosine, which does not bind to the diazepam receptor (*22*), did not suppress food deprivation–induced feeding (Fig. 1B). These data suggest that purines act as satiety factors by way of a specific interaction with the benzodiazepine receptor. Similar findings have been reported for the effect of purines on diazepam-induced exploratory behavior (*7*).

We examined the ability of purines to act as satiety factors in several other feeding models, namely muscimol-induced feeding, insulin-induced feeding, and nocturnal feeding.

Since the benzodiazepine receptor is known to interact with a receptor for GABA and enhance the effects of GABA (*23*), and since central administration of the GABA agonist muscimol induces feeding in sated rats (*24*), it seemed reasonable to postulate that inosine may also suppress muscimol-induced feeding. Stainless steel guide tubes were stereotactically implanted in the lateral ventricle of rats anesthetized with Nembutal at least 5 days before the experiment, as described previously (*25*). Feeding was stimulated in sated rats by intracerebroventricular administration of muscimol (500 ng). Immediately after muscimol injection, inosine (100, 50, or 10 mg/kg) or saline was injected intraperitoneally, the rats were placed in the testing arena, and food intake was measured for 30 minutes. Inosine at the 100 and 50 mg/kg dose, but not at the 10 mg/kg dose, significantly suppressed muscimol-induced feeding (*11, 20*) (Fig. 2).

It is well established that peripheral administration of insulin will induce eating in rats (*26*), with a resultant increase in body weight. In another series of experiments we stimulated feeding in sated rats (six rats per group) by subcutaneous injection of insulin (10 U/kg; Iletin U-100, Eli Lilly). Immediately afterward, inosine (100, 50, and 10 mg/kg) or saline was administered intraperitoneally, the rats were placed in plastic boxes, and food intake was measured for 3 hours. Insulin-induced feeding (2.3 ± 0.3 g per 3 hours) was suppressed by the 100 mg/kg dose of inosine (0.3 ± 0.2 g per 3 hours, $P < .001$), but not by the 50 mg/kg dose (1.4 ± 0.5 g per 3 hours) or the 10 mg/kg dose (1.2 ± 0.5 g per 3 hours).

The ability of inosine to suppress spontaneous nocturnal feeding was next evaluated. Rats were placed into individual cages for at least 1 week before the study. At 2000 hours the rats (ten per group) were injected intraperitoneally with inosine (50 mg/kg) or saline and were returned to their home cages where food intake was measured for 2 hours. Spontaneous nocturnal feeding (3.5 ± 0.2 g in 2 hours) was significantly suppressed by intraperitoneal injection of a 50 mg/kg dose of inosine (2.3 ± 0.3 g in 2 hours, $P < .01$).

We also evaluated the effect of inosine (50 mg/kg) on ingestion of tap water and a 2 percent sucrose solution. Rats (14 per group) were deprived of water for 15 hours before the experiment but were given free access to Purina Lab Chow. After they were injected with the inosine the rats were placed in plastic boxes containing water bottles equipped with sipping tubes that were weighed before and after the study. Water intake over a 60-minute period (5.9 ± 0.9 ml per hour) was unaffected by the 50 mg/kg dose of inosine (6.4 ± 1.0 ml per hour) as was

ingestion of the 2 percent sucrose solution (7.0 ± 0.9 ml per hour compared to 7.1 ± 1.2 ml per hour). This suggests that purines do not suppress food intake because of a nauseating or a general tranquilizing effect.

The results of this study show that the purine inosine not only suppresses feeding known to be related to the diazepam receptor, but also suppresses food deprivation–induced feeding, insulin-induced feeding, and spontaneous nocturnal feeding. Since the effective purines appear to be only those shown to interact with the benzodiazepine receptor, and since the benzodiazepines modulate satiety (*9*), it appears likely that inosine may be an endogenous modulator of satiety acting through the benzodiazepine receptor.

A number of nutrients including carbohydrates, proteins, and fats have been postulated to regulate ingestive behaviors (*27*). On the basis of our study it seems that a purinergic model of appetite regulation can be considered along with the glucostat, aminostat, and lipostat models of appetite regulation.

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Diazepam Impairs Lateral Position Control in Highway Driving

Abstract. *Nine expert drivers operated an instrumented vehicle in tests over a highway at night after being treated with diazepam (5 and 10 milligrams), a placebo, and nothing. They reacted to 10 milligrams of diazepam with increased lateral position variability. Potentially dangerous impairment was inferred from the reactions of some subjects.*

Recent evidence indicates that 20 to 30 percent of drivers in Europe and North America regularly use prescribed psychotropic drugs and that these drivers become involved in serious traffic accidents at a rate five to ten times that of nonusers (*1*). By far the most frequently prescribed drugs are benzodiazepine tranquilizers, of which the most popular is diazepam (Valium or Stesolid). Laboratory, driving simulator, and closed-course driving tests have provided contradictory results but occasionally the suggestion of an adverse diazepam effect on skills and judgment related to actual car driving (*2*). Two studies undertaken in the real environment relied upon post-test observer ratings for demonstrating adverse effects of diazepam on driving in urban or suburban traffic (*3*). The authors of both reports indicated that single observer reliability and interobserver agreement were less than desired. In addition, neither report indicates what performance changes the observers noted in deriving their more-or-less general ratings.

Our study was designed to measure diazepam's effects on aspects of highway driving performance. Test conditions were controlled, and performance was measured objectively. The major purpose was to determine whether single, moderate doses of diazepam impair the driver's fundamental road-tracking ability during uninterrupted high-speed travel. We reasoned that if drivers lose this ability to any significant extent, they can hardly be expected to cope adequately with superimposed task demands.

Subjects were nine healthy, male police driving instructors (ages 24 to 34 years). They were familiar with the road on which they were tested as the result of patrol and teaching duties. Further familiarization with the road, test vehicle, and procedures was provided individually in a preliminary rehearsal. Subjects were informed of the general nature of the experiment, though not of the drug used. Only one recalled having used a prescribed psychotropic drug (diazepam, for 2 weeks, 3 years earlier). Subjects' activities were controlled on test days. They had slept normally, engaged in light work and then fasted for 4 hours before they arrived.

Subjects undertook a 1-hour driving test under five separate conditions, 1 to 3 weeks apart: (i) 10-mg diazepam treatment (D-10), (ii) 5-mg diazepam treatment (D-5), (iii) placebo control (P), (iv) no-tablet control (N), and (v) early-morning control (M). Driving tests began during evening hours (2000 to 2200 hours, with the time constant for a given subject), except in condition M, when the test began at 0100 hours. The order of conditions was different for eight subjects, but one order was inadvertently replicated for the ninth. Drugs and placebo were administered 1 hour before the tests according to a double-blind procedure. Tests were scheduled on consecutive weeknights during the months October to December, but were postponed in

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