References and Notes

1. P. D. Spear, *Prog. Psychobiol. Physiol. Psychol.* **8**, 45 (1979).
2. R. D. Lund, *Development and Plasticity of the Brain* (Oxford, New York, 1978).
3. J. M. Van Buren, *The Retinal Ganglion Cell Layer* (Thomas, Springfield, Ill., 1963); A. Cowey, *Perception* **3**, 257 (1974); R. E. Weller and J. H. Kaas, *Assoc. Res. Vision Ophthalmol. Abstr.* (1980), p. 2.
4. J. T. Dineen and A. E. Hendrickson, *Invest. Ophthalmol. Visual Sci.* **21**, 749 (1981).
5. R. E. Kalil, *Soc. Neurosci. Abstr.* **4**, 633 (1978); *ibid.* **6**, 790 (1980).
6. H. E. Pearson, D. R. Labar, B. R. Payne, P. Cornwell, N. Aggarwal, *Brain Res.* **212**, 470 (1981).
7. R. E. Weller, J. H. Kaas, A. B. Wetzel, *ibid.* **160**, 134 (1979).
8. Physiological studies have identified three functional classes of ganglion cells in the cat and monkey retina. These classes are termed X (or brisk sustained), Y (or brisk transient), and W (or sluggish). The cells are distinguished on the basis of their receptive field organization, dynamic response properties, and axonal conduction velocities. See R. W. Rodieck [*Annu. Rev. Neurosci.* **2**, 193 (1979)] for a review.
9. R. Otsuka and R. Hassler, *Arch. Psychiatr. Z. Gesamte Neurol.* **203**, 212 (1962).
10. Details of recording and sampling procedures are described by P. D. Spear, K. R. Jones, S. R. Zetlan, E. E. Geisert, and S. E. Kornguth [*J. Neurophysiol.* **47**, 1174 (1982)].
11. About half the W-cells had linear spatial summation and about half had nonlinear summation. Therefore, this property could not be used to distinguish W-cells from X- or Y-cells.
12. Recordings from surviving lateral geniculate neurons in cats whose visual cortex was removed at birth show that many of these cells develop abnormally large receptive fields [E. H. Murphy and R. E. Kalil, *Science* **206**, 713 (1979)]. It was originally proposed that these large receptive fields arise from a widespread convergence of retinal afferents onto surviving geniculate cells. The present results support this proposal, since no retinal ganglion cells with abnormally large receptive fields were found in cats given lesions as neonates.
13. P. D. Spear, R. E. Kalil, L. Tong, *J. Neurophysiol.* **43**, 851 (1980).
14. D. B. Bowling and C. R. Michael, *Nature (London)* **286**, 899 (1980); Y. Fukuda and J. Stone, *J. Neurophysiol.* **37**, 749 (1974); K.-P. Hoffmann, *ibid.* **36**, 409 (1973); R.-B. Illing and H. Wässle, *J. Comp. Neurol.* **202**, 265 (1981).
15. J. E. Rose and C. N. Woolsey, in *The Biological and Biochemical Bases of Behavior*, H. F. Harlow and C. N. Woolsey, Eds. (Univ. of Wisconsin Press, Madison, 1958), pp. 127–150.
16. W. M. Cowan, in *Development and Aging in the Nervous System*, M. Rockstein, Ed. (Academic Press, New York, 1973), pp. 19–41.
17. R. E. Kalil and G. Scott, *Soc. Neurosci. Abstr.* **5**, 791 (1979); D. A. Winfield and T. P. S. Powell, *Proc. R. Soc. London Ser. B* **210**, 197 (1980).
18. K. L. Chow and J. H. Dewson, *J. Comp. Neurol.* **128**, 63 (1966).
19. R. E. Kalil, L. Tong, P. D. Spear, *Assoc. Res. Vision Ophthalmol. Abstr.* (1979), p. 157; D. R. Labar, N. E. Berman, E. H. Murphy, *J. Comp. Neurol.* **197**, 639 (1981).

20. We thank K. Vielhuber and S. Miller for excellent technical assistance. Supported by Public Health Service postdoctoral fellowship EY05256 (L.T.), research grants EY01916, EY02545 (P.D.S.), and EY01331 (R.E.K.), and research career development award EY00089 (P.D.S.).

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Ontogeny of Gastric Acid Secretion in the Rat: Evidence for Multiple Response Systems

Abstract. *Gastric acid secretion has been thought to depend on histamine stimulation of the parietal cell. However, in the 2-week-old rat neither exogenous histamine nor the H-2 receptor agonist impromidine stimulates acid secretion, whereas pentagastrin and the cholinergic agent bethanechol are potent stimuli. At this age, the effect of pentagastrin on acid secretion is not blocked by the H-2 receptor antagonist cimetidine, nor is it potentiated by impromidine. These data suggest that, in the rat pup, the acid secretory response to pentagastrin and cholinergic agents occurs before the histamine-mediated system is functional and operates independently of the actions of histamine.*

The role of histamine in the regulation of H^+ ion secretion from the gastric parietal cell has been debated for more than 30 years. Early data suggested the hypothesis that histamine is the final common stimulator of H^+ secretion (*1*). Gastrin and cholinergic agonists, which also stimulate acid secretion, were thought to do so by releasing histamine. This view was later supported by the discovery of the histamine-2 (H-2) receptor antagonists burimamide, metiamide, and cimetidine (*2*). These antagonists not only inhibit histamine-stimulated acid secretion but also block the effects of gastrin and cholinergic agents on acid secretion (*3*). However, in the dog, atropine inhibits the secretory response to pentagastrin, 2-deoxyglucose, and liver extract but not to histamine (*4*), an observation that does not support the hypothesis of a common pathway involving histamine.

An alternative hypothesis is that histamine, cholinergic agents, and gastrin act directly on their own parietal cell surface receptors. Each may stimulate H^+ secretion independently and may also potentiate the effects of the others (*5, 6*).

Evidence for the independent action of gastrin, histamine, and cholinergic agents comes from in vitro studies of dispersed canine parietal cells (*5*). Gastrin, histamine, and carbamylcholine can each stimulate oxygen uptake by the parietal cell. H-2 receptor antagonists block only the effects of histamine, atropine blocks only the effects of carbamylcholine, and neither blocks the effects of gastrin on oxygen uptake. This isolated cell system also provides in vitro evidence that potentiating interactions occur between these agents.

However, to date it has not been established in vivo whether gastrin or cholinergic agents directly stimulate H^+ secretion independently of the actions of histamine. In studying the ontogeny of acid secretion in the rat, I found that the secretory response to pentagastrin and bethanechol develops before the response to histamine. Hence gastrin and cholinergic agents appear capable of direct stimulation of H^+ secretion in animals unresponsive to histamine.

Wistar-derived rats were studied under pentobarbital anesthesia. Output of H^+ was determined by continuous saline perfusion of the innervated gastric lumen (*7*); samples of the perfusate were collected every 10 minutes, and each sample was automatically titrated to *p*H 7.0 with 0.01*N* NaOH. Drugs were infused through a jugular cannula. Rectal temperature was maintained at 35.5° ± 0.5°C by external regulation.

Data were analyzed by analysis of variance or linear regression on an IBM 370/158. Both the analyses of variance and linear regressions were corrected for repeated measures at appropriate levels of the analysis.

In 14- to 16-day-old pups, infusion of pentagastrin or bethanechol produced a fourfold to fivefold increse in H^+ output over infusion of saline. By contrast, infusion of histamine diphosphate through a dose range of 2 to 12 mg/kg per hour did not significantly increase H^+ secretion (Table 1) (*8*).

Histamine affects both H-1 and H-2 receptors, although only H-2 receptors are found on parietal cells. To exclude the possibility that interaction with H-1 receptors (such as those on arterioles)

Table 1. Effects of three secretagogues on acid output in 2-week-old rat pups. Values (microequivalents per hour) are means ± standard errors.

Substance	Dose	*N*	Acid output	
			Basal	Stimulated
Histamine	8 mg/kg per hour	10	7.62 ± 1.94	10.06 ± 0.61*
Pentagastrin	120 μg/kg per hour	14	6.78 ± 0.81	23.0 ± 0.3†
Bethanechol	1 mg/kg per hour	8	5.14 ± 0.7	27.1 ± 4.58†

*$P > .10$ (analysis of variance) (*8*). †$P < .01$.

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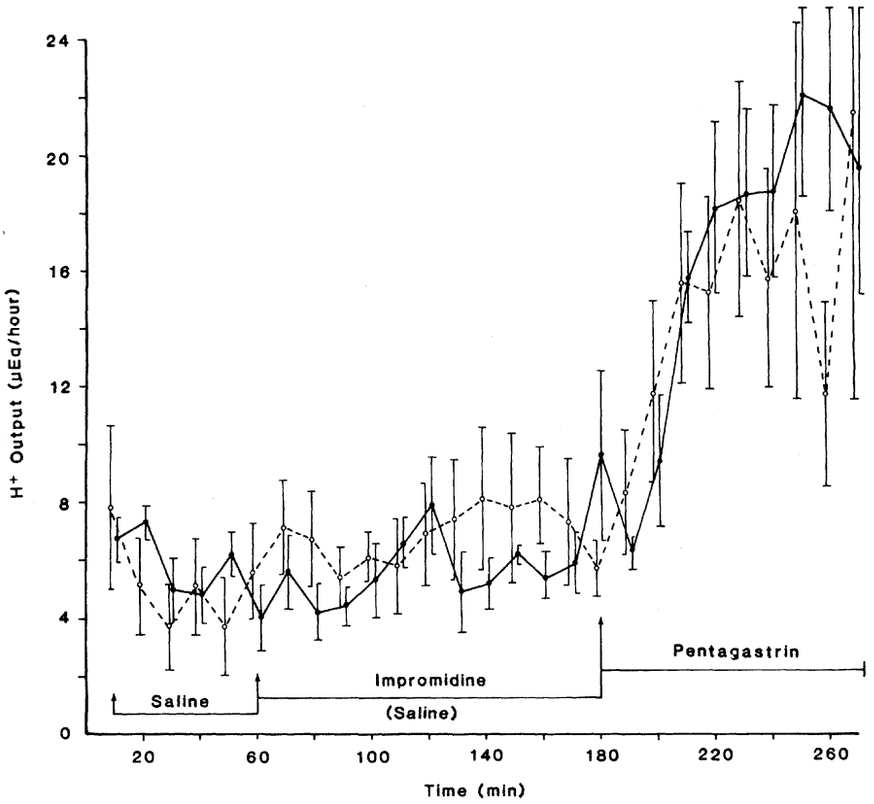

Fig. 1. Effects of impromidine (0.9 μmole/kg per hour) and pentagastrin (120 μg/kg per hour) on H^+ secretion in 13- to 14-day-old rat pups. Rats received saline (solid line; $N = 8$) or impromidine (dashed line; $N = 8$) during the second and third hours of gastric perfusion. Thereafter, all rats received pentagastrin. The effect of pentagastrin is significantly different from that of saline ($P < .0001$, analysis of variance), but the effect of impromidine is not.

attenuated the acid secretory response to histamine, I tested the response to the H-2 receptor agonist impromidine (*9*).

In 13- to 14-day-old pups (24 to 29 g), impromidine (0.9 μmole/kg per hour) did not differ from saline in stimulating H^+ secretion. However, in the same rats, infusion of pentagastrin (120 μg/kg per hour) produced a fourfold increase in acid secretion (Fig. 1). In 16 additional rats of this age, impromidine infused through a dose range of 0.09 to 9.0 μmole/kg per hour produced no consistent differences in H^+ secretion, indicating that the lack of responsiveness is independent of the dose. Infusion of impromidine and pentagastrin in six rats did not potentiate the response to pentagastrin.

Thus, pentagastrin and bethanechol stimulate acid secretion in 2-week-old rat pups. But H-2 receptor agonists do not directly stimulate acid secretion or potentiate the secretory effects of pentagastrin.

I next determined whether an H-2 receptor antagonist can attenuate pentagastrin-stimulated acid secretion at this age. Eight pairs of 13- to 14-day-old littermates (24 to 29 g) were infused with 120 μg of pentagastrin per kilogram per hour. At the time of maximum acid secretory response, cimetidine (6 μmole/kg) or saline was injected blindly by intravenous bolus. A stepwise multiple regression analysis of the results showed that cimetidine did not inhibit pentagastrin-stimulated acid secretion under these conditions. In an additional four pairs of littermates, 12 μmole of cimetidine per kilogram also did not differ from saline in its affect on pentagastrin (*10*).

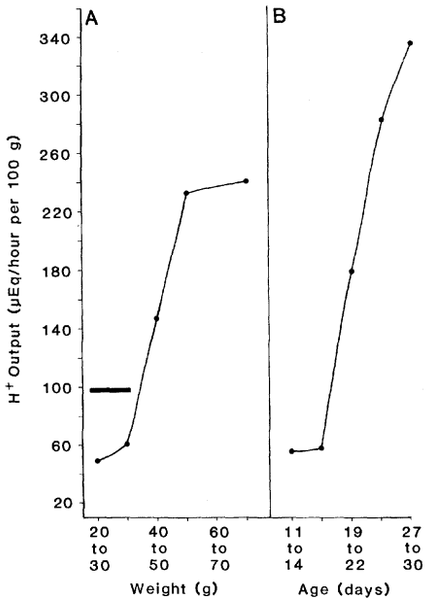

Fig. 2. (A and B) Curves showing the H^+ secretory response to impromidine in developing rat pups. Each point represents the mean maximum H^+ response to impromidine (0.9 μmole/kg per hour) in three to eight rats. [The heavy bar in (A) represents the median maximal H^+ response to pentagastrin in pups weighing 20 to 40 g.] The increase in H^+ response with age is significant [$F(4, 22) = 7.3$, $P < .01$].

By the fourth week (postnatal day 21), impromidine stimulated H^+ secretion and this effect was blocked by the H-2 receptor blocker cimetidine. Overall, the developmental curve for H^+ responsiveness to impromidine is a typical S-shaped power function, with the inflection point occurring between 19 and 22 days of age (40 to 50 g) (Fig. 2).

These results show that, developmentally, H^+ secretory systems responsive to pentagastrin (or bethanechol) are in operation before systems responsive to exogenous histamine or an H-2 receptor agonist. Moreover, the ontogeny of responsiveness to impromidine (Fig. 2) is almost identical with the ontogeny of gastric histidine decarboxylase activity reported by Aures and Hakanson (*11*). Activity of this histamine-forming enzyme begins to increase in the third week of life, reaching adult levels between days 40 and 50.

It is not known why there are no H^+ secretory responses to exogenous histamine or impromidine before the third postnatal week. Parietal cell H-2 receptors may be absent or not fully functional, or H-2 receptor stimulation may not affect the appropriate intracellular response (*12*).

The findings reported here are consistent with the hypothetical model proposed by Soll (*5*), which was based on in vitro studies of dispersed canine parietal cells. Soll proposed that H-2 receptor antagonists (like cimetidine) only appear to block all forms of acid secretory stimuli in vivo. He suggested that, instead, such antagonists selectively block only the direct effects of histamine and the potentiating interactions between histamine and other secretagogues. Against the usual "background" of endogenous histamine in vivo, H-2 receptor blockade attenuates the effects of these other secretagogues by eliminating potentiating interactions with histamine. This model predicts that, in an in vivo preparation which lacks histamine-mediated parietal cell stimulation, pentagastrin and other secretagogues should still be able to stimulate acid secretion and that this

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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References and Notes

1. B. P. Babkin, in *Secretory Mechanism of the Digestive Glands* (Hoeber, New York, 1950), p. 374; C. F. Code, in *Ciba Foundation Symposium on Histamine*, G. E. W. Wolstenholme and C. M. O'Connor, Eds. (Churchill Livingstone, London, 1956), pp. 189–219.
2. J. W. Black, W. A. M. Duncan, C. J. Durant, C. R. Ganellin, E. M. Parsons, *Nature (London)* **236**, 385 (1972); E. J. Durant, J. C. Emmett, C. R. Ganellin, in *Cimetidine: Proceedings of the Second International Symposium on Histamine H-2 Receptor Antagonists*, W. L. Burland and M. A. Simkins, Eds. (Exerpta Medica, Amsterdam, 1977), p. 1.
3. C. F. Code, *N. Engl. J. Med.* **296**, 14 (1977).
4. M. I. Grossman and S. J. Konturek, *Gastroenterology* **66**, 517 (1974).
5. A. H. Soll, *J. Clin. Invest.* **61**, 370 (1978).
6. J. D. Gardner, M. J. Jackson, S. Batzri, R. T. Jensen, *Gastroenterology* **74**, 348 (1978).
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).
9. G. J. Durant, W. A. M. Duncan, C. R. Ganellin, M. E. Parsons, R. C. Blakemore, A. C. Rasmoussen, *Nature (London)* **276**, 403 (1978).
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.
11. D. Aures and R. Hakanson, *Experientia Suppl.* **15**, 666 (1968).
12. L. R. Johnson and co-workers have reported that histamine, but not pentagastrin, produces a small but significant increase in acid output in the 15-day-old rat [K. Takeuchi, W. Peitsch, L. R. Johnson, *Am. J. Physiol.* **240**, G163 (1981)]. Their results and mine may not be comparable because in their study histamine and pentagastrin were used in different animals and body weights were not given. The issue of body weight is important because, as rats become only slightly older and heavier (14 to 16 days old, 33 to 38 g), cimetidine begins to suppress stimulation by pentagastrin and bethanechol [S. H. Ackerman, *Gastroenterology* **76** (No. 2), 1090 (1979)]. Also, these investigators used pyloric ligation to measure acid secretion. Their results may have been confounded by acid stimulation caused by the pyloric ligation itself and by damage to the gastric mucosa during the 4-hour collection period.
13. I thank M. I. Grossman for help in conceptualizing this study, M. A. Hofer and H. Weiner for help in discussing the data, R. Shindledecker for data analysis and technical help, and M. E. Parsons for kindly providing impromidine. Supported by grant R01-AM-18804 from the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases and by research scientist development award K1-MH00077 from the National Institute of Mental Health.

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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

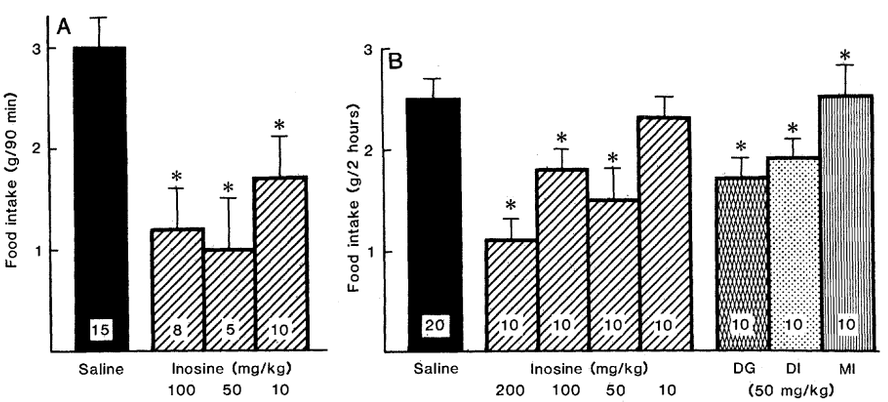

Fig. 1. (A) Effect of inosine on diazepam-enhanced feeding. Rats received diazepam (2.5 mg/kg) intraperitoneally at 2000 hours immediately followed by intraperitoneal administration of inosine or saline. Food intake was measured for the ensuing 90 minutes. The bars represent means ± standard error; the 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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