data from our laboratory indicate that deeper, more mesial discharges may also be recorded neuromagnetically.

These results demonstrate that by neuromagnetic mapping of interictal spikes in the human brain it is possible to identify with precision the three-dimensional location of intracortical sources producing epileptiform discharges. Further analyses of the orientation, polarity, and timing of these fields, coupled with detailed postsurgical histological data, may provide insight about rapidly occurring cellular events underlying interictal spiking.

DANIEL S. BARTH
*Department of Psychology, University of California, Los Angeles 90024*
WILLIAM SUTHERLING
JEROME ENGEL, JR.
*Department of Neurology, University of California, Los Angeles 90024*
JACKSON BEATTY
*Department of Psychology, University of California, Los Angeles 90024*

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10. Subject 1 was a 10-year-old female of normal intelligence with no family history of epilepsy. Focal left facial clonic (partial motor) seizures began at age 9. These occasionally generalized into grand mal (generalized tonic-clonic) seizures. At the time of testing she was receiving carbamazepine (Tegretol) with resulting control of seizures. Subject 2 was a 37-year-old female with recent memory deficits and no family history of epilepsy. Her grand mal (generalized tonic-clonic) and psychomotor (complex partial) seizures began at 19 years of age when she had toxemia. At the time of testing she was receiving phenobarbital and experiencing weekly partial complex seizures and, more rarely, generalized seizures.
11. S.H.E. Corporation, San Diego, California.
12. Standard electrode positions were used depending on the side and location of the focus. Subject 1: FP2-F8, F8-T4, T4-T6, T6-O2, FP1-F7, F7-T3. Subject 2: FP1-T1, T1-T3, T3-T5, T5-O1, FP2-T2, T2-T4. These positions were selected according to the established International 10-20 System [H. H. Jasper, *Electroencephalogr. Clin. Neurophysiol.* **10**, 371 (1958)]. Abbreviations: T, temporal lobe; F, frontal lobe; FP, frontal pole; O, occipital lobe. Odd numbers indicate standard positions over the left hemisphere, and even numbers, over the right hemisphere.
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14. This research was supported in part by the Environmental Physiology Program of the Office of Naval Research on Contract N00014-76-C-0616, by USPHS grant 5-S07 RR07009, and by NIMH training grant 78040-29867-5. We thank J. Perry Jaster for his expertise and assistance in the construction of equipment for this project.

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## Stimulation of Feeding in Rats by Intraperitoneal Injection of Antibodies to Glucagon

Abstract. *Intraperitoneal injections of antibodies to pancreatic glucagon at the onset of the first meal after 12 hours of food deprivation increased meal size 63 percent and meal duration 74 percent in rats. The antibodies also reduced the increase in hepatic vein blood glucose that occurred during meals in control rats, but did not affect the prandial increase in portal vein blood glucose. These results suggest that, under these conditions, pancreatic glucagon is necessary for the normal termination of meals.*

The observation that glucagon injections inhibit feeding in several species (*1–8*) suggests that this hormone, in addition to having extensive metabolic functions (*9*), may be involved in the neuroendocrine control of the behavioral processes that end meals. In rats, glucagon injections that produce glycemic changes in the normal prandial range reduce meal size and duration but do not disrupt the normal behavioral sequence characterizing postprandial satiety (*2, 3*). Glucagon's inhibitory effect is specific for feeding because neither water intake nor body temperature is affected and because no behavioral signs of malaise or aversion are produced (*3, 4*). Exogenous glucagon therefore fulfills the behavioral criteria for a putative endocrine satiety signal (*10*).

The possibility that endogenous glucagon is a physiological satiety signal arises from the observation that plasma glucagon concentrations increase during consumption of mixed-nutrient meals (*11*). If prandial increases in endogenous glucagon are necessary for normal satiety, partial inactivation of circulating glucagon should release feeding from inhibition. We tested this by injecting rats with antibodies to glucagon at the onset of meals. Such injections increased both meal size and length.

Individually caged male Sprague-Dawley rats (260 to 340 g) were maintained for at least 2 weeks on a reversed 12-hour light-dark cycle with unlimited access to a powdered high-carbohydrate diet (*12*). On the test day the rats were deprived of food during the light phase and offered preweighed cups of food at the beginning of the dark phase. One of

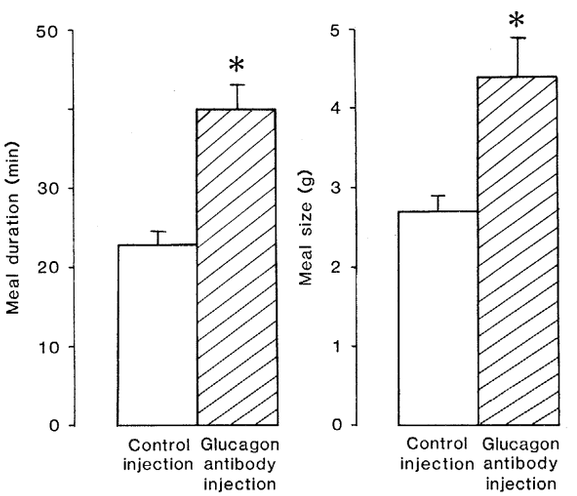


Fig. 1. Meal duration and size in rats deprived of food for 12 hours and injected at meal onset with glucagon antibodies ($N = 18$) or vehicle ($N = 28$). Data are means ± standard errors. Symbol: (*) $P < .002$.


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us observed each rat's behavior once each minute. The rats were divided into treatment groups on the basis of feeding latency and were intraperitoneally injected as soon as they began feeding with rabbit antibodies against purified bovine pancreatic glucagon or with serum from nonimmunized rabbits (*13*). These antibodies are highly specific for pancreatic glucagon (*14, 15*); the injected dose neutralizes about 5 ng of pancreatic glucagon in vitro. The rats were returned to their cages after the injections. They were not noticeably disturbed by this procedure and usually resumed feeding immediately. Meals were considered ended when the final event in the behavioral satiety sequence, resting, was observed (*16*).

Injection of glucagon antibodies dramatically stimulated feeding (Fig. 1). Mean meal size after the antibody injections was 63 percent greater than after the control injections, and meal duration was 74 percent greater. With a lower dose of glucagon antibodies (neutralizing capacity, about 3 ng of pancreatic glucagon), the increase in meal size was 27 percent ($P > .05$, Student's $t$-test) and the increase in meal duration was 32 percent ($P < .02$). The effects appeared specific, since there were no apparent differences in nonfeeding behaviors between experimental and control rats and since both groups showed the postprandial satiety sequence. These results indicate that normal levels of endogenous glucagon are required for normal postprandial satiety. The glucagon released during meals (*11*) may therefore provide an important physiological satiety signal. This is the only direct evidence we know implicating an endogenous hormone released in the gut by food stimuli as a controller of feeding behavior.

In a second experiment, we tested the effect of glucagon antibody injections on hepatic metabolism. Glucagon's most prominent metabolic action is stimulation of hepatic glycogenolysis (*9*), and it has been suggested that the effect of exogenous glucagon on satiety depends on its glycogenolytic and hyperglycemic effects (*4–6*). Rats were maintained as described above, deprived of food on the test day, and observed after presentation of food. Samples of hepatic and portal venous blood and of the left lateral lobe of the liver were obtained under ether anesthesia at meal onset (group O) or 18 to 24 minutes after injection of glucagon antibodies (group A) or control solution (group C) at meal onset. The results of the first experiment indicate that group C rats would be near the end of their meal at the time they were killed, whereas

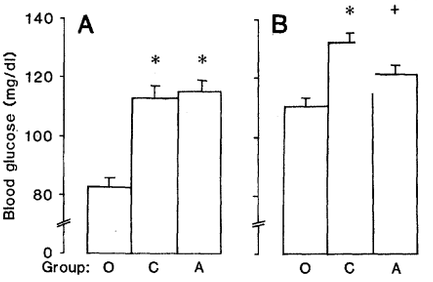


Fig. 2. Effect of glucagon antibody injection on portal vein (A) and hepatic vein (B) blood glucose concentration. Blood samples were obtained at meal onset (group O; $N = 28$) or 18 to 24 minutes after injection of glucagon antibodies (group A; $N = 18$) or control serum (group C; $N = 28$) at meal onset. Symbols: (*) significantly different from group O ($P < .01$); (+) significantly different from group C ($P < .05$).

group A rats would be near the middle of their meals. Blood glucose and liver glycogen were obtained and assayed as described by Geary *et al.* (*2*).

Mean food intakes were similar in groups A and C (2.6 ± 0.2 and 3.0 ± 0.2 g, respectively). Portal vein blood glucose also increased similarly in groups A and C over levels measured at meal onset (Fig. 2A), indicating that glucose was absorbed during feeding. In contrast, hepatic vein blood glucose increased more in group C than in group A (Fig. 2B). The difference in hepatic glucose output may have been due to a blockage of hepatic glycogenolysis by glucagon antibodies, since the content of hepatic glycogen decreased 17 percent ($P < .05$) in group C rats and only 10 percent in group A rats, although this difference is not statistically significant. We previously showed that hepatic glycogenolysis and hepatic vein hyperglycemia accompany rats' meals under these and unrestricted feeding conditions (*17*).

Assuming that the normal prandial glycogenolysis and hepatic vein hyperglycemia are at least partially the result of prandial increases in circulating endogenous glucagon (*11*), the relative hepatic vein hypoglycemia seen after glucagon antibody injection indicates that sufficient endogenous glucagon was neutralized to block the metabolic effects. This is independent evidence of the specific action of the antibody. Glucagon antibodies did not always have glycemic effects in previous studies, perhaps because systemic rather than hepatic blood samples were taken (*18*).

The two effects we observed after injecting glucagon antibodies at mealtime—increased meal size and duration and decreased prandial hepatic vein hyperglycemia—suggest that the increase in circulating glucagon that accompanies meals provides a physiological satiety signal. Our findings are also consistent with the hypothesis that glucagon's satiety effect is mediated by its glycogenolytic and hyperglycemic effects (*4–6*). Our results do not prove this, however, because glucagon antibodies probably oppose glucagon's other biological actions equally effectively. Further, glucagon's hyperglycemic and satiety effects have been dissociated in several experiments (*7, 8, 19*). Thus, the mechanism and the site of glucagon's satiety effect remain to be identified.

W. Langhans
U. Zieger
E. Scharrer
N. Geary

*Institut für Physiologie, Physiologische Chemie und Ernährungsphysiologie, Tierärztliche Fakultät, Ludwig-Maximilians-Universität München, D-8000 München 22, West Germany*

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13. Glucagon antibodies diluted in 1 percent normal rabbit serum in 0.01*M* phosphosaline buffer (*p*H 7.5) containing 0.01*M* EDTA and Trasylol (500 kallikrein inactivated units per milliliter) were purchased from Radioassay Systems Laboratories, Inc., Carson, Calif. For injection, 0.25 ml of this solution was diluted to 1 ml with Krebs Henseleit buffer.
14. Mammalian pancreatic glucagon is a 29–amino acid peptide. The so-called highly specific glucagon antibodies bind the carboxy terminal (amino acids 24 through 29) of purified pancreatic glucagon. They may also bind small amounts of a larger molecular form of glucagon that is suspected to be a glucagon precurser. The highly specific glucagon antibodies do not bind the class of peptides known as gut-type glucagons.

Those enteroglucagons show glucagon-like immunoreactivity only to "nonspecific" antibodies, which bind a part of the amino terminal of glucagon. Although some gut-type glucagons apparently contain the complete glucagon amino acid sequence, the antigenic site at amino acids 24 through 29 is not exposed [J. M. Conlon, in *Glucagon: Physiology, Pathophysiology, and Morphology of the Pancreatic A Cells*, R. H. Unger and L. Orci, Eds. (Elsevier, New York, 1981), p. 55; J. J. Holst, *Digestion* **17**, 168 (1978); J. B. Jaspan, K. S. Polonsky, A. H. Rubenstein, in *Glucagon: Physiology, Pathophysiology, and Morphology of the Pancreatic A Cells*, R. H. Unger and L. Orci, Eds. (Elsevier, New York, 1981), p. 77; A. J. Moody, M. Jacobson, F. Sundby, in *Gut Hormones*, S. R. Bloom, Ed. (Churchill Livingstone, Edinburgh, 1978), p. 369].
15. Radioassay Systems Laboratories gives the cross-reactivity of this antibody (as a percentage of its reactivity to pancreatic glucagon) as 0.0014 to gut-type glucagon, 0.0005 to porcine insulin, and 0.0003 to porcine or synthetic human gastrin.
16. Resting was defined as a stationary posture, with the abdomen supported by the cage floor and with no other behaviors displayed.
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20. Supported by the Deutsche Forschungsgemeinschaft.

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## Larval Testes of the Tobacco Budworm: A New Source of Insect Ecdysteroids

Abstract. *Testes of last-instar larvae of the tobacco budworm release five times more ecdysteroid into incubation medium (judged by radioimmunoassay) in 2.5 hours than is found in testis homogenates. Incubation of testicular components indicates that the testis sheath may be the site of ecdysteroid synthesis. Fractionation of hemolymph, testis homogenate, and incubation medium by high-performance liquid chromatography produces a distinct ecdysteroid pattern in each case. Thus, released testis ecdysteroids are probably converted to other forms for use, sequestration, or general circulation. Their functions are unknown.*

Ecdysteroids, particularly ecdysone and 20-hydroxyecdysone, are currently accepted as the molt and development promoting hormones in insects (*1*). The sources of ecdysone in immature insects are reported to be the prothoracic glands and, to a limited extent, oenocytes associated with epidermis or fat body (*2*). Anomalies, in which ecdysteroid-dependent development occurs in segments experimentally separated from the prothoracic glands, have been reported in at least four insect orders (*3*) and have prompted a search for other organs capable of considerable ecdysteroid synthesis. By the time insects reach adulthood the prothoracic glands degenerate (*1*), but ovaries of some adult females then become capable of secreting ecdysteroids into the circulating hemolymph (*4–8*). Even though testes of adult males contain ecdysteroids, their blood generally holds little or no detectable hormone (*9*). We report now that there is extensive ecdysteroid synthesis and release by isolated testes of late last-instar larvae of the tobacco budworm, *Heliothis virescens*.

Testes of laboratory reared *H. virescens* (*10*) were excised from last-instar larvae just before the pupal molt, at or just before fusion of the paired testes, and washed in sterile insect Ringer solution (*11*) with garamycin (250 mg/ml) (Schering). The osmolality of the Ringer solution was approximately the same as hemolymph of animals at this stage in development (285 mOsmole/kg) (*12*). Testes were separated into components by pulling them apart with forceps in medium and agitating them to expel germinal cells (spermatocysts) and follicle fluid from the sheaths. Sheath and the spermatocyst fractions were rewashed prior to incubation in the Ringer medium. Follicle wash-fluid was saved for analysis by radioimmunoassay (RIA) (*13, 14*). At the end of the incubation period, all tissues and medium samples were processed for RIA. Other tissues (fat body, jaw muscle, hemolymph) were taken simultaneously from the testis donors; brains were taken from 4th-instar larvae. Ecdysteroids were secreted into the medium by intact testes and testis sheaths (Fig. 1) but not by spermatocysts (with or without follicular fluid), muscle, fat body, or brain (Table 1).

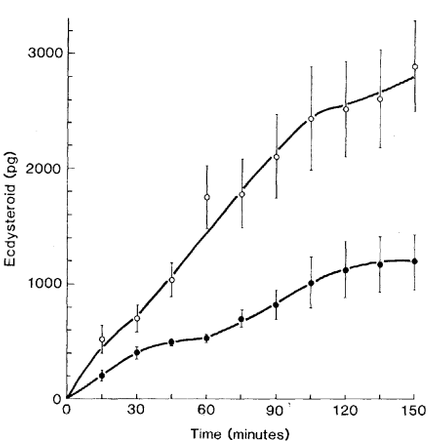


Fig. 1. Ecdysteroid in medium surrounding incubating pairs of whole testes (open circles) and testis sheath-pair equivalents (closed circles). Ecdysteroid content of 100-μl samples, withdrawn every 15 minutes, was determined by RIA (*13*). Fused and nonfused testes were pooled before the experiment; two nonfused testes or one fused testis, incubated in 200 μl of medium, were considered a testis pair. The ordinate represents cumulative data at each time interval. Error bars represent standard errors determined for each point in six separate experiments.

Intact testes released approximately twice as much RIA-detectable ecdysteroid as sheath preparations during the incubation period. More than half of the RIA-sensitive ecdysteroid of whole testes ($N$ = 26) was found in testis follicular fluid (means ± S.D.: 53 ± 7 percent); 20 ± 8 percent was in sheath preparations, and 8 ± 2 percent appeared in washed spermatocysts. The ecdysteroid synthesis tissue appears to be part of the sheath of the testis; it may therefore be analogous to the ecdysteroid-synthesizing follicular epithelium of adult female insect ovaries (*6–8*). High-performance liquid chromatography (HPLC) fractionation (*15*) of methanol extracts of hemolymph, whole testes, and whole testis incubation medium (2.5 hours) followed by RIA of resulting fractions, elicited the ecdysteroid patterns shown in Fig. 2. Peaks E, G, H, and I were common to all preparations. Peaks E and G cochromatographed in the same solvent system with makisterone A and 20-hydroxyecdysone, respectively. Peaks H and I probably correspond to 20,26-dihydroxyecdysone and a highly polar component, respectively (*16, 17*). Hemolymph and whole testes also showed peak C (cochromatographed with ecdysone in our system), peak $F_1$ (cochromatographed with 26-hydroxyecdysone), and peak A (unidentified). Hemolymph and testis ecdysteroid patterns are similar to those described at various times in the life cycles of other insects and as metabolites of ecdysone (*7, 16–18*).

Although the antibody used for RIA in this study is highly sensitive to ecdysteroids, it does not bind each analog to the same degree (*14*) so that the height of each ecdysteroid analog peak in Fig. 2 may not reflect the actual amount of analog. However, the relative titers of corresponding peaks may be compared. Peak $F_1$ is about twice as high in the testis extract as in the hemolymph, while peak G is about half as high. Therefore the proportion of hormones in testis tis-


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