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7. Cathodal d-c current (0.5 ma) was passed for 20 seconds through a 90 percent platinum–10 percent iridium electrode with the uninsulated tip implanted 1 mm anterior to the ear bars (level of oculomotor nucleus), 1.5 mm lateral to the midsagittal sinus, and 6.7 mm below and perpendicular to the surface of the cortex (A1.0, L1.5, D6.7). In a preliminary experiment, histology performed on rats shortly after lesions were made revealed an area of complete tissue loss slightly larger than the ventral noradrenergic bundle, as mapped by Ungerstedt (*4*). Final histological examination of all experimental animals verified correct lesion placements.
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10. A single dose of 8.0 μg of 6-OH-DA hydrochloride dissolved in 0.8 μl of saline (containing ascorbic acid, 0.2 μg/μl) was bilaterally injected at 0.4 μl/min through a stereotaxically lowered 27- or 32-gauge cannula, which was withdrawn 4 to 5 minutes after completion of the injection; coordinates were as in (*7*). Placement was verified in all rats.
11. Coordinates: A0.2 (level of the trochlear nucleus), L1.5, D6.9; same procedure as in (*10*).
12. Five of six additional rats treated with more dilute 6-OH-DA (8 μg/4 μl) also became hyperphagic; the mean increase for all six was 38.8 percent.
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16. Mann-Whitney U test. For differences termed nonsignificant, $P > .10$; for those termed significant, $P < .05$ or less as noted.
17. Coordinates: A2.8, L0.85, D5.6; procedure as in (*10*).
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19. In a more recent series of rats, one animal markedly depleted of noradrenergic fluorescent varicosities in the diencephalon and telencephalon failed to become hyperphagic. Evidently, norepinephrine depletion is necessary for the hyperphagia syndrome reported here but may not always be sufficient for it. T. Maedo and H. Shimizu [*Brain Res.* **36**, 19 (1972)] described a second, smaller noradrenergic projection to the hypothalamus in addition to the ventral bundle. Since our histological examination confirmed that electrolytic lesions leading to hyperphagia spared the region through which this bundle projects, we conclude that its destruction is not necessary for feeding increases. However, whether damage to this pathway contributes to the hyperphagia is undetermined.
20. Assays for all three neurotransmitters were performed on homogenate fractions of the same brains by a slight modification of the techniques of C. L. Chang [*Int. J. Neuropharmacol.* **3**, 643 (1964)]; R. P. Maickel, R. H. Cox, J. Saillant, F. P. Miller [*ibid.* **7**, 275 (1968)]; and D. R. Haubrich and J. S. Penzer (*Anal. Biochem.*, in press).
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25. Since the ventral noradrenergic bundle also projects to extrahypothalamic areas, the present results may reflect norepinephrine loss in these regions as well as in the hypothalamus.
26. An α-adrenergic (norepinephrine-elicited) satiety mechanism has been proposed by D. L. Margules [*Life Sci.* **8**, 693 (1969); *J. Comp. Physiol. Psychol.* **73**, 1 (1970)]. It is not clear how our findings relate to theories of β-adrenergic (isoproterenol-elicited) satiety [D. L. Margules, *J. Comp. Physiol. Psychol.* **73**, 1 (1970); S. F. Leibowitz (*3*); H. W. Goldman, D. Lehr, E. Friedman, *Nature* **231**, 453 (1971)] unless mediated by norepinephrine [S. F. Leibowitz, *Proceedings, 79th Annual Convention of the APA* (American Psychological Association, Washington, D.C., 1971), p. 741].
27. An abstract of some of this work has appeared (*13*). Later experiments are part of the dissertation of J.E.A. submitted to the department of psychology, Princeton University. We thank P. K. Randall for performing the monoamine assays.

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Amphibian Pituitary Growth Hormone and Prolactin: Immunochemical Relatedness to Rat Growth Hormone

Abstract. *Growth hormone and prolactin were electrophoretically isolated from amphibian pituitaries and then were tested in a radioimmunoassay with labeled rat growth hormone and antiserum to the same hormone. This isolation and purification of the hormones increased the steepness of the slopes of competitive inhibition in this system when compared to those of crude extracts. Both hormones from most species tested showed high immunochemical cross-reactivity, indicating that amphibian growth hormone and prolactin are structurally related to rat growth hormone.*

The results of comparative immunochemical studies indicated that the growth hormones (GH's) in extracts of pituitaries of nonmammalian tetrapods fall into major immunochemical categories that correspond closely to their phylogenetic relationship to mammals (*1*). Thus there may be common immunochemical molecular features (determinants) among the GH's of all tetrapods, and the number of such common determinants may vary with phylogeny. However, there are several problems in interpreting the results of such studies when they are based on the use of crude pituitary extracts. In particular, we must consider the possibility that the immunological reactivity in the pituitary extracts from certain nonmammalian species may not be due solely to GH.

Comparative bioassays of GH's and prolactins (PRL's) have revealed a significant overlap in the biological properties of these two hormones from diverse tetrapod species (*2*); the occurrence of such overlap is not predictable on the basis of phylogenetic relationships. In addition, the primary structures of these two hormones from mammalian pituitaries are similar (*3*). Either or both hormones may possess common immunologic determinants in some species but the occurrence of these common determinants may be unrelated to phylogeny. We now present evidence that such phylogenetically unrelated cross-reactivities occur among PRL's and GH's which were isolated from pituitaries of diverse amphibian species.

We separated GH and PRL from fresh frozen pituitary glands of several anuran and urodele amphibia by polyacrylamide disc electrophoresis (*2*). The two hormones are well separated by this procedure; the PRL's have a high electrophoretic mobility relative to the GH's of all species (*2*, *4*). The protein bands containing these hormonal activities from extracts of adenohypophyses from several amphibian species were previously identified by bioassays. We used the linear growth test in juvenile toads to identify GH (*2*) and the local pigeon crop sac for PRL (*4*).

In the present study, the hormones were obtained by electrophoretic separation of extracts of pooled glands from adults of both sexes (Table 1). We tested the efficacy of the electrophoretic system for the separation of the hormones by using rat adenohypophyses which were processed similarly to the amphibian adenohypophyses.

We also took a sample of each crude pituitary extract for analysis by radioimmunoassay prior to electrophoresis. Immunochemical studies were performed by a double antibody radioimmunoassay technique with monkey antiserum to rat GH (*1*). Highly purified rat GH (Ellis, 3.0 U.S.P. units per milligram) was used for preparation of standards as well as for iodination with ^{131}I or ^{125}I. We made several dilutions of the polyacrylamide gel eluates and tested each dilution for its ability to compete with labeled rat GH for antibody to rat GH. The slopes of the curves obtained from the data on competitive inhibition of the binding of labeled rat GH to the antibody (Fig. 1) were used to determine the degree of relatedness among the test materials and the rat GH. The relative position of this dilution curve was used to estimate the apparent concentration of GH in any sample (Table 1). Emphasis was placed on the relative reactivities of the GH and PRL from each species rather than on absolute concentrations of hormone.

The results of the test with GH and PRL isolated from rat glands confirmed that the electrophoretic system could effectively separate the two hormones (Table 1). The slope of the eluate prepared from the region of the gel corresponding to rat GH as determined by bioassay (*2, 5*) was indistinguishable from that of the purified rat GH standard. The graph obtained from the eluate prepared from the region of the gel containing PRL bioactivity (*5*) also yielded a slope similar to the rat GH standard but only at a very high concentration. The results obtained from the test of the rat PRL eluate indicate that this fraction had a GH content equivalent to only 0.14 percent of that contained in the GH region of the gel.

In accordance with previous findings (*1*), the crude extracts of all amphibian pituitaries showed significant cross-reactions with the antiserum to rat GH and, in most cases, the immunoreactive substance or substances in the amphibian extracts differed from rat GH, as evidenced by the relatively flat slopes (see data for bullfrog in Fig. 1). However, with extract from the glands of the newt *Taricha* the slope of the inhibition curve was considerably steeper than those observed previously with extracts of either amphibian, reptilian, or avian glands. As was expected from the reactivity of the extracts, eluates obtained from the region of the polyacrylamide gel corresponding to

Table 1. Immunochemical cross-reactivity of various amphibian and mammalian prolactins with monkey antiserum to rat GH.

Source of prolactin	GH immunoreactivity (%)
Electrophoretically isolated	
Rat	0.14
Ambystoma tigrinum	0.16
Necturus maculosus	24.40
Taricha torosa	25.00
Bufo marinus	23.80
Bufo boreas	50.00
Rana pipiens	65.00
Rana catesbeiana	80.00
Purified prolactins	
Rat (Ellis, H-IV 8c)	0.40
Ovine (NIH S-9)	0.05
Bovine (NIH B-2)	1.50
Porcine (Li)	0.80

bioassayable GH (*5*) also inhibited the binding of labeled rat GH to rat GH antibody. However, in practically all cases, the slopes of these curves were considerably steeper than those obtained with the respective crude extracts (Fig. 1). Furthermore, except for the eluates from the tiger salamander (*Ambystoma tigrinum*), significant cross-reaction occurred with eluates from the region of the gel corresponding to the position of the stainable

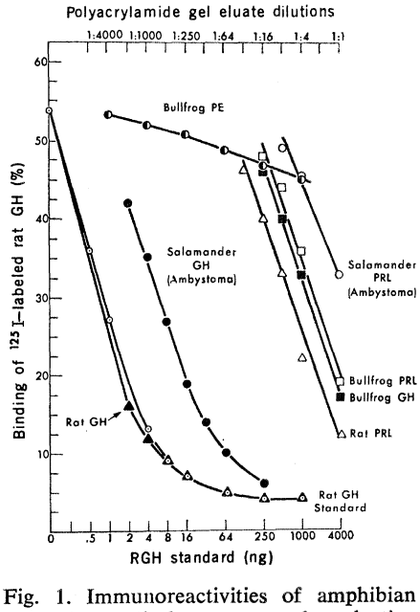

Fig. 1. Immunoreactivities of amphibian and rat growth hormones and prolactins isolated by polyacrylamide disc electrophoresis. The hormones were assayed for their ability to compete with rat GH for antibody to rat GH in a radioimmunoassay. A crude pituitary extract (*PE*) from the bullfrog (*Rana catesbeiana*) is included for reference. The highest concentration tested is represented by 64 μg (wet weight) of tissue. *RGH*, rat GH.

protein band previously shown to have the greatest PRL bioactivity (*4*). In *A. tigrinum*, the PRL region exhibited only about 0.16 percent of the immunoreactivity of the GH region. This is comparable to the low activity observed with PRL from rat glands (Table 1). In all other species of amphibians tested, the apparent GH activity in the PRL region of the gels ranged from 24 to 80 percent of that observed in the respective GH regions (Fig. 1 and Table 1).

Our results indicate that common immunochemical determinants are shared among the GH's of mammalian and amphibian species. However, they raise certain questions regarding the interpretation of earlier findings, at least for the Amphibia, which were based on studies with crude pituitary extracts. First, the apparent correlation between phylogenetic relation and the degree of immunochemical relatedness, as evidenced by the slopes of the inhibition curves, must be reconsidered. The slopes of the curves obtained with the partially purified GH from the amphibians tested are consistently steeper than those obtained with the homologous crude extracts; in fact, they are steeper than the slopes obtained previously with reptilian and avian pituitary extracts. In the case of the newt *Taricha* even the crude extract exhibited a steeper slope than did the reptilian material. A similar increase in slope was also observed when turtle GH was purified (*6*). However, the change in slope with purification is not a general phenomenon since purified duck GH (*6*) and shark GH (*7*) showed no significant increase in the slope of their inhibition curves when compared to the respective crude extracts. Further work with GH's purified from the pituitaries of additional species may enable us to make more meaningful generalizations regarding the slopes of inhibition curves. The basis for the increases in the steepness of the slopes observed in this study with the purified GH's is not apparent at this time.

The second major concern arising from our results is the strong cross-reaction observed with some of the amphibian PRL preparations with the antiserum to rat GH. The monkey antiserum to rat GH showed very low cross-reactivity with purified PRL's of several mammalian species (Table 1). These cross-reactivities could be accounted for by the known contamination of the PRL's with GH. The rela-

tive inactivity of the *Ambystoma* PRL in this immunologic system is consistent with the results with rat glands and confirms the effectiveness of the electrophoretic system for separating amphibian GH and PRL. In contrast is the high immunoreactivity in the PRL zone of the polyacrylamide gels from the other amphibians, especially the two *Rana*. We cannot rule out the possibility that the immunoreactivity shown by most of the amphibian PRL's is due to contamination with GH. However, if such contamination occurs, it must represent a modified form of GH that does not migrate in the usual electrophoretic position. In order to account for this high degree of immunoreactivity by postulating GH contamination, the PRL region would have to contain up to 80 percent of the total GH present in the region identified as having GH bioactivity. Such levels of contamination seem unlikely since our bioassay failed to show significant somatotropic activity in the PRL region from *Necturus* and *Rana catesbeiana* when tested in the toad (*2*). Furthermore, when the PRL eluate was subjected to further electrophoresis under different conditions the *Rana* PRL was homogeneous.

On the basis of available information it appears that PRL from several anuran and urodele amphibia may have a high degree of immunochemical relatedness to amphibian as well as to mammalian (rat) GH. Since the actual amount of protein eluted from the GH and PRL regions has not been determined, it is not possible to compare the specific immunoreactivity of each hormone preparation. Our results provide further evidence of the structural similarities between these two pituitary hormones. In addition they emphasize the need for caution when attempting to use antibodies against mammalian hormones to measure hormones in nonmammalian species, even when the antiserum shows high specificity for the homologous mammalian hormone.

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Lack of Glucagon Response to Hypoglycemia in Diabetes: Evidence for an Intrinsic Pancreatic Alpha Cell Defect

Abstract. *Despite excessive glucagon responses to infusion of arginine, plasma glucagon did not rise in six juvenile-type diabetics during severe insulin-induced hypoglycemia, whereas glucagon in the controls rose significantly. Thus in diabetics pancreatic alpha cells are insensitive to glucose even in the presence of large amounts of circulating insulin. An intrinsic defect common to both alpha and beta pancreatic cells—failure to recognize (or respond to) plasma glucose fluctuations —may be operative in juvenile diabetes.*

Juvenile-type diabetes mellitus is generally thought to result mainly, if not exclusively, from deficient insulin secretion by pancreatic beta cells (*1*). Recent studies (*2–10*), however, suggest that pancreatic alpha cell dysfunction may also play an important role. Plasma glucagon levels are often elevated in diabetes (*3–5*). There is lack of suppression of glucagon by glucose (*6*), and glucagon responses to intravenous arginine are excessive (*7*). Nevertheless, it is unclear whether these abnormalities represent an intrinsic defect in alpha cells (*8*) or are merely the result of insulin deficiency (*9*). In dogs made

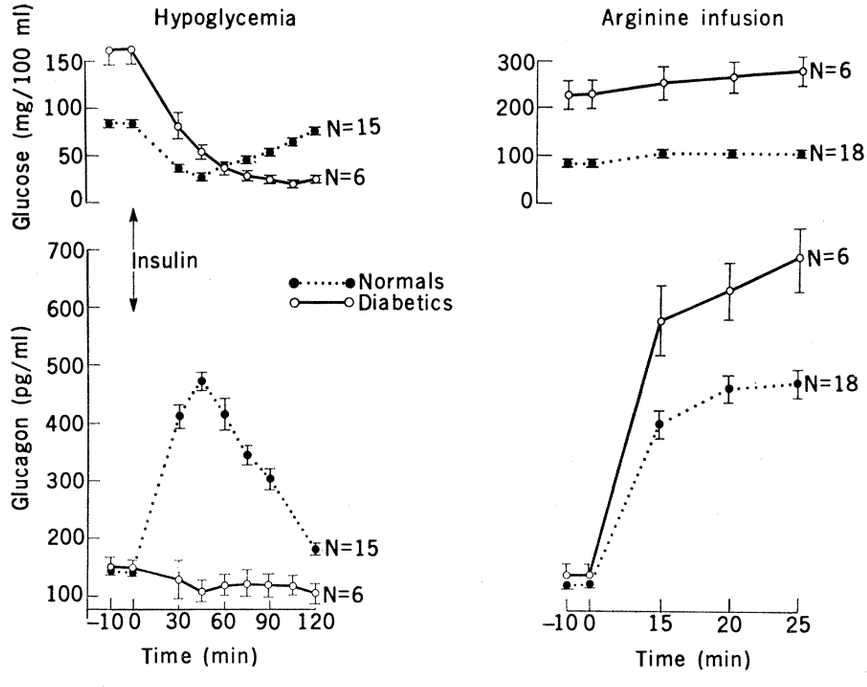

Fig. 1. Plasma glucose and glucagon responses to insulin-induced hypoglycemia (left) and to intravenous arginine infusion (right) in juvenile-type diabetics (——) and in normal controls (- - - -). Vertical bars indicate standard errors; N is the number of patients.