comparatively large size and nondeformability of avian erythrocytes, at equal tube diameters and hematocrits the relative viscosity of avian erythrocyte suspensions is four to six times greater than in mammalian erythrocyte suspensions (*7*). In apparent compensation, the fraction of tissue volume occupied by brain capillaries is about twice as high in birds as in mammals (*8*). In a tissue like the brain, contained in an inelastic skull, further increases in capillary density would increase O_2 delivery, but the resulting increase in tissue volume occupied by capillaries would reduce neuronal space. It therefore seems plausible that further evolutionary improvements in O_2 delivery would be brought about through increases in the diffusion gradient; this could be achieved by means of the proposed mechanism.

We conclude that the P_{O_2} is increased and the P_{CO_2} is decreased in a portion of the arterial supply to the pigeon brain, thereby facilitating diffusion of O_2 into and CO_2 out of brain tissue by increasing the respective gradients. Blood O_2 affinity is lower in birds than in similar-sized mammals, and arterial blood is not likely under most conditions to be saturated with O_2 (*9*). We therefore suggest further that, in raising arterial P_{O_2}, the mechanism must also increase hemoglobin O_2 saturation. This would (i) sustain steady-state O_2 delivery at lower cerebral blood flows than would otherwise be necessary, and (ii) increase brain O_2 delivery under conditions in which an increase in cerebral blood flow alone could not meet the O_2 demand, such as at high altitudes.

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Induction of Pituitary Lactotrope Differentiation by Luteinizing Hormone α Subunit

Abstract. *Addition of gonadotropin releasing hormone to cultures of fetal rat pituitary induced differentiation of lactotropes as revealed by immunocytochemistry. Antiserum to luteinizing hormone (LH) (recognizing native LH), but not antiserum to LH-β (recognizing both native LH and its β subunit), inhibited this induction. Further addition of highly purified LH-α subunit in culture medium also induced lactotrope differentiation. Thus, the α subunit may have a specific biological activity of its own with probable practical use in clinical investigations.*

Induction of pituitary lactotrope differentiation by gonadotropin releasing hormone (GnRH) has been reported (*1*). This effect of GnRH is possibly subsequent to a primary action on gonadotropes and induces a further release of stimulating factor or factors. Such a chain of events has been described for the stimulation of the release of prolactin from adult rat lactotropes (*2*). Moreover in human anencephalic fetuses, where hypothalamic GnRH is lacking, lactotropes are numerous and active (*3*). In these types of fetuses, gonadotropes are known to store the α subunit almost exclusively (*4*, *5*). A possible relation between the abundance of this subunit and the proliferation of lactotropes was therefore considered. We now describe the role of the α subunit as mediator in the action of GnRH and in the differentiation of lactotropes.

Adenohypophysial primordia were explanted from 13-day-old rat fetuses and cultured in a synthetic medium as described (*1*, *6*). Cultures were maintained for 8 days to reach the expected term of gestation when the first lactotropes would normally appear in vivo (*7*). The culture medium was renewed every 2 days.

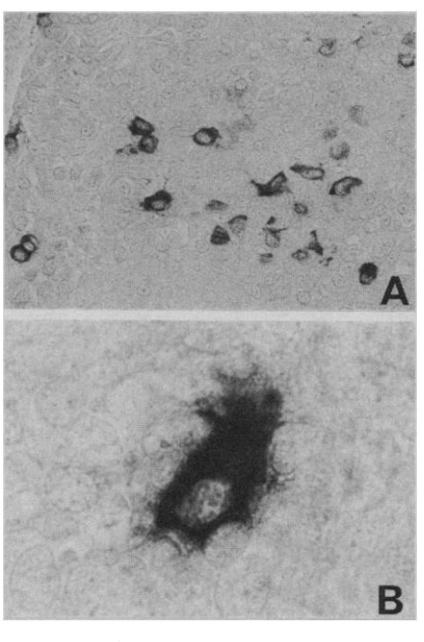

Fig. 1. Immunocytochemistry with antiserum to rat prolactin diluted 1:800 at 4°C overnight (light microscopy). This antiserum has been studied and used previously (*1*). Typical primordium cultured in medium containing LH-α ($10^{-7}M$) (the experiment was repeated four times, and a total of 20 primordia was studied). (A) Several immunoreactive cells are scattered in the cultured tissue (×250). (B) The immunoreactive cells are irregularly shaped and intensely stained (×1200).

When GnRH (Roussel-Uclaf) was added at a concentration of $10^{-9}M$ during the first 24 hours of culture, the lactotropes differentiated in all the primordia tested, and the mean number of immunoreactive cells was relatively high (Table 1). The role of GnRH is to induce the synthesis and release of gonadotropins. Follicle-stimulating hormone (FSH) is undetectable in rat fetal pituitary gland at the end of gestation (*8*), while luteinizing hormone (LH) is assayable from day 18 of gestation. Therefore, the possible effect of LH and its subunits was investigated in our system.

Primordia were cultured with GnRH as described except that an antiserum to pure rat LH (10 percent by volume) was also added throughout the culture period. Under these conditions, the number of immunoreactive lactotropes decreased (see Table 1). The antiserum used binds intact LH but not intact FSH or thyroid-stimulating hormone (TSH) (*9*) and recognizes the common α subunit (*10*). Because the action of GnRH was totally abolished under these conditions, GnRH may act either by the intact LH or by its subunits.

In contrast, when antiserum to porcine LH-β (10 percent by volume) was added instead of antiserum to rat LH, the number of differentiated lactotropes was not significantly altered (see Table 1). The specificity of this antiserum for the β subunit of LH has been shown (*4*); the antiserum also binds with intact LH of several species including rat (*10*) but not with the α subunit. Thus, these data suggest that the free α subunit mediates GnRH induction of lactotropes. Moreover, intravenous injection of GnRH in humans is known to stimulate the release of α subunit before intact LH (*11*).

Another series of experiments was designed to test the effects of the α subunit on lactotrope differentiation. Highly purified porcine LH-α was added to the cultured primordia in three doses throughout the culture period instead of GnRH. The α subunit was purified as described and exhibited less than 0.5 percent contamination with LH and LH-β (*12*). The induction of immunoreactive lactotropes was dose-dependent: at $10^{-12}M$ there was no differentiation of lactotropes; at $10^{-9}M$ immunoreactive lactotropes were detected in all the cultured primordia; and at $10^{-7}M$ the number of immunoreactive cells was significantly increased (220 percent compared to $10^{-9}M$) (Table 1) and the cells were strongly immunoreactive (Fig. 1). This dose dependency supports the specificity of the action of the α subunit. The ultrastructure of immunoreactive lactotropes (Fig. 2) was found to be similar to that of cells induced by GnRH (*1*), thus implying their identity.

The α subunit is common to all glycoprotein hormones (LH, FSH, TSH, and chorionic gonadotropin), and its association with one of the specific β subunits is mandatory for the expression of the usual different hormonal activities (*13*). However, our study shows that the glycoprotein α subunit may have a biological role of its own without association with one of the specific β subunits.

Although our results show that GnRH induction of lactotropes is mediated through the glycoprotein α subunit, it must be pointed out that the number of lactotropes in cultures treated with LH-α was significantly lower than the number present after GnRH treatment. This difference may be due to the porcine origin of the LH-α; porcine LH may therefore be less active on rat hypophysis. Moreover, adherent junctions have been described between gonadotropes and lactotropes in the adult rat (*14*). If these junctions exist in the fetal pituitary gland, they may facilitate the passage of the stimulating factor induced by the GnRH from the gonadotropes directly to

Table 1. Differentiation of lactotropes. In evaluating the efficacy of each of the culture conditions, the number of immunoreactive lactotropes per square millimeter was estimated as follows (*9*). Whole cultured primordia were serially sectioned throughout the experiments, and the number of sections per primordium depended on the size and orientation of the block. For each primordium, every fourth section was drawn with a projecting prism (Wild-Leitz), the surface area was measured with the use of an image analyzer (Kontron), and the number of immunoreactive cells was counted. The total number of cells per primordium was divided by the total surface area to obtain the cell density. The values for t are statistically significant at the $P < 0.05$ level (Satterthwaite approximation used to account for the inequality of variance and Bonferroni correction used for multiple comparisons versus GnRH). For all experiments $n = 4$ except GnRH plus antiserum to porcine LH-β, where $n = 3$.

Additions	Concentration (M)	Immunoreactive lactotropes* (mean ± standard error of the mean)	Difference from $10^{-9}M$ GnRH (± standard error)	t
None		0		
GnRH	10^{-9}	325.0 ± 50.2		
+ antiserum to rat LH		5.0 ± 2.4	320 ± 50.3	6.4
+ antiserum to porcine LH-β		244.0 ± 6.2	81.0 ± 50.6	
Porcine LH-α	10^{-9}	47.5 ± 9.9		
Porcine LH-α	10^{-7}	156.8 ± 23.4	168.2 ± 55.4	3.0
			(109.3 ± 25.4)†	(4.3)

*Numbers per square millimeter of cultured tissue. †Difference from porcine LH-α at $10^{-9}M$.

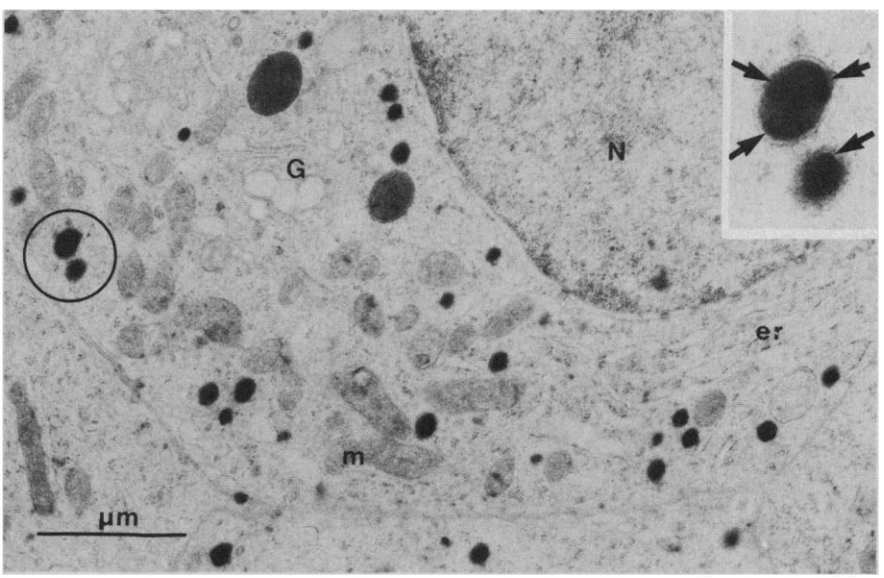

Fig. 2. Ultrastructure of immunoreactive lactotrope in primordium cultured with LH-α. Tissues were treated as described (*20*). For the immunocytochemical reaction, antiserum to rat prolactin (see legend to Fig. 1) was used at 1:800 overnight. Peroxidase-antiperoxidase complexes (arrows) were found over the secretory granules, which were up to about 200 nm in diameter. The ultrastructure is the same as that observed when the lactotropes are differentiated under the effect of GnRH. Abbreviations: N, nucleus; m, mitochondrion; er, endoplasmic reticulum; G, Golgi complex (×20,000). Inset, ×50,000.

the latent lactotropes, thus avoiding unnecessary waste in the extracellular spaces.

Such a biological activity for the free α subunit may explain hitherto unconnected observations. For example, in human anencephaly the large number of lactotropes in the absence of hypothalamic GnRH (*3*) would be due to the exceptionally high concentration of α subunit in pituitary because of the almost complete absence of intact hormone (*4, 5*). In cultures of human fetal pituitaries, the secretion of α subunit but not intact LH persists and even increases after several weeks (*15*). This could occur in connection with an increase in the secretion of prolactin observed under similar conditions (*16*).

In clinical investigation a role of the α subunit should be considered. Indeed, in some pituitary adenomas with markedly elevated concentrations of serum α subunit, the amounts of serum prolactin were also elevated (*17*). Injection of GnRH gave a greater increase in the concentration of serum α subunit in these hyperprolactinemic patients than in normal controls (*18*). In contrast, bromocriptine therapy, which reduces serum prolactin, also reduced the peak serum concentration of α subunit (*18*). As yet, a possibly direct relation between the abnormal secretion of α subunit and hyperprolactinemia has not been considered. These variations should be studied further to appreciate fully their clinical implications.

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Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans in Sediments from Siskiwit Lake, Isle Royale

Abstract. *Polychlorinated dibenzo-*p*-dioxins and dibenzofurans were found in sediment from Siskiwit Lake on Isle Royale, Lake Superior, a location which can receive only atmospheric inputs. The source of these compounds is the atmospheric transport of dioxins and furans formed by combustion of domestic and chemical waste.*

The reputation of chlorinated dioxins and dibenzofurans as extremely toxic chemicals to man (*1*) is largely based on the ability of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and -furan to kill guinea pigs at very low doses (0.6 μg/kg) (*2*). Dioxins and dibenzofurans have several sources and can thus enter the environment either through the atmosphere or through the water. Examples of the atmospheric path include the explosion of a trichlorophenol reactor at Seveso, Italy (*3*), and the incineration of chlorinated waste chemicals (*4*). Examples of the aquatic path include direct industrial discharge into a river (*5*) and leaching at a hazardous waste facility (*6*). Distinguishing between these paths has obvious regulatory implications but is not easy.

We have investigated the atmospheric path by measuring polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) in sediments from Siskiwit Lake, which is located on Isle Royale in northern Lake Superior. This island has been a national

Table 1. Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans in Siskiwit Lake sediment and in two air particulate samples. The three sediment depths correspond to depositional dates of 1982 for 0 to 0.5 cm, 1953 for 5 to 6 cm, and 1935 for 8 to 9 cm. Abbreviations for the various PCDF and PCDD are: tetrachlorofuran, TCDF; pentachlorofuran, PnCDF; hexachlorofuran, HxCDF; heptachlorofuran, HpCDF; octachlorofuran, OCDF; tetrachlorodioxin, TCDD; pentachlorodioxin, PnCDD; hexachlorodioxin, HxCDD; heptachlorodioxin, HpCDD; and octachlorodioxin, OCDD. Concentrations are given as parts per dry weight extracted; ppt, parts per trillion; ppb, parts per billion.

Compound	Sediment (ppt)			Air (ppb)	
	0 to 0.5 cm	5 to 6 cm	8 to 9 cm	Washington, D.C.	St. Louis
TCDF	15	18	N.D.*	1.3	0.2
PnCDF	5	2	N.D.	1.2	0.2
HxCDF	2	2	N.D.	0.8	0.3
1,2,3,4,6,7,8-HpCDF	8.2	12	1.6	9	4.3
1,2,3,4,6,8,9-HpCDF	11	5	N.D.	8.5	7.4
1,2,3,4,7,8,9-HpCDF	1	0.4	N.D.	0.5	0.4
OCDF	4	3.2	1.1	6.2	0.5
TCDD	26	12	N.D.	0.5	1.1
PnCDD	12	11	N.D.	6.4	0.2
HxCDD	10	8	N.D.	1.6	1.2
1,2,3,4,6,7,9-HpCDD	32	20	3.7	9.2	11
1,2,3,4,6,7,8-HpCDD	38	26	4.5	12	14
OCDD	560	390	54	200	170

*Not determined (N.D.), <0.4 ppt.