that glucose utilization in the deeper laminae of the superior colliculus are unaltered by unilateral enucleation (Table 2 and Fig. 1) are consistent with present knowledge of the functional organization of this structure.

The administration of apomorphine to the unilaterally enucleated animals maintained in the dark increased glucose utilization only in the superficial layer of the superior colliculus to which the visual pathway remained intact (Table 2 and Fig. 1). In contrast, apomorphine stimulated glucose utilization equally in the deeper laminae of both superior colliculi, and the response was unaffected by the unilateral enucleation (Table 2 and Fig. 1). Similarly, the alterations in glucose utilization produced in nonvisual structures of the CNS by apomorphine administration were not influenced by the unilateral enucleation.

Although various agents—particularly dopamine, acetylcholine, and γ-aminobutyric acid—have been implicated as possible neurotransmitters in the retina (*10*), the complex synaptic organization of this tissue has impeded the elucidation of the role of each of these agents in the receipt and processing of visual information. The dopamine-containing amacrine cells ramify extensively within the inner plexiform layer and form complex synaptic interconnections with both dopaminergic and nondopaminergic processes (*4*). A dopamine-sensitive adenyl cyclase has been characterized in retinal tissue in vitro (*5*), and the biochemical pharmacological responsiveness of the dopaminergic system in the retina appears to be similar to that of other regions of the CNS (*5*). In contrast, the superficial layer of the superior colliculus has extremely low concentrations of dopamine (*11*), and there is no evidence that it contains dopaminergic receptor systems. Electrophysiological examination of the action of dopamine in the retinal systems has been limited, although it provides some evidence that electrical activity in the retina may be modified by dopamine (*12*). The relation of the retinal dopaminergic system to visual stimuli is not clearly understood, but the rate of synthesis of the amine and its release from the retina are known to depend on experimental lighting conditions (*13*).

We have demonstrated both under ambient laboratory lighting conditions and in the dark that the administration of the putative dopaminergic agonist apomorphine results in increased metabolic activity in the superficial and deep layers of the superior colliculus but not in other primary visual structures in the CNS. Removal of most of the retinal input to one side of the brain by unilateral enucleation abolishes the effect of apomorphine only in the superficial layer of the superior colliculus of the deafferentated side without altering the metabolic effects of apomorphine in the deep layer and elsewhere in the CNS. These results point to the retina as the locus of the action of apomorphine resulting in effects in the superficial layer. They also suggest that the retinal dopaminergic systems function mainly in the retinal pathways to the superior colliculus and not to other primary visual areas.

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Placental Luteinizing Hormone–Releasing Factor and Its Synthesis

Abstract. *The synthesis of a placental luteinizing hormone-releasing factor (pLRF), which is immunologically, physiochemically, and biologically indistinguishable from synthetic LRF, was demonstrated. The incorporation of ^{3}H-labeled leucine by human placental tissue in vitro into pLRF was determined by purification on carboxymethyl-cellulose and specific immunoprecipitation of the ^{3}H-labeled pLRF. The specific activity of the pLRF released into the medium increased 100-fold from day 1 to day 2 of culture and attained a concentration of 2.84 microcuries per microgram. These data indicate that the pLRF that was released initially was endogenous, whereas that released subsequently reflected synthesis.*

Luteinizing hormone-releasing factor (LRF) is a decapeptide synthesized and stored in the hypothalamus. It acts on the pituitary gland to stimulate the synthesis and release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (*1*). Recently we found immunoreactive LRF in human placenta by radioimmunoassay and immunofluorescence microscopy (*2*). The immunofluorescence technique revealed that the placental LRF (pLRF) was present in the cytotrophoblast but not the syncytiotrophoblast of the placental villi. The radioimmunoassay indicated that the concentration of pLRF in human placentas varied according to the duration of pregnancy but not the sex of the fetus (*2*). The enormous quantities of pLRF in the placenta led us to suggest that the placenta may be an extrahypothalamic source of this factor during human pregnancy. This hypothesis is supported by a study showing that when placental tissue is cultured in vitro there is a 5- to 40-fold net increase in the concentration of pLRF in the culture medium after 4 days (*3*).

The studies described herein provide direct evidence of the synthesis of pLRF in vitro by the term placenta. Tritiated leucine was incorporated by the human placenta in vitro into a peptide biochemically, immunologically, and biologically identical to LRF.

Human placentas were obtained immediately after normal term deliveries. Placental villi were dissected free of membranes and maternal basal plate.

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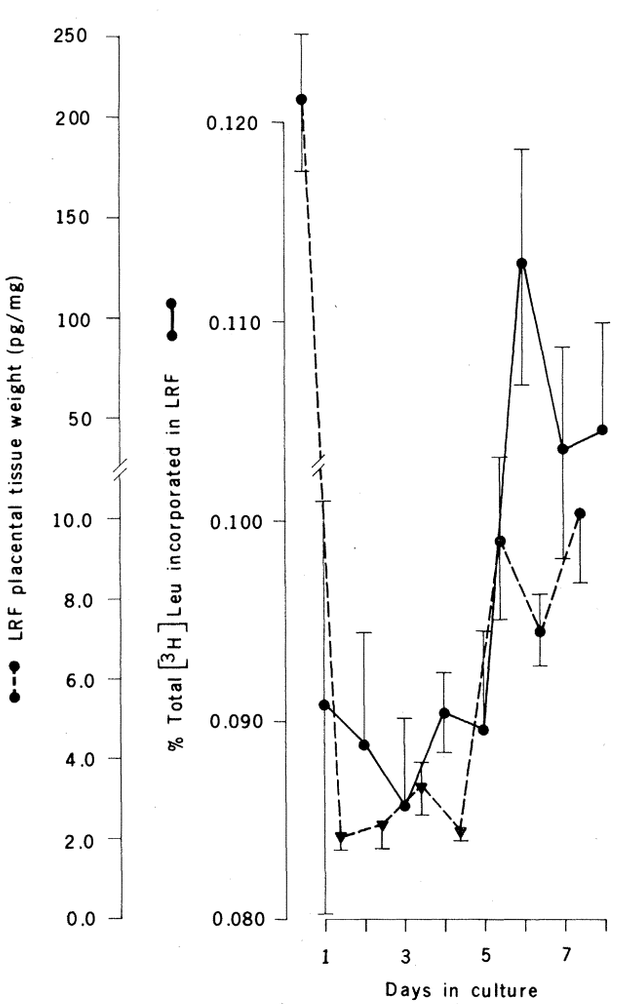
The villous tissue was cut into fragments of ≤ 1 mm^3. An average of 30 mg (net weight) of tissue explant was used in each culture dish. The tissue fragments were placed on an organ culture grid so that they touched the surface of the tissue culture medium but were not immersed in it. Each petri dish had 1 ml of Medium 199 containing [^{3}H]leucine (1 μCi/ml; specific activity, 115 Ci/mmole), penicillin and streptomycin (200 U of each per milliliter), and fungizone (0.025 μg/ml). Five flasks were incubated at 37°C in humid chambers with an atmosphere of 5 percent CO_2 and 95 percent air. The media were changed daily for 8 days. All the samples were kept frozen at −4°C until the hormone assays were done. The media from each culture and for each day of culture were analyzed for pLRF (*3*), α- and β-human chorionic gonadotropin (hCG) (*4*), and human chorionic somatomammotropin (hCS) (*5*) by specific radioimmunoassays.

The largest amount of pLRF was released during day 1 of culture; release of pLRF declined from days 2 to 5 and then increased again on the last 3 days of culture (Fig. 1). The release of hCS declined rapidly after day 1 and remained low throughout the remaining culture period. The release of both α- and β-hCG was similar to that for pLRF, but the increase in α- and β-hCG during the last days of culture lagged behind that of LRF by about 1 day.

The synthesis of pLRF was demonstrated by adding [^{3}H]leucine to the culture medium and observing its incorporation into a molecule immunologically, physiochemically, and biologically indistinguishable from the releasing factor. Portions of tissue culture media were chromatographed on carboxymethyl (CM)-cellulose as described previously (*2*). The [^{3}H]leucine eluted in the same areas as hCS, the hCG's, free leucine, and a second peak in the LRF area (Fig. 2). Portions of the pLRF fractions contained 90 percent of the immunoreactive pLRF of the original culture medium.

The total amount of [^{3}H]leucine in the LRF fractions was calculated for each of the five replicate cultures and averaged for each day of culture. The percentage of [^{3}H]leucine incorporated into pLRF was constant during the first 5 days, then it increased significantly on days 6 to 8 (Fig. 1). When compared to the immunoreactive pLRF released into the culture medium, a similar pattern was noted from days 2 to 8 and a constant specific activity for pLRF released from days 2 to 8 was observed. These data demonstrate that after day 1 of culture, the rate of release reflected the rate of synthesis.

Using immunoprecipitation with a specific antiserum to LRF (UZ-2) (*3*), we precipitated the pLRF in a portion of each culture medium. The [^{3}H]leucine in the precipitated pLRF was determined, and these values were converted to the total amounts of ^{3}H-labeled pLRF in each culture medium sample. The specific activity of the labeled pLRF, which was calculated by dividing the labeled pLRF by the immunoreactive pLRF, increased 100-fold from day 1 to day 2 and attained a maximum constant specific activity of 2.84 μCi per microgram of pLRF thereafter. This suggests that the pLRF released on the first day of culture was predominantly endogenous and that released thereafter was predominantly newly synthesized pLRF. The calculations of the total amount of leucine (unlabeled and tritiated) in the medium also



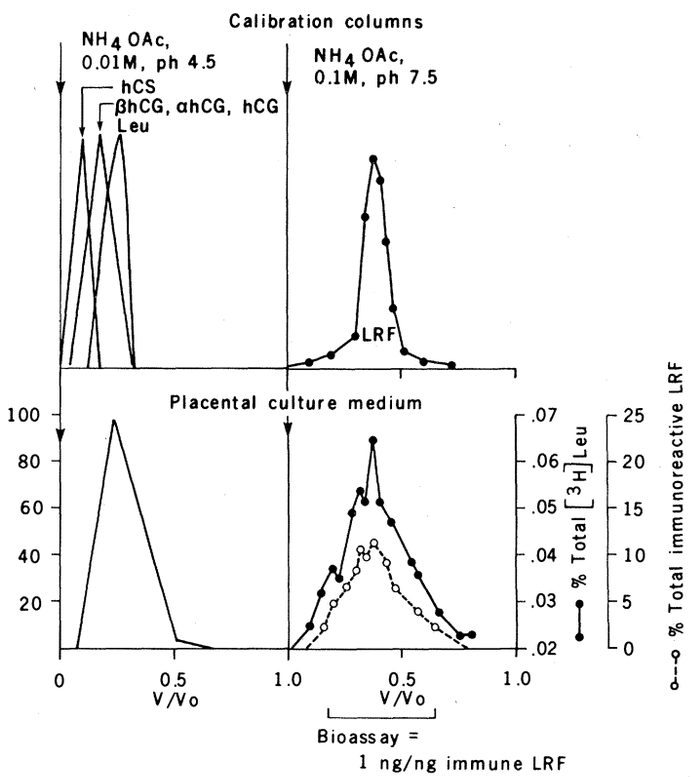


Fig. 1 (left). The release of immunoreactive pLRF into the medium by placental explants (●--●) is compared with the incorporation of [^{3}H]leucine into pLRF (●—●) as determined by CM-cellulose chromatography. Solid triangles indicate that the pLRF was less than or at the limit of detectability in some of these cultures. Fig. 2 (above). The elution pattern of [^{3}H]leucine and immunoreactive pLRF in spent placental culture medium (lower graph) is compared with that of pure hCS, α- and β-hCG, hCG, leucine, and synthetic LRF (upper graph). The biologic activity of the pLRF eluted in the indicated area was similar to that for synthetic LRF (V/V_0, ratio of elution volume to void volume).

indicate that after the first day of culture the pLRF released was newly synthesized. These findings are consistent with previous studies showing that placental tissue in vitro releases its initial content of immunoreactive LRF during the first 24 hours of culture (*3*).

The remaining eluates of pLRF from the CM-cellulose chromatography were pooled and concentrated by Diaflo filtration. The pLRF of the resulting concentrate was measured by radioimmunoassay and doses of 1.5, 2.0, 5.0, 7.0, and 9.3 ng were injected intravenously into five adult male rats. An increase in LH release was observed. Similar doses of synthetic LRF were injected into another group of male rats and this elicited an LH response similar to that of the pLRF. The total net increase of LH (minus the baseline) was calculated from 0 to 60 minutes after the injection of either pLRF or synthetic LRF. Linear regression analysis of the total net LH release stimulated by similar doses of pLRF and synthetic LRF yielded a correlation coefficient of .944 ($P < .002$). Thus the pLRF obtained by CM-cellulose chromatography was biologically equipotent to synthetic LRF.

This study demonstrates that the human placenta synthesizes a pLRF that is immunologically, physiochemically, and biologically indistinguishable from hypothalamic LRF. We have postulated that the function of this pLRF may be to control hCG secretion and thus may affect steroidogenesis during pregnancy. We demonstrated previously that synthetic LRF can stimulate α- and β-hCG release by the human placenta in vitro and that the stimulation of β-hCG occurs in a dose-response manner (*2, 5*); these findings were confirmed by other investigators, as was the stimulation of adenosine 3′,5′-monophosphate production by synthetic LRF (*6*). Recently, we found that circulating chorionic gonadotropin in the pregnant monkey increased after the administration of synthetic LRF (*7*). The amino acid sequence, chemical nature, and biological significance of the pLRF synthesized by the placenta remain to be determined.

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Toward a Functional Architecture of the Retina: Serial Reconstruction of Adjacent Ganglion Cells

Abstract. *Twenty adjacent ganglion cells in cat retina were partially reconstructed from electron micrographs of serial thin sections. Cells were classified by size and by dendritic branching patterns as α, β, or γ cells. The α and β cells were further subdivided by differences in the laminar distribution of their dendrites in the inner plexiform layer. The distribution of synaptic contacts on the cells was distinctive for each of the five major classes. Contacts on the α and β cells were mainly on the dendrites in the sublamina in which a cell's major dendritic arborization was contained.*

There has been substantial progress in correlating the anatomical structure of cat retinal ganglion cells with their electrophysiological properties. Based on Golgi impregnations of single neurons, Boycott and Wässle (*1*) divided the ganglion cell population into α cells (large somas and broad, radiate dendritic trees), β cells (medium-sized somas and narrower, bushy dendritic trees), and γ cells (small somas, varied dendritic geometry). [The α, β, and γ cells seem to correspond to Y, X, and W cells, respectively, in the current physiological scheme (*2*).] Ganglion cells have been further subdivided anatomically according to the height in the inner plexiform layer (IPL) at which their dendrites arborize. Cells that branch in the outer third of the IPL have off-center receptive fields, whereas cells that branch in the inner two-thirds of the IPL have on-center receptive fields (*3*).

Table 1. Diameter, surface area, and volume for each of 20 reconstructed ganglion cell bodies. Differences in surface area and volume for somas of the same diameter reflect differences in shape.

Size category	Diameter (μm)	Surface area (μm^2)	Volume (μm^3)
α	> 31*	1,705	10,049
	> 25*	1,654	9,250
β	19	1,117	4,532
	19	1,032	4,120
	18	855	2,931
	17	937	3,475
	16	855	2,957
	16	757	3,558
	15	931	2,956
γ	12	123	934
	12	434	1,085
	10	564	1,261
	10	337	671
	9	242	468
	9	347	713
	9	350	547
	8	165	338
	8	166	222
	7	162	221
	6	158	211

*Minimum value; cell did not reach its maximum diameter within series.

Little is known about the distribution of synaptic contacts on ganglion cells. We lack such basic information as whether the contacts are on the cell bodies or the primary dendritic shafts, and whether [as the data of Nelson *et al.* (*3*) would suggest] the contacts are restricted to the dendritic branches in the sublamina of each cell's major arborization. Nor is much known about the biophysically relevant parameters for each cell type, such as soma surface area. The latter is particularly important, since it can be a major determinant of a cell's input impedance (*4*). Finally, the distribution of the five cell classes within a small patch of retina is unknown because the Golgi method, which defines each class by soma size and branching pattern, stains only a few of the neurons in a given region.

Reconstruction from electron micrographs permits study of the microdistribution, dendritic and synaptic patterns, and biophysically relevant parameters of specific ganglion cell types. We have begun to gather such information by partially reconstructing a group of adjacent ganglion cells from 150 serial sections photographed with the electron microscope. The series encompassed a block of tissue (about 15 by 150 by 200 μm) taken from within 1° to 2° of the area

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