the central retina of the largest surface prejuvenile captured (Fig. 2D) but were not observed in older juveniles or adults. Thus the loss of single cones in this species may occur through fusion of two single cones to form a double cone or by fusion of two single cones and a double cone which might then divide to form two double cones. This latter process may explain the disorganization of the cone pattern and the presence of intermediate triple and quadruple cones in transitional specimens (Fig. 2D).

Interspecific comparisons correlating cone type and pattern with behavior and ecology of teleosts have suggested that species with well-developed patterns of single and double cones feed on fast-moving prey and that these patterns may improve perception of movement (*2*), possibly providing a structural basis for high temporal and spatial resolution (*12*). Double cones and poorly developed patterns, on the other hand, are associated with less acute vision in deep water. The intraspecific changes in cone type and pattern observed in *S. diploproa* in association with changing habitat and environmental light confirm past observations on the function of single and double cones and cone patterns based on interspecific comparisons.

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7. Surface prejuveniles may grow to 45 to 55 mm standard length (SL), although specimens as large as 58.7 mm SL have been captured. The smallest observed benthic juvenile is 41.9 mm SL, but the smallest specimens normally encountered are 45 to 50 mm SL. The migratory season for this species lasts about 4 months and may include an intermediate midwater habitat.
8. Although I examined the retinas of benthic adult *S. diploproa* (eye diameters up to 21.3 mm), they were not included in this analysis. Determination of the density of single and double cones requires analysis of tangential sections of retina, since a section of one member of a double cone might be mistaken for a single cone in retinal cross section. Density determinations further require that the retinas be fully light-adapted, so that all cones are on the same level within the visual cell layer and consequently appear in the same tangential section. Cross sections of adult retinas revealed incomplete and variable states of light-adaptation. I examined serial tangential sections from adults, however, and determined that single cones were not present.
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Prolactin Synthesis by Human Chorion-Decidual Tissue: A Possible Source of Prolactin in the Amniotic Fluid

Abstract. *Explants of human chorion-decidual tissue obtained at delivery from normal, full-term pregnancies synthesize and secrete prolactin. This hormone is indistinguishable from pituitary prolactin by chromatographic, electrophoretic, immunologic, and receptor assay techniques. These results suggest that chorion-decidua may be the source of the large quantities of prolactin in amniotic fluid.*

Prolactin (PRL), a polypeptide pituitary hormone, influences lactation, water and electrolyte transport across membranes, fertility, and growth in a variety of species (*1*, *2*). Human PRL concentrations in the maternal circulation rise steadily during pregnancy becoming tenfold higher than those in the nonpregnant state; concentrations in the amniotic fluid exceed those in the maternal or fetal circulation by 100-fold early in pregnancy and by five- to tenfold at term (*3*, *4*). Biologically, chemically, and immunologically, amniotic fluid human PRL is similar or identical to the pituitary hormone (*5–7*), yet its origin and physiologic function are unknown. An extra-pituitary source of PRL in amniotic fluid is suggested by experiments with pregnant rhesus monkeys in which neither maternal hypophysectomy nor fetal death decreased amniotic fluid PRL concentration (*8*). Friesen *et al.* (*9*) demonstrated that explants of chorionic tissue released small amounts of human PRL during a 24-hour culture, and suggested that chorion might be the source of amniotic fluid PRL. However, Riddick and Kusmik (*10*) reported that tissue composed predominantly of decidual cells secreted human PRL during 18 hours of culture. We report here studies of the synthesis and secretion of human PRL in vitro by placental trophoblast, amnion, chorion, and decidua.

Human placentas and the associated membranes from uncomplicated, full-term pregnancies were obtained within minutes of delivery. Membrane pieces were dissected from several locations peripheral to the placenta and rinsed with Earle's balanced salt solution. The amnion was carefully removed from the closely attached chorion-decidua, but attempts to separate the chorion manually from decidua were unsuccessful. The tissues were then cut into explants (5 by 5 mm) and pooled. Placental tissue was prepared for culture as described elsewhere (*11*). Explants of placenta, amnion, or chorion-decidua with an average wet weight of 100 mg each were cultured in separate incubation flasks with 5 ml of minimal essential medium containg 15 percent fetal calf serum, penicillin-G (25 unit/ml), and streptomycin at 25 μg/ml at 37°C in an atmosphere of 95 percent O_2 and 5 percent CO_2 for periods of up to 6 days with medium changes every 24 hours. The media removed at the end of each day were centrifuged at 1000*g* for 10 minutes at 4°C and stored at −20°C. At the end of the culture period, tissues were homogenized in 3 ml of ice-cold 0.1*M* ammonium bicarbonate, *p*H 9.2, with 1 m*M* α-toluenesulfonyl fluoride and 0.5 percent Triton X-100. Each homogenate was rapidly frozen and thawed five times to disrupt cell membranes, and then centrifuged at 500*g* for 10 minutes at 4°C. The resulting pellet was washed with 2 ml of homogenizing solution, and the combined supernatants were centrifuged at 12,000*g* for 30 minutes at 4°C. The clear supernatant was stored at −20°C until assayed. Concentration of human PRL in the incubation media and tissue homogenates were measured by homologous radioimmunoassay (*12*). [The human PRL used as the standard and for iodination (VLS-2) and the rabbit antiserum to human PRL were provided by the Hormone Distribution Program, NIAMDD.] Iodination of PRL was performed by the lactoperoxidase method (*13*), and the reaction was stopped by addition of sodium azide (*14*). Iodine-125-labeled human PRL was separated from the free ^{125}I and damaged hormone by chromatography on Sephadex G-150.

Cultures of the amniotic layer or placental tissue released less than 10 ng of prolactin during the first 24 hours of incubation and none thereafter. However, the explants of chorion-decidua secreted human PRL over the entire 6-day culture period at a relatively constant rate (Table 1). The average daily secretion was 294 ± 34 ng/100 mg (wet weight of tissue) and the total amount of human PRL released over the 6-day period exceeded by 1800 percent the amount in the tissue before culture. Addition of cycloheximide in concentrations of 0.05 m*M* to chorion-decidual explants for 24

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hours resulted in a 68 ± 5 percent decrease in the human PRL released to the medium and an 89 ± 3 percent decrease in human PRL content of the tissue when compared to explants cultured without cycloheximide. These results suggest that chorion-decidua synthesizes and secretes PRL.

Synthesis and secretion was demonstrated by the incorporation of labeled amino acids into human PRL. Explants with a total weight of 1.5 g were cultured in 15 ml of Earle's balanced salt solution with 200 μCi of a ^{3}H-labeled amino acid mixture (New England Nuclear). After 20 hours of incubation at 37°C, the media were centrifuged at 1000*g* for 10 minutes. The clear supernatant was concentrated two- to threefold at 0°C by ultrafiltration with a molecular separator (Millipore) having a nominal cutoff of 10,000 daltons, and subjected to gel chromatography on Sephadex G-150 (Fig. 1). Effluent fractions were assayed for human PRL by radioimmunoassay, radioreceptor assay, and immunoprecipitation. In the radioreceptor assay rabbit mammary tissue was used as a source of receptor and ^{125}I-labeled human PRL was used as tracer molecule (*15*). Immunoprecipitation was performed by a method similar to that described for the immunoprecipitation of human placental lactogen and human chorionic gonadotropin (*11*), with rabbit antiserum to ovine PRL (*16*). Samples of effluent fractions after gel chromatography were incubated for 48 hours at room temperature with 20 μl of antiserum to ovine PRL and 500 ng of ovine PRL as carrier under conditions previously demonstrated to precipitate over 80 percent of a trace amount of ^{125}I-labeled human PRL. After incubation, the tubes were centrifuged at 2000*g* for 30 minutes at 4°C and the precipitate was washed three times with tris-HCl buffer containing unlabeled amino acids. The precipitates were dissolved in 0.1*M* sodium hydroxide, neutralized with 15 percent ascorbic acid, and radioactivity was counted in Aquasol II. To account for nonspecific binding of ^{3}H-labeled protein to the incubation tubes, samples of the column effluent were incubated with nonimmune rabbit serum, and the counts in these tubes were subtracted from those containing the ovine PRL immunoprecipitates.

Table 1. Amounts of human PRL by radioimmunoassay in the incubation media and tissues of chorion-decidua explants at the end of each day of culture. Results are expressed as the mean ± standard deviation of at least three 100-mg tissue samples (wet weight). In the radioimmunoassay, the displacement of ^{125}I-labeled human PRL by serial dilutions of culture medium paralleled the displacement by pituitary human PRL. Degradation of human PRL during culture was less than 15 percent over a 24-hour period.

Day of culture	Human PRL	
	Released into medium (ng)	In tissue (ng)
0		33 ± 4
1	320 ± 11	51 ± 2
2	314 ± 13	46 ± 2
3	319 ± 7	41 ± 4
4	314 ± 32	44 ± 3
5	242 ± 45	49 ± 2
6	260 ± 27	37 ± 4

In the radioimmunoassay more than 95 percent of the human PRL eluted as a single symmetrical peak that coincided with elution position of ^{125}I-labeled human PRL (Fig. 1). Analysis of column effluent by radioreceptor assay showed more than 95 percent of the receptor binding activity eluted in the same position. Immunoprecipitation of effluent fractions revealed a single symmetrical peak of ^{3}H-labeled material in the elution position of ^{125}I-labeled human PRL.

The immunoprecipitated ^{3}H-containing material was further characterized by electrophoresis in sodium dodecyl sulfate-polyacrylamide slab gels (*17*). Fluorography of the dried gel (*18*) revealed a single band of ^{3}H-containing material with a mobility identical to that of ^{125}I-labeled human PRL (Fig. 2).

This study demonstrates the in vitro synthesis and secretion by chorion-decidual tissue of human PRL which is indistinguishable from human PRL by chromatographic, electrophoretic, immunologic, and receptor assay techniques. Since chorion-decidual tissue is located in close proximity to the amniotic fluid, these results suggest that chorion-decidua is the source of the prolactin in amniotic fluid.

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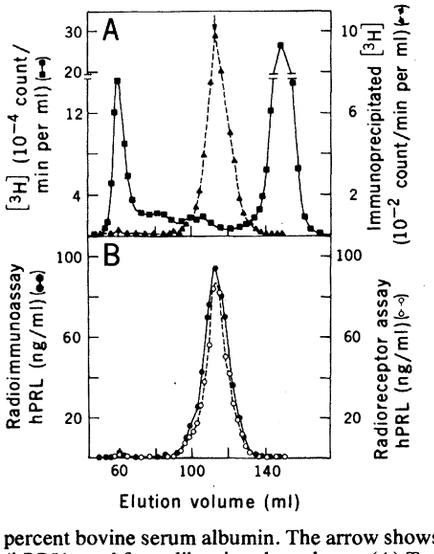
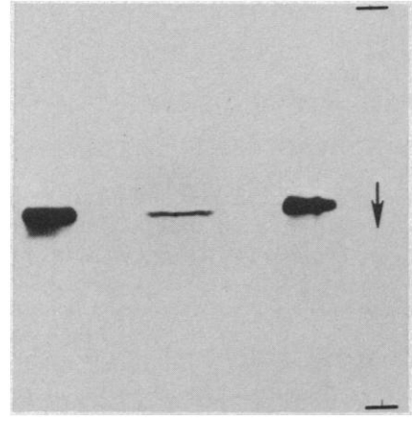


Fig. 1 (left). Chromatography of culture medium on a 1.5 by 85 cm column of Sephadex G-150, equilibrated and eluted at 4°C with 25 m*M* tris-HCl, *p*H 7.6, with 10 m*M* $CaCl_2$ and 0.1 percent bovine serum albumin. The arrow shows the elution position of ^{125}I-labeled human PRL (*hPRL*) used for calibrating the column. (A) Total ^{3}H counts in column effluent (■) and counts of ^{3}H-labeled human PRL obtained by immunoprecipitation (▲). (B) Human PRL measured by radioimmunoassay (●) and by radioreceptor assay (○). Fig. 2 (right). Fluorography of ^{3}H-containing material obtained by immunoprecipitation (center), and pituitary ^{125}I-labeled human PRL (the right and left) after electrophoresis. Immunoprecipitates were incubated for 2 minutes at 100°C in 60 m*M* tris-HCl buffer, *p*H 6.8, with 1.25 percent sodium dodecyl sulfate (SDS). 1.5 percent 2-mercaptoethanol, 12.5 percent glycerol, and 6 μg of bromophenol blue, and applied with identically incubated ^{125}I-labeled human PRL to a discontinuous polyacrylamide slab gel prepared according to Laemmli (*17*). The separating gel was 12.5 percent polyacrylamide, 0.1 percent SDS, and 125 m*M* tris-HCl, *p*H 6.8; the stacking gel contained 5 percent polyacrylamide. Electrode buffer was 25 m*M* tris-HCl, *p*H 8.6, 0.1 percent SDS, and 0.2*M* glycine. Electrophoresis was performed at room temperature for 6 hours at 20 mA. The gel was fixed in 10 percent trichloroacetic acid with 25 percent isopropyl alcohol for 1 hour and prepared for fluorography by impregnation with dimethyl sulfoxide with 22.2 percent PPO (*18*). Kodak X-omat RP-5 film was exposed to the dried gel for 10 days at −70°C.



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Tumor-Promoting Phorbol Esters Inhibit Binding of Epidermal Growth Factor to Cellular Receptors

Abstract. *Tumor-promoting phorbol esters and related plant macrocyclic diterpenes inhibit the binding of epidermal growth factor to its receptors on HeLa cells. This effect shows marked structural specificity and correlates with other biological effects of these compounds on mouse skin and in cell culture systems. The active compounds inhibited binding of ^{125}I-labeled epidermal growth factor with a 50 percent effective dose in the range of 10^{-8} to 10^{-9}*M. *Inhibition appears to be due to a decrease in the number of available epidermal growth factor receptors rather than a change in receptor affinity. These results suggest that certain biologic effects of tumor promoters may result from alterations in the function of cell surface receptors involved in growth regulation.*

Tumor-promoting agents are an intriguing class of compounds since, although they do not themselves induce cancer, they markedly enhance the production of skin tumors when repeatedly applied to mouse skin previously exposed to a low dose of a chemical carcinogen (*1*, *2*). Elucidation of the molecular action of tumor promoters would aid in the design of rapid screening systems for the detection of such agents in the human environment and would facilitate an understanding of the enigmatic multistage carcinogenic process. The most potent tumor promoter is 12-*O*-tetradecanoyl phorbol-13-acetate (TPA). It is the active principle of croton oil (*1*, *2*), which was originally used as a promoting agent in the two-stage mouse skin carcinogenesis system developed by Berenblum and co-workers [see (*2*)].

Recent studies on mouse skin and with cell cultures have revealed a number of novel biologic and biochemical effects of TPA and related compounds (*1*-*3*). In cell culture systems TPA induces several phenotypic changes which resemble those seen in cells transformed by viruses or chemical carcinogens (*3*). This mimicry of transformation includes altered cell morphology and increased saturation density, alterations in lipid metabolism and cell surface glycoproteins, enhanced membrane transport of 2-deoxyglucose and possibly other nutrients, induction of the enzymes plasminogen activator and ornithine decarboxylase, and induction of prostaglandin synthesis (*4*). TPA also enhances the transformation of cells in culture previously exposed to a chemical carcinogen (*5*).

Another category of effects of TPA in cell culture is inhibition of terminal differentiation. This has been seen in cell cultures of diverse species and types and extends to a variety of programs of differentiation (*6*). Studies on mouse skin and epidermal cultures also suggest that TPA interferes with epidermal differentiation (*1*, *2*, *7*).

These and other findings have led us to postulate that TPA acts by usurping the function of a cell receptor or receptors whose normal function is to mediate the action of a yet to be identified endogenous growth regulator or hormone (*3*). Consistent with this hypothesis are (i) the low concentration at which TPA acts in cell culture (approximately 10^{-8} to $10^{-10}M$); (ii) the remarkable similarity in structural requirements seen when a variety of active and inactive phorbol esters and related macrocyclic diterpenes are tested in diverse systems; and (iii) the highly pleiotropic and reversible effects of these compounds. Since the earliest effects of TPA appear to occur at the cell membrane (*4*, *8*), we further postulated that the putative receptors are on the cell surface and the growth regulator may be a polypeptide hormone.

A possible candidate for the polypeptide hormone is epidermal growth factor (EGF), since it shares a number of biologic effects with TPA. These include stimulation of proliferation of both epidermal and mesodermal cells, increase in deoxyglucose transport (*9*), and induction of ornithine decarboxylase (*9*) and prostaglandin synthesis (*10*). In addition, EGF has been reported to promote tumor induction on mouse skin (*11*). We have recently found that like TPA, it is also a potent inducer of plasminogen activator production (*12*). EGF has been purified from both mouse and human sources. Mouse EGF has been extensively characterized. It contains 53 amino acids of known sequence (*13*). Like several other polypeptide hormones, EGF exerts its effects by binding to specific cell membrane receptors (*9*, *13*). Mouse and human EGF are not species-specific and compete with each other in receptor-binding studies, although they are antigenically different (*14*). The physiological role of EGF is not fully understood.

In the present study we have found that TPA and related macrocyclic diterpenes of plant origin are extremely potent inhibitors of EGF binding to intact mammalian cells.

The effects of various test compounds on the binding of ^{125}I-labeled EGF to HeLa cells are given in Fig. 1 and Table 1. Binding was almost completely inhibited by low concentrations (10 to 20 ng/ml) of unlabeled EGF, indicating that we were dealing with a specific saturable receptor. We found that extremely low concentrations (0.25 to 50 ng/ml) of TPA and related compounds produced a dose-dependent decrease in [^{125}I]EGF binding

Table 1. Effects of tumor promoters on cellular binding of [^{125}I]EGF. Binding assays were done with HeLa cell cultures essentially as described in Fig. 1. Test compounds were added at a concentration of 100 ng/ml to the binding buffer containing 0.225 ng of [^{125}I]EGF.

Test compounds	[^{125}I]EGF bound (count/min)
None	6149
12-*O*-tetradecanoyl phorbol-13-acetate (TPA)	255
Phorbol	5792
4-*O*-Me TPA	5510
Phorbol didecanoate	1349
Phorbol dibenzoate	2160
4αPDD	5956
Mezerein	620
Gnidipalmin	5457
Gnidimacrin 20-palmitate	5409
Gnilatimacrin	491
Gnidilatin	1042
Anthralin	6076

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