the dygynic embryos developed better since 44 percent implanted; seven embryos, albeit retarded, were found. These were confirmed as dygynic embryos homozygous for glucose phosphate isomerase (GPI-aa). Significantly, in triploid eggs where the genetic constitution was restored to normal after the removal of one of the two female pronuclei, development continued to term. Over 50 percent of such embryos implanted and 16 (36 percent, with dark eyes and GPI-ab) developed to term. The unoperated triploid embryos also implanted (38 percent) and 30 embryos with a variety of abnormalities were obtained on day 11 of pregnancy, similar to a previous study (*18*). Development of hemizygous diploid gynogenetic and dygynic embryos from inbred C57BL/6 and BALB/c (from Olac, Bicester, England) and (C57BL × CBA)F_1 mice was also examined (Table 2). Although a number of them implanted, only 11 sites contained dygynic embryonic derivatives.

The poor development of hemizygous diploid embryos can be most easily explained by the effects of the expression of recessive lethal genes, although there are also likely to be fewer cells in these implanting blastocysts because of the suppression of the first cleavage division. However, even dygynic embryos developed to about the same extent as diploid parthenogenones (*3, 12*). Because of recombination events during the first meiotic division, the two haploid sets during the second polar body extrusion can be dissimilar. Hence, suppression of the polar body extrusion could lead to heterozygosity at some loci. This would result in some dygynic embryos having a chance of overcoming the effects of recessive lethal genes and might partly account for their relatively better development. In outbred amphibians a few viable dygynic adults have been obtained (*19–21*). In mammals there is a great excess of genes and chance combination leading to viable dygynic genotype is perhaps far less. However, in some tissues, such as trophectoderm, there is preferential inactivation of the paternal X chromosome (*22, 23*). Hence, errors in X-inactivation after implantation may occur which could provide an explanation for the developmental failure of both gynogenones and parthenogenones, since they lack a paternal X chromosome, although X-inactivation does occur in extraembryonic membranes of parthenogenones (*24*). We have consistently noticed very meager development of trophoblasts in parthenogenones and gynogenones.

The role of the extragenetic contribution from spermatozoa in development still needs explanation, since a few eggs with nuclei from parthenogenetic embryos of strain LT/Sv transplanted to enucleated fertilized eggs develop to term (*7*). This result suggests that some of the dygynic embryos from the four strains of mice used in the present study should also develop to term, but thus far they have not. Therefore, the role of cytoplasmic factors in the development of gynogenones and parthenogenones remains to be determined.

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Phorbol Ester Receptors: Autoradiographic Identification in the Developing Rat

Abstract. *Autoradiography with ^{3}H-labeled phorbol dibutyrate was used for the light microscopic detection of phorbol ester receptors in rat fetuses. In 15- and 18-day fetuses, as well as in adult rats, receptors were found to be concentrated in the central nervous system. The localization of receptors in the ventral marginal zone of the fetal neural tube, the lens of the eye, and other sites suggests a role for phorbol ester receptors in cellular process extension and cell-cell interaction.*

Phorbol esters are among the most potent tumor-promoting agents known. Their effects are mediated by specific cell membrane receptors that can be labeled with [^{3}H]phorbol-12,13-dibutyrate ([^{3}H]PDBu) (*1*). These receptors are distributed ubiquitously in animal tissues but their highest concentrations are in the brain (*2*). Solubilized phorbol ester receptors copurify with protein kinase C, a calcium- and phospholipid-dependent enzyme (*3*) that is stimulated by phorbol esters (*4*). Phorbol ester receptors may be relevant to normal cell growth and differentiation, since phorbol esters induce a number of enzymes—including epidermal transglutaminase and ornithine decarboxylase (*5*)—which participate in rapid tissue growth (*6*). We now report selective localizations of phorbol ester receptors in rat fetuses. Our findings suggest that phorbol ester receptors have a role in ontogenetic process extension rather than in cell division.

We used light microscopic autoradiography (*7*) to determine the locus of [^{3}H]PDBu (New England Nuclear) binding to slide-mounted, unfixed tissue sections (8 to 10 μm) derived from 15- and 18-day rat fetuses and adult rat brain. Sprague-Dawley rats were obtained from Charles River. We assigned gestational age by using the appearance of the vaginal plug as day 0 of gestation. Unfixed tissue was used to avoid altering the properties of the [^{3}H]PDBu binding sites. Slide-mounted tissue binds [^{3}H]PDBu with an affinity of 10 n*M*, and 4β-phorbol 12-myristate 13-acetate inhibits binding at 50 to 100 n*M*. Equilibrium is attained within 30 minutes. These properties are consistent with the [^{3}H]PDBu sites labeled in studies with tissue homogenates (*2*). Slide-mounted sections were incubated with 2 n*M* [^{3}H]PDBu in 100 m*M* NaCl and 50 m*M* tris-HCl, *p*H 7.7, for 1 hour at room temperature. Separate sections were incubated with 2 n*M* [^{3}H]PDBu and 1 μ*M* 4β-phorbol 12-myristate 13-acetate (Sig-

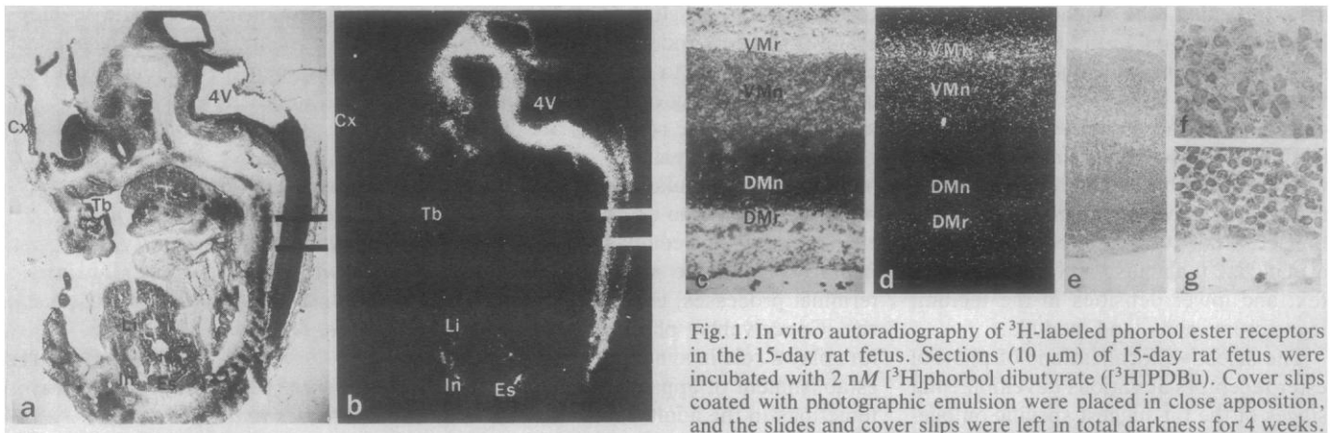

Fig. 1. In vitro autoradiography of ^{3}H-labeled phorbol ester receptors in the 15-day rat fetus. Sections (10 μm) of 15-day rat fetus were incubated with 2 n*M* [^{3}H]phorbol dibutyrate ([^{3}H]PDBu). Cover slips coated with photographic emulsion were placed in close apposition, and the slides and cover slips were left in total darkness for 4 weeks. (a) Rat fetus stained with pyronine Y. (b) [^{3}H]PDBu-derived silver grains viewed under dark field. Slides incubated in the presence of [^{3}H]PDBu and 1 μ*M* 4β-phorbol 12-myristate 13-acetate showed no specific silver grains under dark field viewing. *Cx*, cerebral cortex; *4V*, fourth ventricle; *Tb*, tooth bulb; *Li*, liver; *In*, intestine; *Es*, esophagus. Selective high densities of receptors occurred over the posterior brain and spinal cord. The dark bands in (a) and the light bands in (b) delineate the region of the spinal cord viewed under higher magnification in (c) (light field) and (d) (dark field). *VMr*, ventral marginal zone; *VMn*, ventral mantle zone; *DMn*, dorsal mantle zone; *DMr*, dorsal marginal zone. Grain density is selectively enriched over the ventral marginal zone, which is actively extending processes. (e) Staining with toluidine blue reveals the cell nuclei and substantiates identification of the marginal and mantle zones. This section is aligned with that shown in (d) and enables the identification of cell bodies within the mantle zones. (f) Higher magnification of the ventral mantle zone stained with toluidine blue, showing nuclei and demonstrating the morphological integrity of the sections. (g) Higher magnification of the dorsal mantle zone stained with toluidine blue.

ma) to define nonspecific interactions. After the incubations, the slide-mounted sections were washed four times in ice-cold buffer for 1 minute each time. The slides were then dried, and cover slides coated with photographic emulsion were placed in apposition to the sections (*7*). The ratio of specific to nonspecific binding of [^{3}H]PDBu to tissue sections was approximately 8 in fetal tissues and approximately 15 in adult brains. Differences in the observed [^{3}H]PDBu binding may be due to alterations either in the number or the affinity of these receptors during development.

In the 15-day rat fetus, [^{3}H]PDBu binding was highest in the posterior portion of the brain and spinal cord (Fig. 1, a and b). Among the various layers of the neural tube, grains were most highly concentrated in the ventral marginal layer (Fig. 1, c to g). Patches of grains were located in putative hematopoietic centers in the liver, in the developing foregut and intestine, and in the tooth buds. Low grain density was observed in the heart, the lung, the cerebral hemisphere, and the bulk of the liver.

In the 18-day fetus (Fig. 2a), binding of [^{3}H]PDBu was highest in the posterior portion of the brain and in the developing meninges, calvarium, and basicranium, whereas label density was low in the cerebral cortex. In comparison with findings in the 15-day fetus, label density at 18 days was greatly augmented in the ventricles of the heart, the submucosal and mucosal layers of the intestine, and the thymus. Within the developing eye, the lens and extraocular connective tissue exhibited high grain densities; substantially lower densities were observed in the retina and developing choroid (Fig. 2b).

In the adult rat, our biochemical as-

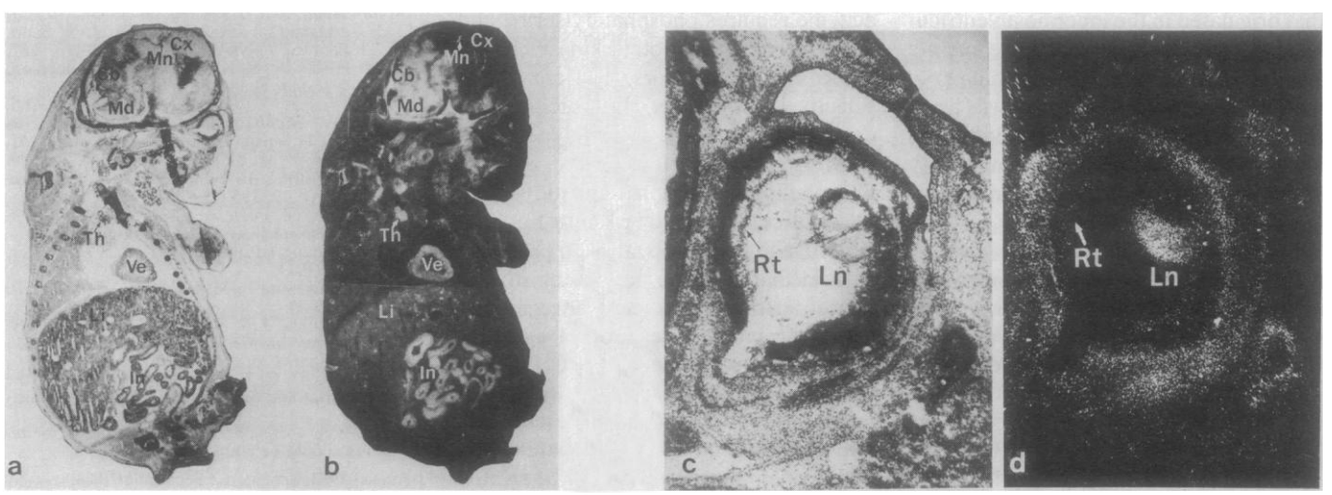

Fig. 2. In vitro autoradiography of ^{3}H-labeled phorbol ester dibutyrate in the 18-day rat fetus. Sections (10 μm) of 18-day rat fetus were incubated with 2 n*M* [^{3}H]PDBu. Cover slips coated with photographic emulsion were placed in close apposition, and the slides and cover slips were left in total darkness for 4 weeks. (a) Rat fetus stained with pyronine Y. (b) The same slide viewed in dark field, revealing the silver grains resulting from [^{3}H]PDBu. Slides incubated in the presence of 1 μ*M* unlabeled 4β-phorbol 12-myristate 13-acetate showed no specific silver grains in dark field. High grain densities occur in the posterior portion of the brain, over the ventricle of the heart, the thymus, and the intestine. The mottled appearance of the liver suggests localization on specific cell subtypes, possible hematopoietic centers. *Cx*, cerebral cortex; *Mn*, meninges; *Cb*, cerebellum; *Md*, midbrain; *Th*, thymus; *Ve*, ventricle of the heart; *Li*, liver; *In*, intestine. (c) Higher magnification of the eye stained with pyronine Y. (d) The same slide viewed in dark field shows silver grains predominantly over the developing lens and extraocular connective tissue. *Ln*, lens; *Rt*, retina.

says revealed the highest levels of [^{3}H]PDBu binding in the brain (data not shown), confirming the findings of Blumberg and his associates (*2, 8*). Our autoradiographic analyses showed [^{3}H]PDBu-associated grains to be highly concentrated throughout the adult neuropil, with especially high densities in the cerebellar cortex, hippocampus, basal ganglia, thalamus, and cerebral cortex, and lower densities in the tectum, tegmentum, pons, and medulla.

The selective localizations of phorbol ester receptors at various developmental stages of the rat suggest specific ontogenetic functions. Localizations in the developing eye and brain imply a role in cell process elongation and membrane turnover rather than cell division. For example, in the eye of the 18-day fetus, the germinal layers of the lens contain mitotic cells. These cells divide and move through the equatorial zone, where they undergo elongation and enter the body of the lens. In the body of the lens, the cells undergo continued elongation, accumulate crystallins, and become incorporated into more organized layers of fiber cells in the posterior body of the lens. High densities of grains were found in the equatorial region and posterior body of the lens, with much lower densities occurring in the germinative region of the anterior lens. Similarly, within the neural tube in the 15-day fetus, the marginal layers are rapidly expanding zones, containing numerous cell processes but few cell bodies; the latter are located instead in the mantle zones (Fig. 1, e to g). The dorsal and ventral mantle layers contain many postmitotic neuroblasts that originated in the innermost ventricular layer and migrated into the mantle layer to become part of the forming gray matter. Very low grain density was associated with the mantle and ventricular layers, and extremely high grain density occurred in the ventral and dorsal marginal layers.

The absence of high-affinity phorbol receptors in those layers of the developing nervous system and lens that undergo rapid mitosis indicates that the receptors do not play a major role in cell division. The greater binding seen in extending cellular processes of both the lens and the marginal layer of the developing spinal cord suggests a role in process extension, possibly related to membrane formation and turnover. This increase may be due to increased numbers or increased affinity of sites. Within the mature brain, these receptors are highly concentrated in synaptic zones. Most neuronal cell populations in the adult brain never undergo cell division. However, one dynamic cellular activity of the mature brain is the plasticity of axonal terminal processes, especially at synapses. Conceivably, phorbol ester receptors play a role in synaptic plasticity and establishment of appropriate cellular interactions in the adult brain.

The phorbol ester receptor may serve a different function in peripheral tissues than in the central nervous system. Since the phorbol ester receptors may be protein kinase C (*3*), the functional role of these receptors in different tissues may depend on tissue-specific substrates for the kinase. Diverse cellular responses to phorbol esters may derive from differences in substrates for the protein kinase C that subserve divergent functional and metabolic roles. Thus, one cannot exclude the possibility that, in the gut and heart, these receptors may be related to mitosis at some particular stage of development. However, it is also possible that the phorbol ester receptor plays a general role in cell-cell interaction and recognition in peripheral tissues similar to the proposed role in process extension in the central nervous system.

The recent finding that solubilized phorbol ester receptors copurify with protein kinase C (*3*) suggests that autoradiography with [^{3}H]PDBu may also identify the loci of protein kinase C. This enzyme requires phosphatidylserine and differs from other protein kinases in the nature of its activators. The enzyme is selectively stimulated by calcium and diacylglycerol (*3, 4*). Diacylglycerol derives from the action of the enzyme phospholipase C on phosphatidylinositol. The conversion of phosphatidylinositol to diacylglycerol has been proposed as a mediator of the actions of several neurotransmitters and cellular regulators (*9*). Conceivably, the phorbol ester receptor and its protein kinase C function mediate the influences of phosphatidylinositol turnover on cellular function via phosphorylation (*10*). In the mature nervous system these events may be related to synaptic actions, while in the developing spinal cord and lens they may reflect the establishment of cellular processes or cell-cell interactions.

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