core extends into the cytoplasm. In addition, the cytoplasm contains a network of photon-absorbent material that surrounds vacuoles, connects with dense bodies, and, in some cases, appears to be continuous with the pseudopod core. Platelets fixed in glutaraldehyde before the preparation of whole mounts show similar morphological detail in both dense bodies and pseudopods.

The fact that resist dense bodies appear to consist of concentric rings (Fig. 3) raises the question of whether this ultrastructure is unique to the resist, exists in the living state, or is introduced in the whole mount during the air-drying procedure. It probably is not an artifact of the exposure and development processes, since other regions of high photon absorption (such as the pericytoplasmic rim and the pseudopod core) are reproduced in the resist as smooth structures. In addition, TEM examination of the same platelets before and after x-irradiation for periods of 8 to 100 hours showed no obvious structural differences. If the lamellar arrangement exists in vivo, it may occur by sequential precipitation of secreted material as the dense body is formed in the megakaryocyte. An explanation of why it is not visible by TEM in the actual whole mount is lacking, however.

Two other features visible in the resist but not in the whole mount may owe their prominence in the resist to differences in the cross section for electron scattering versus photon absorption. In passing from carbon to sulfur (atomic numbers 6 to 16), the total electron scattering cross section for 100-keV electrons rises by a factor of approximately 3, while the absorption cross section for 4.4-nm photons rises by a factor of 50. Thus a PO_4 group looks like approximately eight carbon atoms to 100-keV electrons but looks like more than 50 carbon atoms to 4.4-nm photons. Platelets contain large quantities of actin, a molecule associated in its polymerized form with one adenosine diphosphate molecule. Pseudopods in fixed and sectioned platelets appear to contain a filamentous core, which is believed to be composed of actin, although a specific connection with cytoplasmic elements has not been convincingly documented (*5*). If the cytoplasmic network, the periplasmic rim, and the pseudopod core represent adenosine diphosphate–associated actin relatively rich in phosphorus and oxygen, they would be essentially indistinguishable to 100-keV electrons when embedded in a proteinaceous matrix, but significantly more absorbent than their surround to 4.4-nm photons.

Transmission electron microscope images of x-ray resists made from other cells or tissues may provide new insights into subcellular organization, since they can highlight features not readily visible with conventional electron microscopy (*6*). Resists may now be examined with a resolution approaching 5 nm and, with proper equipment, can provide qualitative information about elements that may be present in small cellular areas. Perhaps the most important application of the new technique, however, will result from accurate quantitation of photon absorbance in areas of resists made with x-rays just below and above the absorption edges of elements of interest (*7*). By superimposition or subtraction of images obtained in this fashion, contact x-ray microscopy may provide a valuable tool for the quantitative imaging of the distribution of elements in biological specimens.

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6. In preliminary experiments, replicas made by TEM from whole mounts of fixed *Acanthamoeba castellani* show vacuoles and bundles of filaments. Cultured cells, lymphocytes, and bacteria have also been examined.
7. Quantitation can be achieved by simultaneous exposure of a biological specimen and a wedge composed of a step series of Mylar films of known thicknesses. The electron scattering power of areas in the developed resist corresponding to the wedge series of Mylar films can then be used to derive the photon absorbance of the specimen.

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Estradiol and Progesterone Profiles Indicate a Lack of Endocrine Recognition of Pregnancy in the Opossum

Abstract. *Concentrations of estradiol and progesterone in blood collected during the 12.5-day gestation period of the Virginia opossum were not significantly different from those during equivalent days of the estrous cycle. Progesterone was correlated with an index of corpora luteal mass. Ratios of estradiol to progesterone were highest 3 to 4 days before estrus and on the day of parturition.*

As representatives of evolutionary lines that have been separate for over 80 million years (*1*), members of the infraclasses Metatheria (marsupials) and Eutheria (all other viviparous mammals, for example, primates, rodents, or ungulates) exhibit fundamentally different strategies for gestation and lactation. Eutherians deliver large, well-developed neonates after a relatively long gestation period that interrupts the estrous cycle and effects major alterations in maternal physiology. The corpus luteum of pregnancy is usually maintained beyond its normal life-span in the estrous cycle (*2*), and progesterone concentrations remain high until near parturition. Likewise, profiles (concentrations plotted over time) of circulating estrogens in eutherian gestation are high and qualitatively different from those seen during the estrous cycle. In contrast, marsupials give birth to extremely small, embryonic young after a relatively short gestation and lactate for an extended period, several times longer than the period of gestation. In all but a few macropods (kangaroos and wallabies), the gestation period of marsupials is shorter than the estrous cycle, and if nurslings are taken from the mother after birth, estrous cycles continue unaltered (*3*).

Sharman (*3*) suggested that the estrous cycle might be hormonally equivalent to gestation in marsupials, a hypothesis based on anatomical and histological comparisons of reproductive tracts, embryo transfer experiments (*4*), and the temporal coincidence of estrous and gestational cycles. Our test of the equivalence hypothesis predicted no differences between these two reproductive

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states in profiles of circulating estradiol or progesterone, that is, no endocrine recognition of pregnancy.

The Virginia opossum *(Didelphis virginiana)* is an ideal model for the study of marsupial reproductive endocrinology. The 12.5-day gestation period is confined to the luteal phase of the 29-day estrous cycle (*5, 6*), and uteri from equivalent days of the estrous cycle and gestation are virtually identical in size, appearance, and biochemical composition (*6–9*). Furthermore, the embryonic trophoblast does not become implanted in or alter the underlying uterine mucosa (*6, 10*). *Didelphis virginiana* is a member of the ancestral family Didelphidae and is the only marsupial in temperate North America, the geographic center of origin for marsupials (*1*). Thus, from an evolutionary perspective, the opossum is valuable for comparison with the more advanced and diverse marsupials of Australia.

The study described here demonstrates that endocrine recognition of pregnancy does not occur in the Virginia opossum. Although a few Australian marsupials have been examined in this regard (*11–13*), to our knowledge the equivalence hypothesis has not previously been tested in a didelphid, nor in any marsupial on a day-by-day basis of comparing progesterone and estradiol profiles.

Adult opossums, captured in the wild, were caged individually in a semienclosed facility and maintained on a diet of dry dog food, fish, and water. Estrous cycles were monitored daily by vaginal smear cytology, and pregnancy was confirmed through observation of neonates or embryos (*14*). Blood samples were collected at 0700 to 1000 hours, 6 days prior (−6 to −1) to estrus (day 0), on days 0 through 14, and on day 18 of the estrous cycle and on equivalent days of gestation (days 0 through 13 and days 1 and 5 postpartum). Not all animals were bled on a daily basis, but all were sampled on day 0, day 3 (arrival of eggs or embryos in the uterus), day 7, and day 11. Samples were obtained from 25 adult females during 33 estrous cycles, and 22 animals were bled during 28 pregnancies. Estradiol and progesterone in serum samples were measured by radioimmunoassay according to previously described procedures (*15*).

Profiles of circulating estradiol and progesterone observed during the luteal phase of the estrous cycle and gestation were very similar (Fig. 1). Day-by-day *t*-tests (*16*) of mean estradiol and progesterone concentrations on equivalent days of the estrous cycle and gestation revealed no significant differences except on day 2, when estradiol concentrations were higher in the pregnant animals ($P < .05$). This difference might reflect stimuli associated with copulation, but it occurred too early to implicate true recognition of pregnancy by the uterus. Furthermore, both individual and averaged concentrations of estradiol after estrus fluctuated widely, with no apparent pattern regardless of reproductive status. Thus we regard the difference on day 2 to be trivial compared to the overwhelming similarity and statistical equivalence of the profiles for estradiol and progesterone and of the ratios of estradiol to progesterone (E : P) throughout the estrous cycle and gestation.

Estradiol profiles of the estrous cycle in other marsupials are not available, but our data (Fig. 1A) followed the general pattern seen in eutherians (*17*). Proestrus concentrations (days −6 to −1) increased to a high of 23 ± 2 pg (mean ± standard error) per milliliter of serum and then dropped to 9 ± 1 pg/ml at estrus when progesterone concentrations were at their lowest point in the cycle. This surge of estrogen is apparently a requisite among mammals for the onset of estrus and one in a sequence of endocrine signals leading to ovulation (*17*). Estradiol fluctuated between 5 and 16 pg/ml during the luteal phase of estrous cycle (days 1 through 13), the pattern and range of values being similar to those in sheep (*18*) and very close to the extremes of 3.8 and 15.9 pg/ml in plasma from pregnant tammar wallabies (*Macropus eugenii*) (*19*).

Profiles of circulating progesterone were unimodal and similar in the two reproductive states (Fig. 1B). Peak concentrations occurred on day 8 of the estrous cycle (13.6 ± 1.3 ng per milliliter of serum) and on day 9 of gestation (17.8 ± 3.4 ng/ml). The rapid decline of progesterone to low concentrations on days 11 and 12 was very similar in pregnant and nonpregnant females.

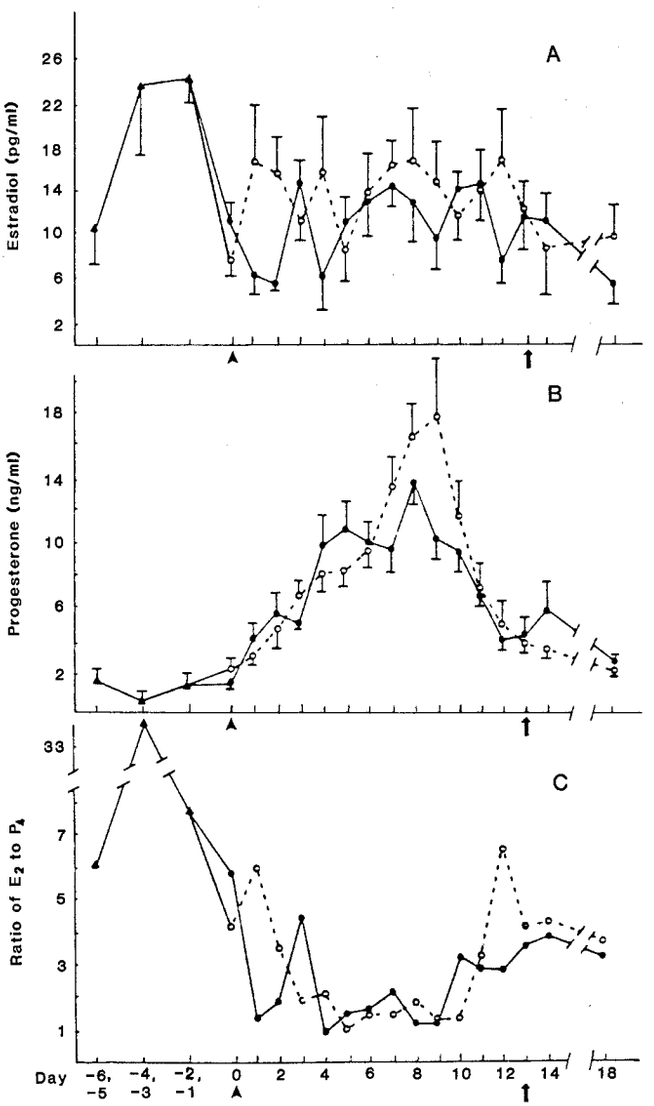

Fig. 1. Concentrations of (A) estradiol and (B) progesterone, and (C) the ratios of estradiol (E_2) to progesterone (P_4) (picograms per nanogram) in the peripheral serum of female Virginia opossums during the estrous cycle and gestation. Symbols: ●, nonpregnant; ○, gestation. The number of animals sampled on each day of the estrous cycle or gestation ranged from 5 to 25. A minimum of 11 were sampled on days 0 (estrus, indicated by arrowheads), 3, 7, 11, 12, and 13 (parturition, indicated by arrows). Means are presented ± 1 standard error.

Progesterone concentrations were significantly correlated ($y = 0.27x - 15.9$, $r^2 = 0.86$, $P < .01$) with an index of luteal mass, the sum of the maximum diameter of all corpora lutea in both ovaries. Thus, the relatively high progesterone concentrations found here and in a previous study (12 ng/ml) (*20*) were probably the result of the high luteal mass in the Virginia opossum (30.6 ± 1.1 corpora lutea per ovary per cycle in our study). In contrast, maximum average concentrations of luteal phase progesterone in monovular (one corpus luteum per cycle) Australian marsupials were much lower: 4.5 ng per milliliter of plasma in *Trichosurus vulpecula* (*11*), 2.5 ng/ml in *Setonix brachyurus* (*13*), and 902 pg/ml in *Macropus eugenii* (*19*).

Estrogens and progesterone do not act independently in the control of reproductive processes; rather, their effects can be synergistic or antagonistic depending on their relative concentrations in the blood. Consequently, the E:P ratio (in picograms per nanogram) provides an integrated view of hormonal control at any point in the reproductive cycle. The maximum E:P ratio just before estrus (Fig. 1C) is typical of many, but not all mammals (*17*). The E:P ratio remained low during both the midluteal phase of the estrous cycle and midgestation and then increased rapidly just prior to parturition, an increase known to be a critical signal for the onset of parturition in many eutherians (*21*). However, despite these biologically important dynamics, no significant daily differences ($P > .05$) in the E:P ratios of pregnant and nonpregnant animals occurred at any point from days 0 to 14 (*22*), providing further support for the equivalence hypothesis in this marsupial.

Parturition is an enigma in the context of the equivalence hypothesis, because it has no functional counterpart in the estrous cycle. Also, it might be controlled by endocrine signals and hormones not investigated in our study (*21*). These must include, however, ovarian hormones, because parturition did not occur in ovariectomized opossums (*23*). Indeed, analysis of the E:P ratio profiles (*24*) of pregnant animals revealed a significant ($P < .05$) peak on day 12 compared to other samples obtained around parturition (days 10 to 14, Fig. 1C). Thus, a rapidly increasing E:P ratio from days 10 to 12 might be an important endocrine signal leading to uterine contractions and parturition in the Virginia opossum.

Our data indicate that endocrine recognition of pregnancy did not occur, but this conclusion should not be extended to all marsupials. Metatherians vary considerably in length of gestation relative to the estrous cycle and in the extent of embryo-uterus interactions (*25*), and recently a difference was reported between progesterone concentrations of pregnant and nonpregnant quokkas (*Setonix brachyurus*) (*13*). Furthermore, other hormone profiles could have been measured. Estradiol and progesterone were selected because they play central roles in the regulation and maintenance of the estrous cycle and gestation, but further tests of the equivalence hypothesis involving gonadotropins, oxytocin, prostaglandins, and relaxin are needed to understand the evolutionary and physiological significance of marsupial reproduction.

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Diethylstilbestrol Induces Neoplastic Transformation Without Measurable Gene Mutation at Two Loci

Abstract. *The frequency with which diethylstilbestrol induces neoplastic transformation and somatic mutation was measured concomitantly in Syrian hamster embryo cells. While diethylstilbestrol was as active as benzo*[a]*pyrene in inducing transformation, it failed to induce mutations at two conventionally studied loci. These results suggest that diethylstilbestrol may transform cells in the absence of gene mutations.*

Diethylstilbestrol (DES) is a synthetic estrogen known to be a carcinogen in humans and rodents (*1*, *2*). Because DES has been used as a food additive for cattle and as a gynecological medication for women (*1*, *2*), it is of interest in studies of the mechanism of cancer induction.

The observation that most carcinogens are mutagens in bacterial assays such as the Ames test provides strong support for the somatic mutation theory of carcinogenesis (*3*). However, DES is not active as a mutagen in such tests (*4*) and thus represents an important exception to the relation between mutagenesis and carcinogenesis. This observation and the known estrogenic activity of DES may indicate that DES induces cancer by a mechanism that differs from that of other environmental carcinogens. However, the failure of DES to cause mutagenic activity in prokaryotic assay systems may be due to the absence of adequate metabolic activation, as has been shown previously for other carcinogens. Metzler and McLachlan (*5*) demonstrated that DES can be oxidatively metabolized to produce electrophilic intermediates that interact with cellular macromolecules.

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