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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Using a cellular system for the study of somatic mutation and neoplastic transformation in the same cells (*6*), we found that DES induces neoplastic and morphological transformations of Syrian hamster embryo cells in culture at a frequency similar to that observed with the carcinogen benzo[*a*]pyrene. Under conditions that result in transformation, DES fails to induce somatic mutation at two genetic loci, whereas benzo[*a*]pyrene is mutagenic. This is an example of definitive dissociation of these two processes measured in the same cellular system.

When Syrian hamster embryo cells were treated with DES (0.01 to 10 μg/ml), colonies were morphologically transformed in a dose-dependent manner. The morphology of the DES-transformed colonies was indistinguishable from that observed after treatment with benzo[*a*]pyrene and other carcinogens in these and previous studies (*7*). Morphological transformation occurred after treatment with noncytotoxic doses of DES (0.01 to 0.1 μg/ml for 48 hours). Treatment of the cells with larger doses of DES (1 μg/ml for 48 hours) (82 percent survival) in a respreading assay (*8*) resulted in morphological transformation of 0.7 percent of the surviving colonies (Table 1). This is comparable to the amount of transformation induced by benzo[*a*]pyrene at 1 μg/ml for 48 hours (Table 1). No morphological transformation was observed in control cultures treated with solvent only [0.1 percent dimethyl sulfoxide (DMSO)]. Cultures treated with benzo[*a*]pyrene or DES gave rise to tumorigenic cell lines, whereas control cultures were nontumorigenic and senesced (*9*).

Induction of somatic mutations at the loci for hypoxanthine-guanine phosphoribosyl transferase (HPRT) and for sodium- and potassium-dependent adenosinetriphosphatase (Na^+,K^+-ATPase) was measured by the frequency of occurrence of 6-thioguanine–resistant (TG^r) and ouabain-resistant (Oua^R) colonies, respectively. The conditions used are suitable for such studies (*8*). Benzo[*a*]pyrene at 1 μg/ml for 48 hours induced at least a 72-fold increase in the frequency of mutations at these two loci, with optimal expression of mutations 4 days after treatment (Fig. 1). No mutants were observed in 10^6 cells from any of the solvent-treated control cultures at any of the expression times after treatment. Likewise, no mutants were observed from cultures treated with DES (1 μg/ml) for 48 hours (Fig. 1), although morphologically transformed colonies were seen in the cloning assay from the same cultures at every expression point of the experiment. Similar results were obtained in four separate experiments with DES at 1 μg/ml and other doses.

Table 1. Comparison of morphological transformation and somatic mutation by benzo[*a*]pyrene and diethylstilbestrol. Morphological transformation is computed as the number of morphologically transformed colonies divided by the total number of colonies scored.

Compound	Concentration	Percent survival*	Morphological transformation		Somatic mutation†	
			Percent	Ratio	Oua^R	TG^r
Benzo[*a*]pyrene	1 μg/ml	69	0.9	20/2291	9.4×10^{-5}	7.2×10^{-5}
DES	1 μg/ml	82	0.7	17/2472	$< 10^{-6}$	$< 10^{-6}$
Solvent only	0.1 percent DMSO	100	0.0	0/4092	$< 10^{-6}$	$< 10^{-6}$

*From a direct clonal assay. †Measured at the peak of the expression curve.

Our studies show that DES can induce morphological transformation of Syrian hamster embryo cells in cultures without inducing measurable somatic mutations at the conventionally studied HPRT and Na^+,K^+-ATPase loci. These results suggest that DES can transform cells through a mechanism other than a point mutation, a frameshift mutation, or a small deletion. Mutations at the chromosome level—for example, the loss or acquisition of one or more chromosomes by a mechanism such as nondisjunction—would not be detected by ouabain resistance or 6-thioguanine resistance. Ouabain is cytotoxic because it inhibits the activity of Na^+,K^+-ATPase, an essential enzyme, and loss of the ATPase gene does not result in ouabain resistance (*10*). The HPRT enzyme is not required for cell viability. Loss of this gene function results in 6-thioguanine resistance, but loss of the entire X chromosome is lethal because there is only one active X chromosome in a cell (*11*).

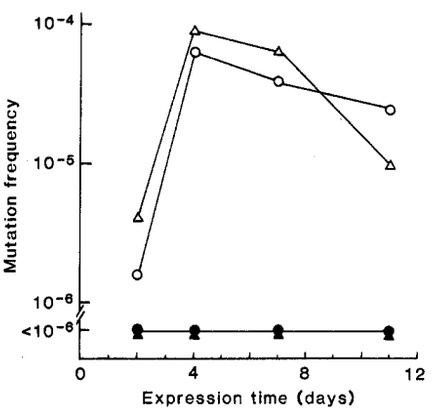

Fig. 1. Expression curve for induction of somatic mutations. Syrian hamster (Lakeview Hamster Colony, strain LVG/LAK) embryo fibroblasts were established and grown under the conditions previously described (*6–8*). Third-passage cells from cryopreserved stocks were plated in 75-cm^2 flasks at a density of 2.5 to 5 × 10^5 cells per flask. After 18 hours the cells were treated with solvent only (0.1 percent DMSO), benzo[*a*]pyrene (1 μg/ml) (open symbols), or diethylstilbestrol (1 μg/ml) (closed symbols) in medium with 10 percent fetal calf serum. The cells were incubated for 48 hours with the chemical, washed twice with phosphate-buffered saline to remove the chemical, and allowed to continue to grow for an additional 2 days. At that time the cells were subcultured by the procedure described (*8*) and were assayed for cytotoxicity and morphological transformation by plating 5 × 10^3 cells in each of five 100-mm dishes with normal complete medium; for ouabain resistance (△, ▲) by plating 10^5 cells in each of ten 100-mm dishes in complete medium containing 1.0 m*M* ouabain, and for 6-thioguanine resistance (○, ●) by plating 10^5 cells in each of ten 100-mm dishes in complete medium and thioguanine (3.0 μg/ml). All dishes were incubated to form colonies for 8 days. The mutation frequencies were calculated as previously described (*8*).

Genetic damage resulting from chromosome loss can be induced by DES. Roa and Engleberg (*12*) showed that DES causes chromosome nondisjunction in HeLa cells. Chrisman and co-workers observed aneuploidy in 8-day-old mouse embryos when DES was administered to pregnant mothers (*13*) and in bone marrow cells when DES was administered to male mice (*14*). Sawada and Ishidate (*15*) demonstrated that DES has colchicine-like effects on mammalian cells in vitro. Parry and Sharp (*16*) showed that DES causes mitotic aneuploidy in yeast. Preliminary results indicate that DES can cause chromosome polyploidization and nondisjunction in Syrian hamster embryo cells (*17*). The recent report (*18*) correlating aneuploidy with vaginal cellular dysplasia in young women exposed prenatally to DES suggests that DES-induced aneuploidy is important in neoplastic development in DES target tissues and provides a parallel between our results in vitro and studies in human subjects.

Thus, some carcinogens may be capable of inducing neoplastic transformation in the absence of somatic mutation, or neoplastic transformation may result from a mutational event at the chromosome level. Studies of the direct transformation of cells in vitro by DES may

yield new insight into the mechanism of DES oncogenicity in target tissues such as the uterus and vagina.

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Cancer Incidence in the Love Canal Area

Abstract. *Data from the New York Cancer Registry show no evidence for higher cancer rates associated with residence near the Love Canal toxic waste burial site in comparison with the entire state outside of New York City. Rates of liver cancer, lymphoma, and leukemia, which were selected for special attention, were not consistently elevated. Among the other cancers studied, a higher rate was noted only for respiratory cancer, but it was not consistent across age groups and appeared to be related to a high rate for the entire city of Niagara Falls. There was no evidence that the lung cancer rate was associated with the toxic wastes buried at the dump site.*

The carcinogenic potential of environmental contamination from chemical wastes has focused public attention on the dumping sites that contain these wastes. Love Canal is among the most important of these sites. We report data on cancer incidence in the census tract surrounding the Love Canal for the period 1955 to 1977 (*1*).

Eighty chemicals were originally identified at the landfill (*2*), and the number has grown. These include benzene, which can induce leukemia in humans, seven compounds including trichloroethylene, which are carcinogenic in animals, and several toxic chemicals. Studies of the effects of benzene and halogenated hydrocarbons on animals and humans suggest that, if the chemical contamination at Love Canal has resulted in actual human exposure, the most likely resulting cancers would be liver cancer, lymphoma, and leukemia (*3–5*). Because of the uncertainty about exposure and the large number of chemicals dumped, we examined rates of all categories of cancer as well as the three most likely cancers.

The census tract surrounding the Love Canal (Fig. 1) provided a distinct population base from which to calculate cancer incidence rates. According to the 1970 census, the tract contained 4897 people. The census tract is bounded by streams to the north and west, by the Niagara River and a parkway to the south, and by a sparsely populated rural area to the east. The residences located on and adjacent to the dump site include approximately 225 households and 700 individuals. This area, referred to as rings 1 and 2 in an earlier report (*2*), is darkly outlined in Fig. 1.

Waste burial started in the 1920's and ended about 1953. Aerial photographs show that housing development increased after the dumping stopped, population of the tract grew rapidly. Major public concern about health hazards associated with the buried wastes started in early 1978. Because this concern marked the beginning of substantial changes in the size of the tract population and may also have resulted in changes in disease reporting, we analyzed data through 1977 only.

The source of the cancer incidence data was cases reported to the New York Cancer Registry, which has collected reports since 1940. In general, the data from 1966 on are of better quality than the earlier data; for example, before 1966 the reference date for each case was the date of the report, and since then it has been the date of diagnosis. We examined rates for all sites of cancer for the period 1966 to 1977; rates for the cancers of special interest (liver cancer, lymphoma, and leukemia) were examined for the period 1955 to 1965 as well.

The populations on which incidence rates were calculated were estimated from the two decennial censuses and a special census in 1967. We used the 1970 census count for the years 1970 to 1977; the 1960 and 1967 census count for those years; linear interpolation for all other years from 1960 to 1969; and extrapolation of the resulting trend for the years 1955 to 1959.

We calculated age- and sex-specific cancer incidence rates for ten major cancer sites and five age groups for each of the 25 census tracts in the city of Niagara Falls. Although the number of cases in each age- and sex-specific category was quite small, this analysis helped rule out the occurrence of large increased risks in subgroups of the study population. (Such a procedure seems essential to epidemiological investigations where the etiological mechanism underlying the disease is poorly understood, as is the case with environmentally caused cancer.) For each of these 50 age-site groups, the 25 rates were arrayed in rank order by census tract for males and females. Rates for the Love Canal tract appeared in the highest quintile 9 out of 50 times for males, and 8 out of 50 times for females. All rates in the highest quintile were examined further. For example, the bladder cancer rate among males 65 to 74 years old from the Love Canal tract was in the highest quintile, but this elevated rate was confined to that age group and was based on only three cases. In four instances for males and two for females the occurrence of one case put the Love Canal tract in the upper 20 percent of tracts. In our judgment there were no unusual patterns among the age-specific rates.

Standardized incidence ratios (SIR's) (*6–8*), based on registry data and census tract population estimates, were calcu-

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