vations made previously for the early development of the chick, rat, and a number of amphibian and invertebrate species (*4, 10, 11*). These increases precede, or occur simultaneously with, increases in DNA, RNA, and protein synthesis (*2, 3*), and strongly imply a fundamental role for ODC and the polyamines in the phase of rapid growth associated with early embryonic development. Our data in this report support this conclusion in that they show that α-DFMO, an irreversible inhibitor of ODC, suppresses the increases in ODC and the polyamines associated with early murine gestation and arrests embryonic development at that point. However, it has recently been emphasized that the validity of such evidence depends entirely on proof of the specificity of the inhibitory drug for the particular metabolic pathway involved (*12*). On mechanistic grounds specificity would be predicted since α-DFMO works through the principle of substrate-induced inhibition (*5*). Thus, α-DFMO does not directly inhibit ODC irreversibly. Rather it is accepted by the enzyme as a substrate (*6*) and decarboxylated to yield a highly reactive intermediate which alkylates the enzyme and inactivates it irreversibly. In practice, the pattern of polyamine biochemical changes produced by α-DFMO is entirely consistent with selective inhibition of ODC. Further, α-DFMO does not inhibit other 1-carboxylyases such as glutamic acid decarboxylase, histidine decarboxylase, or aromatic L-amino acid decarboxylase (*6*), or SAM-DC (Fig. 1). Finally, the compound has no acute pharmacological activity and is essentially nontoxic, there being no untoward effects following a single oral dose of 5000 mg/kg. There is, therefore, no apparent explanation for the contragestational effects of α-DFMO other than inhibition of ODC. The close temporal correlation between inhibition of the peak rise in enzyme activity and arrest of embryonic development further supports this conclusion.

Barkai and Kraicer (*13*) found that substantial increases in ODC were associated exclusively with the maternal decidual reaction in the rat. We have confirmed this observation for the mouse (*14*), but we do not know whether the decidualizing tissue or the embryo itself is the site where the inhibition of ODC is functionally important. The histological evidence (Fig. 2) suggests that the decidual reaction takes place normally despite treatment with α-DFMO. Further, in recent experiments, contragestational effects have been obtained by administration of α-DFMO on day 8 of gestation only, at which time decidualization is established (*15*). The embryo, therefore, seems to be the functionally important site for inhibition of ODC. Decidualization may represent an example of a growth process which is associated with, but not dependent on, an increase in ODC activity.

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Phenobarbital Exposure in Utero: Alterations in Female Reproductive Function in Rats

Abstract. *Phenobarbital administration to pregnant rats from day 12 to day 19 of gestation suppressed body weight gain and produced significant effects on reproductive function in their offspring. These effects included delays in the onset of puberty, disorders in the estrous cycle, and infertility. Moreover, the animals exposed to phenobarbital in utero showed altered concentrations of sex steroids, gonadotropic hormones, and estrogen receptors. These findings suggest that phenobarbital treatment during prenatal development can produce permanent alterations in sexual maturation.*

Psychotropic agents such as tranquilizers and sedatives represent the major class of drugs prescribed to the pregnant woman (*1, 2*). Traditionally, investigators have studied the effects on fetuses of exposure to drugs and chemicals during organogenesis, since such exposure can cause malformations. Prenatal exposure to drugs, however, may also produce functional disturbances. We have used rats to study the long-term effects on reproductive function of exposure to phenobarbital in utero. This drug, which is widely used during human pregnancy, is known to block ovulation (*3–5*) in the adult rat, and treatment of neonatal rats with phenobarbital can alter adult sexual behavior (*6*). Our results show that phenobarbital has profound effects on reproductive function in female rats exposed to the drug in utero. These effects include a delay in the onset of puberty, disorders in the estrous cycle, and infertility.

Phenobarbital was administered to pregnant rats (Sprague-Dawley, CD strain) in a single subcutaneous injection in the morning from day 12 to day 19 of pregnancy. Each rat received 40 mg of the drug per kilogram of body weight per day. Control pregnant rats received injections of saline. Day 0 of gestation was the day on which a vaginal smear positive for sperm was first obtained. Six pregnant animals were assigned to each treatment group in every experiment. At birth the number of offspring in each litter was randomly adjusted to 8 or 10, with equal numbers of each sex in every group. Animals were weighed only once every week and weaned at 21 days of age. Food and water were constantly available. The onset of puberty was determined from the time of vaginal open-

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ing and appearance of the first estrus smear. The estrous cycle was examined when the rats were about 60 days of age, smears being prepared every day for 12 consecutive days. Fertility was determined by mating females at 80 to 90 days of age with normal males over a period of 5 to 15 days until there was evidence of successful mating, as determined by the presence of spermatozoa in the vagina; the presence of implantation sites was verified by cesarean section. To determine the concentrations of estrogen, progesterone, and luteinizing hormone (LH) in the plasma by radioimmunoassay (*7–9*), we used rats killed at approximately 3 months of age when they were in estrus. Estrogen receptor affinity and concentration were assayed in target tissues according to methods previously described (*10*).

The administration of phenobarbital during pregnancy had no effect on litter size. All newborn rats appeared normal by gross examination. Body weights, although similar to controls during the neonatal period, were depressed significantly in the phenobarbital-treated rats as puberty approached (Fig. 1). In addition, phenobarbital delayed the onset of puberty. Table 1 shows that a high percentage of animals exposed to phenobarbital in utero had persistent estrus or constant estrus and were infertile; however, the incidence of infertility decreased when the animals were mated over a longer period of time. Animals with persistent estrus conceived when they were allowed to remain with a normal male for a longer period of time, but animals with constant estrus did not conceive under such conditions.

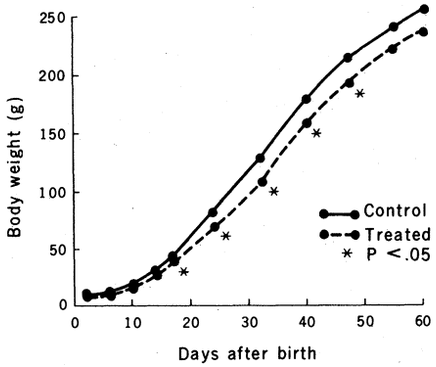

Fig. 1. Effect of prenatal treatment with phenobarbital on body weight gain. The data were analyzed by Student's *t*-test (day-by-day comparisons).

To investigate the mechanism for the reproductive dysfunction, we assayed hormone concentrations in plasma and found significant increases in both estrogen and progesterone and a decrease in the concentration of LH in the phenobarbital-treated rats (Table 2). The concentrations of cytoplasmic estrogen receptors in uteri were significantly increased in phenobarbital-treated animals (Table 2). However, no differences were observed in the brain, hypothalamus, and pituitary (data not shown), and the dissociation constant (K_d) for these receptors was unaltered.

These findings demonstrate that prenatal exposure to phenobarbital produced long-term defects in the onset of puberty, vaginal cyclicity, and fertility, all of which may be related to altered concentrations of both sex and gonadotropic hormones. In normal cycling rats, an increased concentration of LH is associated with an increased concentration of estrogen. In that regard, our observations of increased estrogen in the presence of lower values of LH in treated animals is surprising but not unusual. A similar pattern in LH and estrogen has been reported (*11, 12*) in animals injected with androgen during critical periods of sexual development (which induced sterilization). At present, we have no explanation for such observations.

The subnormal values of LH in the plasma of animals exposed to phenobarbital in utero suggest a defect in hypothalamic-hypophysial regulation of tropic hormone secretion. This phenomenon has been described in a previous study with adult rats (*4, 13*). However, this effect in the adult could be reversed once the drug was withdrawn, whereas the effects that we report here were present even 3 to 4 months after prenatal exposure to phenobarbital.

Increased estrogen receptor concentration in the uteri of treated animals suggests that phenobarbital also interfered with the development of steroid receptors, thereby altering the responsiveness to estrogen, which in turn resulted in reproductive dysfunction. Of course, increased estrogen receptor may be a

Table 1. Effect of phenobarbital on reproductive function in female offspring. The data (means ± standard deviation) were pooled from three separate experiments. Numbers in parentheses show the numbers of animals used. The onset of puberty is defined by vaginal opening and the first appearance of estrus smear. Normal cycle refers to 2 days of estrus followed by 2 to 3 days of diestrus. Animals with persistent estrus exhibited estrus smears during 70 to 80 percent of the cycle. Animals with constant estrus showed no diestrus smear at the time of examination.

Group	Average age of onset of puberty (days)	Estrous cycle (percentage of total)			Fertility (percentage of total)		
		Normal	Persistent	Constant	Mated for 5 days	Mated for 8 days	Mated for 15 days
Control	34.1 ± 1.5 (35)	96 (30)	4 (30)	0 (30)	100 (5)	100 (7)	100 (8)
Phenobarbital	36.5 ± 1.8 (47)*	42 (45)†	38 (45)†	20 (45)†	40 (5)*	62 (8)*	75 (8)

*$P < .01$. †$P < .001$, by Student's *t*-test or by χ^2-test.

Table 2. Effect of prenatal exposure to phenobarbital on concentrations of hormones in the plasma and estrogen receptors in the uterus. The data are expressed as means ± standard deviation. Numbers in parentheses indicate the number of animals used. 17β-Estradiol was assayed according to the method described by Korenman *et al.* (*7*); progesterone was measured with antiserum from Miles Laboratory by the procedure of Lindner *et al.* (*8*); and LH was analyzed with the radioimmunoassay kit distributed by NIAMDD program according to the procedure described by Niswender *et al.* (*9*). Estrogen receptors were determined according to the method of Gupta and Bloch (*10*) with the following modifications. Portions of 127,000*g* supernatant fractions were incubated with 20 nmole of [^{3}H]estrogen with or without 100-fold excess of unlabeled estrogen for 2 hours at 4°C. Estrogen receptor concentrations were determined from the specific binding of estrogen per milligram of protein.

Group	Hormones (ng/ml)			Estrogen receptor (pmole/mg protein)
	17β-Estradiol	Progesterone	LH	
Control	0.27 ± 0.04 (12)	0.94 ± 0.45 (10)	8.40 ± 1.20 (8)	0.27 ± 0.04 (8)
Phenobarbital*	0.69 ± 0.21 (12)†	1.62 ± 0.45 (12)†	4.50 ± 2.02 (10)‡	0.69 ± 0.21 (10)§

*The animals with reproductive disorders were used for biochemical determinations. †$P < .01$. ‡$P < .001$. §$P < .05$, Student's *t*-test.

secondary effect of the high estrogen concentration observed in these animals, as suggested by King *et al.* (*14*). Alternatively, the increased cytoplasmic receptors may mean that there is a block in the translocation of estrogen receptors from cytoplasmic to nuclear sites with a subsequent buildup of receptors in the cytoplasm (*15*). The preliminary finding of significantly decreased nuclear binding (50 percent) in phenobarbital-treated animals supports the latter possibility.

Other long-term effects of phenobarbital exposure in utero have been suggested by several investigators (*16–22*). Middaugh *et al.* (*19*) found that the development of several neurologic reflexes was retarded in mice, and an increased incidence of congenital malformations was reported in humans (*21, 22*). Recently, a defect in sexual mating behaviors was found in rats exposed to this drug after birth (*6*).

The clinical significance of our results is unknown, but the striking and permanent effects of phenobarbital on reproductive function in animals suggest that this drug should be used with great care in pregnant humans. Many of the other psychotropic agents taken by women during pregnancy may also have delayed effects on the biologic functions of their offspring.

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DNA Polymerases in Parasitic Protozoans Differ from Host Enzymes

Abstract. *Analysis of extracts of the bloodstream forms of* Trypanosoma brucei *showed that both DNA polymerase-α and DNA polymerase-β activities were present. The detection of DNA polymerase-β in* T. brucei *demonstrates the presence of this enzyme in unicellular organisms. DNA polymerase-β is present also in* Leishmania mexicana. *The DNA polymerases in* T. brucei *are immunologically distinct from the host enzymes. The structural differences between the parasite and the host enzymes could be exploited for the development of agents to combat parasitic diseases.*

The existence of DNA polymerase-β (*1*) in animal, plants, and bacteria has been surveyed (*2*). DNA polymerase-β is found only in multicellular animal cells and is not present in free-living protozoans (*3, 4*), yeast (*5*), and slime molds (*6*), and it has not been detected in plants or in bacteria.

Relatively little information exists concerning the molecular biology and biochemistry of parasitic protozoa, which are generally found in a protected environment within the animal host or insect vector and contain the causative agents of diseases in both humans and domestic animals. *Trypanosoma brucei*, for example, is a flagellate protozoan that causes trypanosomiasis in livestock and is closely related to the trypanosomes responsible for human sleeping sickness.

One approach to combating parasitic diseases is to augment the host's immunological defense mechanisms. Another is to administer drugs targeted against specific enzymes and proteins of the parasites (*7*). In trypanosomiasis, large quantities of parasites can be obtained for exploratory biochemical studies by infecting mice or rats with *T. brucei*. We therefore studied enzymes involved in nucleic acid metabolism in *T. brucei* in an attempt to detect differences between the protozoan and mammalian enzymes. We now report the presence of both DNA polymerase-α and DNA polymerase-β in extracts of *T. brucei* (bloodstream form) and show that the enzymes in the parasite are distinct from the host enzymes.

Soluble extracts of *T. brucei* (*8*) were prepared, separated on sucrose gradients, and analyzed for DNA polymerase activities (*9*). The sucrose gradient profile of DNA polymerase activity in *T. brucei* is presented in Fig. 1. Both DNA polymerase-α and DNA polymerase-β are present, and the sedimentation characteristics of each are similar to that for the corresponding mammalian enzyme (*2*).

The detection of DNA polymerase-β activity in *T. brucei* is unusual, and it is necessary to demonstrate that the enzymes extracted from the purified *T. brucei* are not residual host enzymes, that is, rat DNA polymerase-α and rat DNA polymerase-β. This demonstration can be made with antiserums to rat DNA polymerases. The antibody for DNA polymerase-α is a rabbit immunoglobulin G fraction with broad specificity (*10*). This antiserum was prepared against a partially purified polymerase-α from calf thymus terminal deoxynucleotidyltransmerase-α from other mammalian sources as well as mammalian DNA polymerase-β. This antiserum is inactive against DNA polymerase-α from sea urchin, calf thymus terminal deoxynucleotidyltransferase, DNA polymerase-γ from rat liver, and *Escherichia coli* DNA polymerase I. The antiserum to DNA polymerase-β used in this study was obtained by immunization of a rabbit with homogeneous DNA polymerase-β from calf thymus (*11*). The antiserum to DNA polymerase-β inhibits DNA polymerase-β from all mammalian sources tested. However, the titer is lower for DNA

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