white matter became more hypoxic than gray matter. In a model of cerebral oligemia Welsh *et al.* (*15*) observed a disproportionately greater accumulation of lactate in white matter compared to gray matter. These authors postulated that this "failure" of white matter metabolism resulted from a relatively greater reduction in blood flow to white matter. During severe hypoxia, cerebral blood flow may increase up to fourfold (*16*), thereby promoting the delivery of oxygen to the tissues. However, Ginsberg *et al.* (*17*) demonstrated in moderately hypoxic, normotensive "Levine" rats that the rise in blood flow to several gray matter structures was variable, ranging from 40 to 100 percent. Although blood flow data for white matter are presently unavailable, it is conceivable that regional cerebral blood flow to white and gray matter during hypoxia is altered disproportionately to their metabolic needs and that white matter may sustain the more severe insult.

The present findings of regional differences in cerebral glucose metabolism during hypoxia may be relevant to human stroke and other clinical conditions of cerebral hypoxia-ischemia. Postanoxic leukoencephalopathy in man is a condition that is characterized by edema and necrosis of white matter with relative sparing of gray matter structures (*18*). The precise hypoxic insult or combination of insults necessary to reproduce this clinical condition experimentally are unknown. Further elucidation of the biochemical and physiological factors responsible for the greater sensitivity of white matter metabolism to hypoxia may lead to the understanding of the susceptibility of white matter to hypoxic injury.

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Clomid Administration to Pregnant Rats Causes Abnormalities of the Reproductive Tract in Offspring and Mothers

Abstract. *In rats, a single injection of clomiphene citrate (Clomid) during pregnancy causes multiple abnormalities of the reproductive tract in the offspring and mothers. These abnormalities probably result from the ability of Clomid to cause long-term estrogenic stimulation.*

Clomiphene citrate (Clomid) is used extensively for the induction of ovulation in women with secondary amenorrhea (*1*). Injection of Clomid to neonatal rats causes extensive abnormalities of the reproductive tract in adult rats (*2*). In this report we demonstrate that, in rats, a single injection of Clomid during pregnancy results in the development of abnormalities of the reproductive tract in the pups when they become adults, as well as in the mothers. These results indicate the potential danger which may be inherent in the use of this drug in women.

Rats of the Sprague-Dawley strain were mated, and the morning that copulatory plugs were found was designated as day zero of pregnancy. Clomid (2.0 mg/per kilogram of body weight) was injected on days 0, 5, or 12 of pregnancy (*3*). At birth, the number of pups was adjusted to eight per mother rat. The pups remained with the mother rat until the day of weaning (day 21) without further handling except for weekly determinations of body weight. At weaning, males and females were separated and caged in groups of four and checked daily for preputial separation or vaginal opening. Once this was determined, rats were kept undisturbed until they were killed at

Table 1. Incidence of epithelial abnormalities in the organs of rats treated with Clomid during pregnancy. The rats were injected with Clomid (2.0 mg/kg body weight) on day 1, 5, or 12 of pregnancy. Rat mothers (N = 12) and offspring (N = 28) were autopsied 100 days postpartum. Control females received either no treatment or 0.1 ml of oil. Results are expressed as percentages.

Group	Highly disorganized epithelium	Extensive hyperplastic vacuolated atypical epithelium	Extensive metaplastic epithelium	Degenerating epithelium	Cysts	Sloughing of noncornified epithelium
			Vagina			
Control	0	0	0	0	0	0
Mothers	0	10	0	10	24	31
Offspring	0	0	0	0	8	21
			Cervix			
Control	0	0	0	0	4	8
Mothers	12	35	35	12	0	12
Offspring	14	21	18	0	0	14
			Uterus			
Control	0	0	0	0	8	0
Mothers	12	47	47	0	12	12
Offspring	14	46	68	0	25	14
			Oviduct			
Control	0	0	0	0	0	0
Mothers	0	6	0	47	18	18
Offspring	0	11	0	14	0	0

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6, 8, or 15 weeks. The experiment was terminated at 15 weeks. The rat mothers were also killed at 15 weeks. Vaginal smears were obtained on the day the rats were killed, and the ovaries, oviducts, uteri, and vaginas were removed. These were placed in Bouin's fixative for subsequent sectioning and staining by routine hematoxylin and eosin procedures.

Rats that were injected on day 0 did not become pregnant. This is in agreement with the observations of others (*4*); however, when the rats were autopsied, 6, 8, or 15 weeks later, the uteri showed extreme stromal and glandular development with hyalinization and small angular nuclei, an almost obliterated lumen, and severely metaplastic and disorganized luminal epithelium. Two of the five animals had follicular cysts while three had many very large corpora lutea showing a peculiar fatty degeneration or vacuolization. Oviducts of these rats showed fluid distension with destruction of the epithelium in some areas. The incidence of atypical or abnormal epithelial tissues in these animals is shown in Table 1.

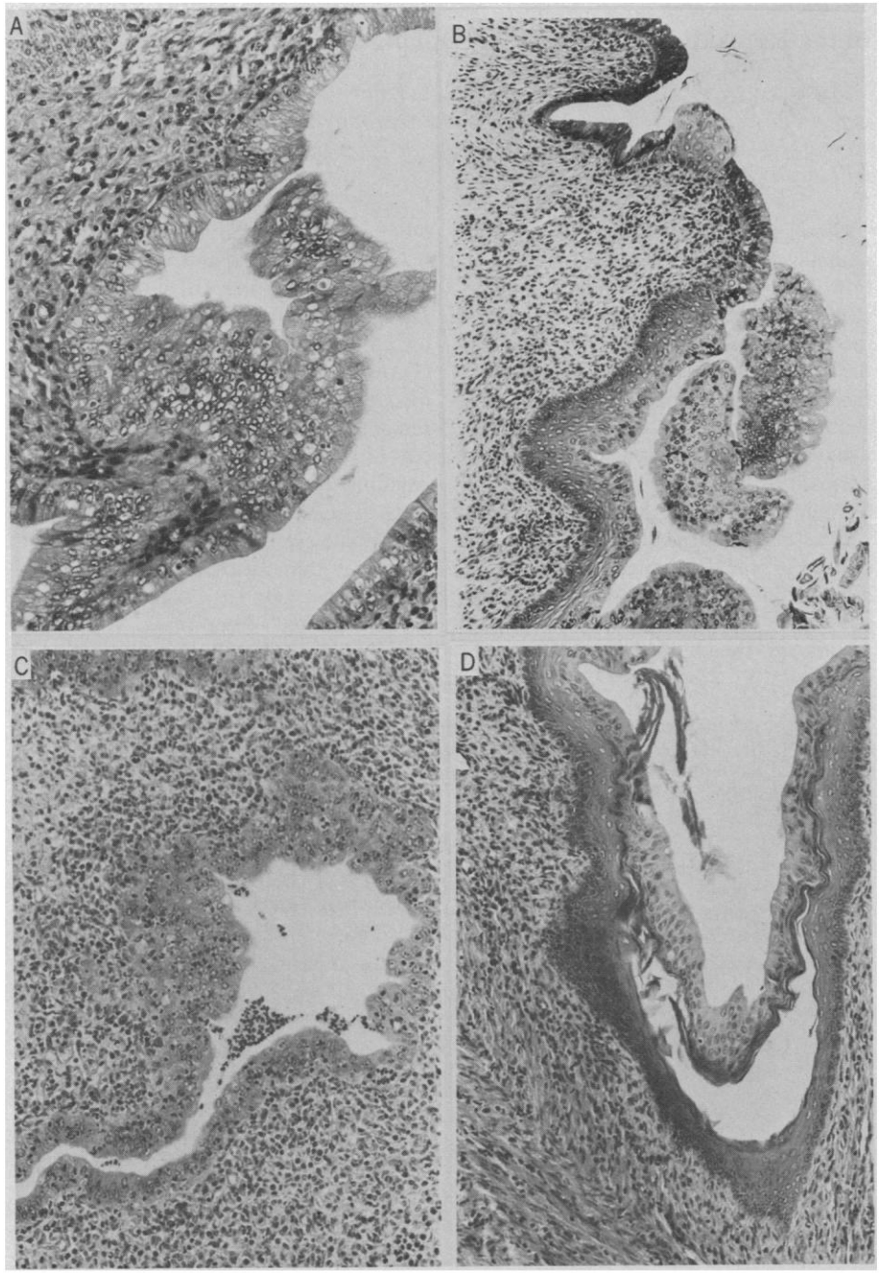

Fig. 1. Abnormal epithelial tissue in Clomid-treated rats. (A) Vacuolated luminal epithelium of the uterus in offspring (×430). (B) Hyperplastic disorganized epithelium at the utero-cervical junction in offspring (×100). (C) Hyperplastic epithelium which appears to be invasive in uterus of rat mother (×100). (D) Cornification in deep layers of vaginal epithelium with apparent sloughing of noncornified cell layers in vagina of mother (×100).

Three of the rats treated on day 5 became pregnant and delivered normal litters on day 21. When the rats were killed 15 weeks later, the reproductive tract of both offspring and mothers showed a remarkable array of abnormal or atypical conditions (Table 1 and Fig. 1). The incidence of disorganized and vacuolated epithelium in the vagina and cervix of the offspring is reminiscent of the vaginal adenosis that has been observed in young girls whose mothers received diethylstilbestrol during pregnancy (*5*). These abnormalities of the epithelium were also observed in the uterus and oviduct of both offspring and rat mothers. Disorganized hyperplastic epithelium, which appeared to be invasive, was observed in the uterus and vagina of offspring and mothers (Fig. 1C). The high incidence of sloughing of noncornified cells (Fig. 1D), cysts (both uterine and ovarian), degenerating epithelium, extensive metaplasia, and hyperplastic vacuolated epithelium indicate the extent of the abnormalities which can be produced by Clomid. In addition, leucocytic infiltration of the intercellular spaces and glands of the stroma and epithelium of the uterus was observed. This was occasionally seen in the follicles and corpora lutea of the ovaries of both offspring and mothers.

In addition to the abnormalities shown in Table 1, the uteri of the two rats that did not have litters contained extremely wide glandular stroma with a few glandular cysts and low atrophic luminal epithelium.

Of the six females injected on day 12, only four had litters successfully. One mother bore only three pups, two of which were stillborn and one which died 2 days later. A second female went 9 days beyond normal delivery date at which time a laparotomy revealed that one uterine horn, ovary, and oviduct was grossly abnormal and contained no conceptuses while the other side had a very large ovarian tumor with adhesions to the oviduct, uterus, intestine, and body wall. This tumor measured 7 by 10 by 6 cm and contained the necrotic remains of at least three pups. Histological examination of the other uterine horn revealed disorganized and possibly invasive epithelium with nests of degenerating stromal cells. The uterine luminal epithelium was metaplastic and cornified throughout the lower half of the uterus. The female pups of these mothers appeared normal at 3 weeks of age, but by 7 weeks areas of luminal epithelial metaplasia had appeared in the uterus. At 15 weeks, the epithelial metaplasia and general extensive stromal development with

glandular hyperplasia was marked. In two females the cervical epithelium showed proliferation, downgrowth, and glandular invasion of the stroma. The ovaries seemed normal, but the oviducts were distended with fluid and contained cysts without epithelium. One of nine male pups had testicular tubules in which spermatogenesis did not proceed beyond secondary spermatocytes. Interstitial cells were abundant. The epididymis was filled only with fluid and cell fragments and showed local cysts with inflammatory reaction. The remaining male offspring appeared normal.

The abnormalities observed in this report may arise from the early and intense estrogenic stimulation of developing fetal tissues by Clomid. Continuous exposure to high concentrations of estrogen during fetal or neonatal life is known to increase the incidence of preneoplastic and neoplastic changes in the reproductive tract (*5*, *6*). Clomid belongs to a class of compounds that has the ability to cause long-term estrogenic stimulation of certain cell types. Clomid causes long-term retention of the estrogen receptor in the nucleus of uterine cells, and this is accompanied by a sustained stimulation of uterotropic activity (*7*). This stimulation is primarily due to the ability of Clomid to stimulate the epithelium of the uterine lumen, whereas, estradiol, a physiological estrogen, causes all tissues of the uterus to grow (*8*). Therefore, Clomid can be a long-acting estrogen in some cell types and may cause hyperestrogenization of fetal tissues which ultimately leads to the expression of abnormal growth.

The abnormalities that were observed in the maternal tissues may also result from a similar hyperestrogenization. We have observed extensive epithelial cell stimulation in adult cycling rats (*9*) and assume that this takes place in the pregnant rat.

The long-acting effects of triphenylethylene derivatives and their ability to cause differential cell stimulation have obvious implications for the use of these drugs in humans. Clomid has been widely used for the past 15 years to induce ovulation in anovulatory women (*1*). For treatment a dose of 50 to 100 mg of Clomid is taken orally for 5 days. If the initial trial fails and no signs of pregnancy are present, the treatment is resumed the following month, about 40 days later. This treatment regimen is often continued for many months, hence the exposure of a woman to Clomid can be extensive. Under these circumstances, Clomid may be stimulating some cell types while acting as an antagonist in others. The eventual effects of such stimulation may remain unknown for many years. It is possible that Clomid and other triphenylethylene derivatives could cause hyperestrogenization of certain cell types in humans and hence great caution should be applied when these drugs are used in humans.

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Inhibition of Mast Cell Histamine Secretion by *N*-Substituted Derivatives of Phosphatidylserine

Abstract. *The structural basis for the highly specific action of phosphatidylserine in enhancing mast cell histamine secretion induced by concanavalin A was investigated by studying the activities of three* N*-substituted derivatives:* N*-acetyl phosphatidylserine,* N*-1-dimethylaminonaphthalene-5-sulfonyl phosphatidylserine, and* N*-4-nitrobenzo-2-oxa-1,3-diazole phosphatidylserine. None of the derivatives was capable of activating concanavalin A–induced histamine secretion at concentrations two to three times that required for maximal activation by phosphatidylserine. Instead, the derivatives were found to inhibit the secretory response of mast cells to the calcium ionophore A23187 as well as to concanavalin A. The inhibition was noncytotoxic, partially reversible by washing, and associated with binding of* N*-substituted phosphatidylserine to the mast cell.*

Upon stimulation of a mast cell with an appropriate secretagogue, its cytoplasmic granules are exocytosed in an energy-dependent process that involves fusion of the cell surface membrane with underlying perigranule membranes (*1*). As a result of mast cell degranulation, histamine stored in the secretory granules is released and is capable of increasing vascular permeability and stimulating smooth muscle contraction (*2*). Phosphatidylserine (PS) potentiates histamine release from mast cells exposed to dextran (*3*), antigen (*4*), or concanavalin A (Con A) (*5*). Other phospholipids—phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, phosphatidic acid, and phosphatidylethanolamine (PE)—have no effect on mast cell secretion (*6*, *7*).

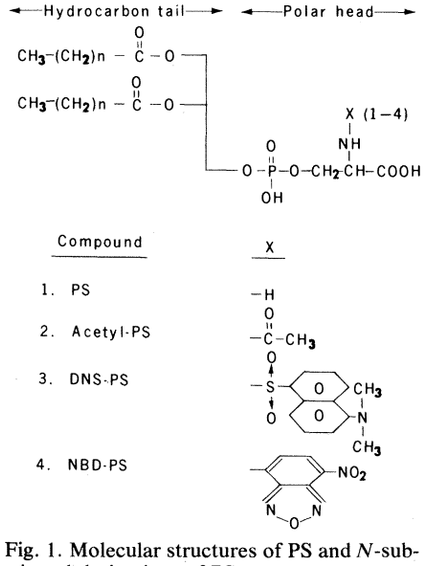

Fig. 1. Molecular structures of PS and *N*-substituted derivatives of PS.

We sought further information on the chemical specificity of the effect of PS on mast cell secretion by examining the properties of *N*-substituted derivatives of PS. Three such compounds were synthesized (*8*) and studied for their effects on mast cell secretion: *N*-acetyl phosphatidylserine (acetyl-PS), *N*-1-dimethylaminonaphthalene-5-sulfonyl phosphatidylserine (DNS-PS), and *N*-4-nitrobenzo-2-oxa-1,3-diazole phosphatidylserine

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