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AN ESTROGEN-DEPENDENT ENDOGENOUS PEROXIDASE IN UTERINE EPITHELIAL CELLS OF NEONATAL RATS*)

By

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ABSTRACT

The endogenous peroxidase synthesis within the uterine epithelial cells during the neonatal period of female rats following the estrogen stimulation was studied by means of the histochemical method, to elucidate the critical period in the initiation of its synthesis and the relation between the peroxidase synthesis and the morphological alteration within these cells.

The critical period was approximately 6 days of age, and it appeared that the period was earlier than that reported in previous biochemical investigations. The period is well in accord with the time when the quantity of estrogen receptor become sufficient to be functionally valid. It is suggested on this basis that the histochemical detection of intracellular peroxidase activity is more practical for clarification of estrogen-sensitivity of the tissue than the quantitative measurement of estrogen receptor in the neonatal rats.

Ultrastructural findings revealed that the initial appearance of intracytoplasmic estrogen-dependent peroxidase activity was most closely correlated to the developmental status of the rough endoplasmic reticulum and that the rough endoplasmic reticulum, the Golgi apparatus and the apical vesicles were involved in the process of peroxidase synthesis and intracytoplasmic transport.

It appeared that peroxidase synthesis was a good marker of estrogen-mediated cell growth, although the exact role of peroxidase in the uterine epithelial cells has been still uncertain.

INTRODUCTION

Since Lucas et al.[1] first reported that ovariectomized rats given an exogenous estrogen exhibited the synthesis of an endogenous peroxidase in the uterine tissue. This particular phenomenon in the uterus has been confirmed either biochemically or histochemically by various investigators including Martin et al.[2], Neufeld et al.[3], Bröckelmann[4], Bröckelmann & Fawcett[5], Lyttle & Jellinck[6] and Churg & Anderson[7].

In 1975, Anderson et al.[8] stated that the synthesis of an endogenous peroxidase induced by estrogen was seen not only in genital organs, such as the vagina, the cervix and the uterus, but also in estrogen-dependent rat mammary tumor. The generality of peroxidase synthesis

*) 井内康輝：新生児期ラットの子宮上皮細胞におけるエストロゼン依存性の内因性ペルオキシダーゼ活性について

following estrogen stimulation in female genital organs, besides the rat, was extended by Lyttle & DeSombre[9] in 1977 to the mouse, the guinea pig, the hamster and the human being. And it has come to the extent that the peroxidase in the estrogen-sensitive tissue can be regarded as a good marker for the estrogen action as well as the estrogen-mediated growth[10].

Up to the present, only a few histochemical investigations[5,7,8] on this particular peroxidase synthesis within the uterine tissue have been carried out. The author previously clarified that the estrogen-dependent peroxidase synthesis in target cells was limited to be induced only within epithelial cells and no evidence of peroxidase activity took place in non-epithelial endometrial stromal cells and myometrial smooth muscle cells under the estrogen influence[11].

Tokuoka[12,13] previously pointed out that following the estrogen stimulation endometrial stromal cells as well as myometrial smooth muscle cells displayed nuclear and cytoplasmic changes, indicating an accelerated collagen synthesis by means of electron microscopic and autoradiographic studies. In other words, it was clarified that estrogen-mediated nuclear and cytoplasmic alteration occurred in not only uterine epithelial cells, but also other components of the uterine wall. This fact that the non-epithelial cells with an estrogen receptor, showed no endogenous peroxidase synthesis under the estrogen influence seemed fully accord with the hypothetic views that peroxidase played a role in termination of estrogen action, which had been proposed by Lyttle & Jellinck[6] and Anderson et al.[8] Recent observations made by Keeping & Jellinck[14] and Jellinck et al.[15] in 1978 also suggested that the proposed hypothesis was debatable.

Even though the role of this particular enzyme in the estrogen-target tissue is still obscure, it is our view that the synthesis of this enzyme can be an excellent marker indicating estrogen-mediated growth activity in the target epithelial cells.

The present histochemical as well as ultrastructural study has been undertaken to elucidate the critical period in the initiation of estrogen-dependent peroxidase synthesis within the uterine epithelial cells during the neonatal period of female rats and ascertain if the parallelism between the peroxidase synthesis and the morphological alteration exists within these cells.

MATERIALS AND METHODS

Experiment I:

Sprague-Dawley female rats, purchased from Charles River Japan, Inc., were mated with male rats of the same strain obtained from the same source. All female neonates born at our laboratory were divided to 16 groups by each litter with a foster mother. Consequently, the number of neonatal female rats in each of these 16 groups became variable from 6 to 12, and the total number of rats submitted in this experiment became more than 150.

All of each two groups were given subcutaneously a single dose of 40 μg estradiol-17β (Sigma) dissolved in 0.05 ml propylene glycol at 1, 3, 4, 5, 6, 7, 14 and 21 days of age, respectively. The control animals composed of one or two animals in each group were given a single dose of 0.05 ml propylene glycol at the same times when the rest of all animals in each group were given estradiol-17β. All of these rats were fed by their foster mothers until the time when they were sacrificed.

Segments of the biocornated uterus in the first one or two rats in each group were excised under pentobarbital anesthesia at either 12 or 24 hours after the treatment, followed by consecutive surgical excisions of the uterus in every one or two rats at every 12 hours until 60 or 120 hours after the treatment.

The removed uterine tissue was fixed in a cold 4% formaldehyde-5% glutaraldehyde solution (Graham & Karnovsky[16], 1966) for 2 hours, and rinsed in 0.1M phosphate buffer (pH 7.4) for at least 12 hours, and freezed thereafter at $-70°$C.

The staining method of endogenous peroxidase in tissue preparation was the same as that described in our previous report[11]. The uterine tissue sectioned with a cryostat with either 4 or 20 μm thick was preincubated in a medium of 0.1% 3,3-diaminobenzidine-tetrachloride (DAB) for 45 minutes and incubated in the same medium containing 0.05% H_2O_2 for subsequent 15 minutes. After the incubation, the tissue slices were rinsed in 0.1 M tris-aminomethane buffer (pH 7.2) and consequently 0.1 M phosphate buffer (pH 7.4).

Tissue slice, 4 μm thick, was placed on a slide-glass and examined under the light microscope. On the other hand, tissue slice, 20 μm thick, was postfixed in 1% osmium tetraoxide for 1 hour, dehydrated in graded ethanol, and embedded in Epon 812. Then, ultra-thin section, either unstained or stained with saturated solutions of uranyl acetate and lead citrate (Sato[17], 1968) was examined with a Hitachi HU7 electron microscope.

Besides, a segment of excised uterine tissues, fixed in the same solution, was dehydrated and embedded in paraffin. Tissue section was stained with hematoxylin and eosin to examine with the light microscope. The height of the endometrial surface epithelium and the diameter of the uterine lumen were measured with eye-piece micrometer. Remaining uterine tissue excised was postfixed in 1% osmium tetroxide and embedded in Epon 812. Ultra-thin section was stained with saturated solutions of uranyl acetate and lead citrate to examine with the electron microscope.

Experiment II:

Neonatal female rats, 6 days of age, obtained with the same manner as the experiment I, were given intraperitoneally a single dose of 40 μg estradiol-17β(Sigma) in 0.05 ml propylene glycol, and the same dose of estrogen was injected twice to all of these rats at each interval of 24 hours. The uterus of each one or two rats was then excised consecutively under pentobar bital anesthesia at 24, 48, 60, 72 and 84 hours after the initial treatment.

Examinations of removed uterine tissue from these rats were the same as those in the experiment I.

RESULTS

Experiment I:

(1) Critical period of the initial peroxidase synthesis in the epithelial cells of the uterus

The results of the histochemical detection of peroxidase activity are as summarized in Table 1.

The rats given estrogen at either 1 or 3 days of age showed no evidence of peroxidase synthesis (Fig. 1). While, a more or less positive evidence of peroxidase synthesis was seen in the rats given estrogen at 5 days of age or thereafter.

Among the rats treated with estrogen at either 4 or 5 days of age, only a few rats became to display a weak positive evidence of peroxidase synthesis in the uterus after an interval time, 72 hours in the former 4 day-old rats and 48 hours in the latter 5 day-old rats. In other words, in the rats given estrogen at 4 through 5 days of age, a positive evidence of peroxidase synthesis in the uterus appeared in some of these rats at 7 days of age. The rest of the rats in the same groups did not show any evidence of peroxidase synthesis in the uterus at all.

Uneqivocal evidence of peroxidase synthesis in the uterus was first seen in all of the rats, treated at 6 days of age. Among these rats, some began to show a weak peroxidase activity at 36 hours after the estrogen administration. The most intensive evidence was noticed in the uterine epithelial cells of the rats at 48 hours after the estrogen administration. And none of the rats in this group remained to show a positive evidence of the synthesis at 72 hours

Table 1. Histochemical Detection of Peroxidase Activity in Rat Uterine Epithelial Cells

Days of age at treatment \ Hours after estrogen treatment	12	24	36	48	60	72	84	96	108	120
1		−	−	−	−					
3		−	−	−	−	−				
4	−	−	−	−	−	±	−	−		
5	−	−	−	±	−	−	−	−	−	−
6	−	−	±	+	−	−	−	−	−	−
7	−	−	+	+	−	−				
14	−	±	+	+	−	−				
21		+		+		+		−		

after the estrogen administration (Fig. 2).

The findings obtained from this group suggested at least that the time sequence of peroxidase synthesis within the uterus was gradually enhanced to reach the maximum around 48 hours after the estrogen administration.

Peroxidase activity in the rats given estrogen at 7, 14 or 21 days of age, was detected between 24 through 72 hours after the treatment. In general, the activity tended to last longer and to be more intensive at the maximum in these groups, with the increase of the neonatal age.

Throughout the present experiment, both the endometrial stromal cells and the myometrial smooth muscle cells were negative at all for peroxidase activity, and the control rats in each experimental group showed no peroxidase activity in the uterine epithelial cells at any stages.

In summary, it became clear that the critical period of peroxidase synthesis in the uterine epithelial cells following the estrogen stimulation was approximately 6 days of age during the neonatal period in the rat according to the present histochemical examination.

(2) Morphological alteration of the epithelial cells of uterus following the estrogen stimulation

The figures of morphological alteration of the uterus in the rats, treated at either 3 days or 6 days of age are shown in Figures 1 and 2. The average height of the surface epithelium of the endometrium and the average diameter of the uterine lumen in each of these rats are summarized in Table 2.

The uterus of the rats, treated at 3 days of age, revealed no evidence of either luminal dilatation or increased height of the surface epithelium. In the rats treated at 6 days of age, however, the uterine lumen gradually dilated to reach a maximum at 48 hours after the treatment and reduced the dilatation again at 60 hours. The cuboidal or flattened surface epithelial cells at first gradually increased the height to be tall-columnar in shape at 48 hours and returned to be cuboidal or flattened again at 60 hours.

Similar morphological alteration of the uterus seen in the rats treated at 6 days of age in the present observation was also noted in the uterus of spayed female rats treated with estrogen in our previous study[11].

In the control rats, no obvious morphological alteration of the uterus was observed, except for a gradual increase of uterine size along the neonatal aging.

(3) Ultrastructural alteration in relation to peroxidase synthesis in the epithelial cells of the uterus

In the rats treated at 6 days of age, the height of epithelial cells was increased and the nuclear-cytoplasmic ratio was decreased at 36 hours after the treatment with estrogen. The microvilli of these cells were increased in numbers and became longer. In apical areas of the cytoplasms many small vesicles were developed (Fig. 3). At 48 hours when peroxidase activity became most intensive in these cells, the nuclear euchromatin was prominent and the nucleolus was obscure. Intracytoplasmic profiles of the rough endoplasmic reticulum

Table 2. Morphological Modulation of Rat Uterus Following Estrogen Treatment at 3 and 6 Days of Age

	Hours after estrogen treatment	12	24	36	48	60	72	84	96
Diameter of uterine lumen (mm)	3 days of age	0.25 × 0.04	0.30 × 0.05	0.36 × 0.07	0.25 × 0.06	0.35 × 0.06	0.25 × 0.07		
	6 days of age		0.35 × 0.11	0.43 × 0.11	0.50 × 0.13	0.37 × 0.03	0.44 × 0.03	0.42 × 0.05	0.44 × 0.07
Height of surface epithelium (μ)	3 days of age	15	20	13	15	13	13		
	6 days of age		25	30	35	15	13	10	8

markedly developed and the cisternaes were irregularly dilated (Fig. 4, 5). However, these alterations did not take place synchronously in all epithelial cells and no obvious development of the rough endoplasmic reticulum was seen in some of the cells.

At 60 hours when peroxidase activity became undetectable, the height of epithelial cells was decreased. The nuclear heterochromatin became prominent with occasional nucleolus. The microvilli of the cytoplasm were decreased in numbers and became shorter, and the small vesicles in the cytoplasmic apex were disappeared. At this stage the most prominent finding was an evidence of extremely reduced distribution of profiles of the rough endoplasmic reticulum. Numerous autophagic vacuoles containing degenerative organelles and lipid vacuoles in basal area of cytoplasm became also conspicuous (Fig. 6).

In the control rats, the epithelial cells revealed no significant development of the rough endoplasmic reticulm and no apical vesicles were seen in the cytoplasm at any experimental stage.

These ultrastructural findings of the epithelial cells suggested that the initial appearance of peroxidase activity was most closely correlated to the developmental status of the rough endoplasmic reticulum.

Ultrastructural localization of peroxidase activity within the epithelial cells of the uterus in rats at 48 hours after the estrogen treatment was specified to profiles of cisternae of the rough endoplasmic reticulum, the Golgi apparatus and the small vesicles in the apical area. In strongly positive cells for peroxidase activity, entire profiles of the rough endoplasmic reticulum appeared to be involved, and in weakly positive cells the nuclear envelope and the rough endoplasmic reticulum only in juxta-nuclear areas were involved in peroxidase synthesis. No evidence of peroxidase activity was seen in remaining organelles including the mitochondria at any stages following the estrogen treatment (Fig. 7, 8).

These findings suggested that the rough endoplasmic reticulum, the Golgi apparatus and the apical vesicles were involved in the process of peroxidase synthesis, intracytoplasmic transport and release.

Experiment II:

The results of histochemical detection of peroxidase activity and morphological alteration in the rat uterine epithelial cells under the continuous stimulation with estrogen are summarized in Table 3. And the histologic appearances of the uterus including figures of peroxidase activity are shown in Figure 9.

Peroxidase activity in the epithelial cells appeared at 48 hours after the beginning of the treatment which was lasted until 84 hours, and its activity was almost similar at any stage.

The dilatation of the uterine lumen following the estrogen treatment was first seen at 48 hours after the beginning of the treatment and lasted until 84 hours. At 24 hours, the surface epithelial cells became columnar in shape and at 48 hours, they became tallest. No reduction of the cellular height took place thereafter.

Ultrastructurally, the surface epithelium was very tall and nuclear-cytoplasmic ratio was low at 48 hours after the beginning of the treatment. The nuclear euchromatin was prominent, while

Table 3. Histochemical Detection of Peroxidase Activity and Morphological Modulation in Rat Uterine Epithelial Cells after Continuous Treatment with Estrogen

	Hours after estrogen treatment	24	48	60	72	84
Peroxidase activity		−	+	+	+	+
Uterine Morphology	Diameter of uterine lumen (mm)	0.40 × 0.06	0.34 × 0.25	0.40 × 0.26	0.38 × 0.18	0.37 × 0.15
	Height of surface epithelium (μ)	28	35	28	23	25

the nucleolus was not prominent. The microvilli were abundant and well developed. Numerous small vesicles were seen in the apical area of the cytoplasm, and also the rough endoplasmic reticulum was prominent (Fig. 10).

At 72 hours after the beginning of the treatment, the same findings as seen at 48 hours were still present. In addition, large autophagic vacuoles containing degenerated organelles became prominent (Fig. 11).

Ultrastructural localization of peroxidase activity in the epithelial cells was confirmed within the cisternae of the rough endoplasmic reticulum, the Golgi apparatus and the apical vesicles at 48 hours after the beginning of the treatment as well as at 72 hours. Autophagic vacuoles showed no peroxidase activity.

DISCUSSION

Previous biochemical study made by Lyttle et al.[18] on an early response of target cells to estrogen stimulation indicated that the uterus of infant rats, as old as 11 to 13 days after birth first became sensitive to either estradiol-17β or stilbestrol stimulation. In these rats, significant increase of the uterine weight as well as the peroxidase concentration in uterine tissue appeared at the stage, 20 hours after the estrogen administration which was consistent with the stage in the more mature rats as described in their previous report[19].

On the contrary to this, the results of the present histochemical investigation on rat uterine tissue clarified an apparent evidence of response to estrogen in neonatal rats first appeared as early as 6 days after birth. However, it took more time to develop the initial response to estrogen, as long as 36 hours following the stimulation. In other words, there was no evidence indicating the response to estrogen at any stage earlier than 36 hours in these rats stimulated at 6 days of neonatal age. And, thereafter, the response to the estrogen stimulation began to appear earlier and last longer with the time sequence through the neonatal period.

In addition, according to Lyttle et al.[18], the maturation of the uterus is not strictly related to the neonatal aging, but is more closely related to the size and weight of the uterus itself reflecting the developmental status of the uterus during the neonatal period. Therefore, it can be said on these phenomena that the detection of estrogen susceptibility in target cells during the neonatal period ought to be done with extreme care at multiple spots covering various stages upon the time sequence following the stimulation.

In previous investigations dealing with biochemical response to exogenous estrogens during the infantile period in animals, the majority of biochemical examinations including RNA content[20] as well as DNA synthesis[21] and phosphorylation of 2-deoxyglucose[22], were made around the period of 12th through 15th neonatal dates, and only a few concerned with estrogen-stimulated biochemical synthetic activity in the uterine tissue during the earlier neonatal period.

Consequently, it is worthy to note that Walker et al.[23,24] detected synthesis of an estrogen-induced protein, called "IP", first described by Notides & Gorski[25], in rats as younger as 5 days of neonatal age. The period of estrogen-induced synthesis of this protein seems to be one of the earliest known biochemical response in neonatal rats, and is in accord with the period that the histochemical evidence of estrogen-induced peroxidase activity initiated in neonatal rats after birth in the present investigation.

From the view point of the first appearance of estrogen receptor within target tissue of neonatal rats, Clark & Gorski[26] stated that the receptor was detected in uterine tissue within the first day of birth. The quantity of uterine receptor in rats of 5 days old was about 2/3, as compared with that in rats of 21 days old. While, the quantity of cytosol receptor in the uterus of 5 to 7 days old rats appeared to be sufficient for the intracellular estrogen translocation from the cytoplasm to the nucleus.

This suggests that the susceptibility of rat uterine tissue to estrogen in the neonatal period initiates at 5th to 7th day after birth. Further, the time of initial appearance of estrogen susceptibility in neonatal rats well matches to the time of initial appearance of peroxidase activity that was detected in the present histochemical investigation, and this histochemical detection of peroxidase activity is more useful for clarification of estrogen sensitivity than the direct demonstration of estrogen receptor in the neonatal period, as described by Jellinck &

Newcombe[27].

Furthermore, it is of my interest to note that the critical period in the present experiment is consistent with that of an experimental induction of the persistent estrous state. Takasugi[28] showed that the critical period to induce persistent estrous state in neonatal female mice by estradiol administration, was 3 days of age, and suggested that there is a good correlation between the cellular differentiation and reponsiveness to steroid hormone. With regard to the mechanism of persistent estrous state, Thrower et al.[29] studied on sex hormone receptors in the hypothalamus and the uterus of rats in persistent estrous status, induced by the administration of testosterone neonatally and pointed out that premature exposure to sex steroid hormone, even though transient, interferes with translocation of estrogen from cytosol to nuclear receptor. From these facts it appears that the cellular differentiation of uterine epithelial cells to be an estrogen-target cells begin at several days after birth. The results of the present investigation well support this view.

Attention has been called to the development of unusual carcinoma, clear cell adenocarcinoma, in the genital tract of young women who were exposed in utero to a synthetic estrogen, DES[30-32]. Because of very specific selectivity to affect only Müllerian duct epithelium[33], Metzler & McLachlan[34,35] payed an attention to peroxidase activity in target tissue whether it plays a significant role in DES carcinogenicity. The hypothesis they offered was that peroxidase mediated oxidation of DES and binding of oxidized DES to nuclear DNA and protein. A recent knowledge that the male hamster kidney that is susceptible to carcinogenic effect of DES[36], shows much higher peroxidase activity than the kidney of rats and mice, less susceptible to DES carcinogenic effect[37], might well support the above-described hypothesis of DES carcinogenicity.

To confirm the relationship of peroxidase activity with carcinogenic or teratogenic effect of DES to the female genital tract in utero, it is thought to be the most important to ascertain whether or not this enzyme can be induced prenatally by DES treatment to maternal animals. However, the result of the present investigation that the critical period of peroxidase synthesis following the estrogen stimulation during the neonatal period was around 6th day after birth does not accept this hypothesis. Moreover, it was observed in our preliminary experiment that the maternal placenta had no peroxidase activity and the neonatal female rats immediately after birth, delivered from maternal rats treated estrogen during pregnancy, also showed no evidence of peroxidase activity.

A microelectrophoretic study of uterine peroxidase in rats revealed that it was in the range of 100,000 in molecular weight and was divided into two subunits, 70,000 and less than 20,000 in each molecular weight[38]. As to the functional significance of uterine peroxidase, Klebanoff & Segal[39] showed that estradiol was inactivated by horseradish peroxidase or myeloperoxidase in mice, and Bröckelmann[4] indicated that following radioactive estradiol-17β injection to the uterus of rats the radioactivity was detected over intracytoplasmic localizations of peroxidase activity. Moreover, Lyttle & Jellinck[6,40] suggested that uterine peroxidase of rats can catalyse estrogen to water-soluble by-products and may function to terminate estrogen action in the target tissues.

However, recent investigations[14,15] indicated that even though uterine peroxidase of rats can catalyse the metabolism and covalent binding of estradiol to protein in vitro, it is unlikely to limit the duration of estrogen action in vivo. Also it has been suggested that the female reproductive tract peroxidases of rats or other species function as germicides, fungicides or spermicides in halogenation reactions[41], but the ultimate significance of intrauterine peroxidase has not been specified yet.

Ultrastructural observations of intracytoplasmic peroxidase in the rat uterus have been made by Bröckelmann & Fawcett[5], Churg & Anderson[7] and Anderson et al.[8] The localization of the peroxidase was seen within profiles of the cisternae of rough endoplasmic reticulum including the nuclear envelope, the Golgi apparatus and the secretory granules, suggesting that the peroxidase could be transported to the uterine lumen by an intracellular route, although further investigations in regard with its synthesis, intracellular transport and release are necessary[42].

Nilsson[43-47] indicated that an ultrastructural modulation of epithelial cells in the mouse

uterus took place under an estrogen influence, which was represented by evidences including the development of rough endoplasmic reticulum, the increase of small vesicles in the apex, the elongation of microvilli and the decrease of lipid during the estrous stage. Kang et al.[48] showed a histological modulation of the uterine epithelium in immature female rats by estradiol-17β treatment. Topographical changes of the luminal surface of uterus in rats under the scanning electron microscope was demonstrated by Anderson et al.[49]

All of these findings appeared to be coincident with those seen in the present observation following the estrogen stimulation. It is strongly suggested, therefore, that peroxidase synthesis in uterine epithelial cells of these animals is not only closely related to the development of rough endoplasmic reticulum during the estrous state, but also highly indicative to be a good marker of estrogen-mediated cell growth, although the exact role of peroxidase is still uncertain.

It is worthy to note that the significance of endogenous peroxidase activity within carcinogen-induced mammary tumors in small rodents has been also confirmed by various investigators[50-52] as a reliable indicator of the tumor growth that depends upon estrogen influence. Similar good parallelism of endogenous peroxidase synthesis and estrogen-receptor activity has been elucidated by Duffy & Duffy[53] and Collings et al.[54,55] in human mammary cancers.

Intracellular localization of peroxidase under the electron microscope has clarified by means of cytochemical application of 3, 3'-diaminobenzidine (DAB) to various mammalian cells, such as neutrophils[56-59], eosinophils[60,61], peritoneal macrophages[62,63], Kupffer cells of liver[64,65], thyroid[66,67], larynx[68], large intestine[69,70], salivary glands[71-77], lacrimal gland[78,79] and lactating mammary gland[80]. It is known that one of these peroxidases, the lactoperoxidase, 82,000 in molecular weight[81], can be synthesized in epithelial cells of the mammary gland in prelactation through lactation stages and of the salivary gland as well as the lacrimal gland. It is said that this particular peroxidase acts as a bacteriocidal agent in the milk and in other extracellular fluids. Ultrastructural localization of its activity in epithelial cells is known to be restricted within profiles of the cisternae of the rough endoplasmic reticulum including the nuclear envelope, the Golgi apparatus and the secretory granules. The localization of peroxidase in uterine epithelial cells is similar to this lactoperoxidase.

In regard with endogenous peroxidase synthesis during fetal and/or neonatal periods in rats, only those in the salivary and lacrimal glands have ever been investigated. According to Yamashita & Barka[75], peroxidase activity in the rat salivary gland first appeared within profiles of the rough endoplasmic reticulum including nuclear envelope of epithelial cells at the 17th gestation date. While, peroxidase in the lacrimal gland, the activity first appeared at 6 hours of the neonatal period[79]. These observations suggest that the estrogen-independent synthesis of various endogenous peroxidases in various organs appears at variable stages during the fetal through the neonatal period and some of these appear earlier than the estrogen-dependent synthesis in the neonatal uterine epithelium.

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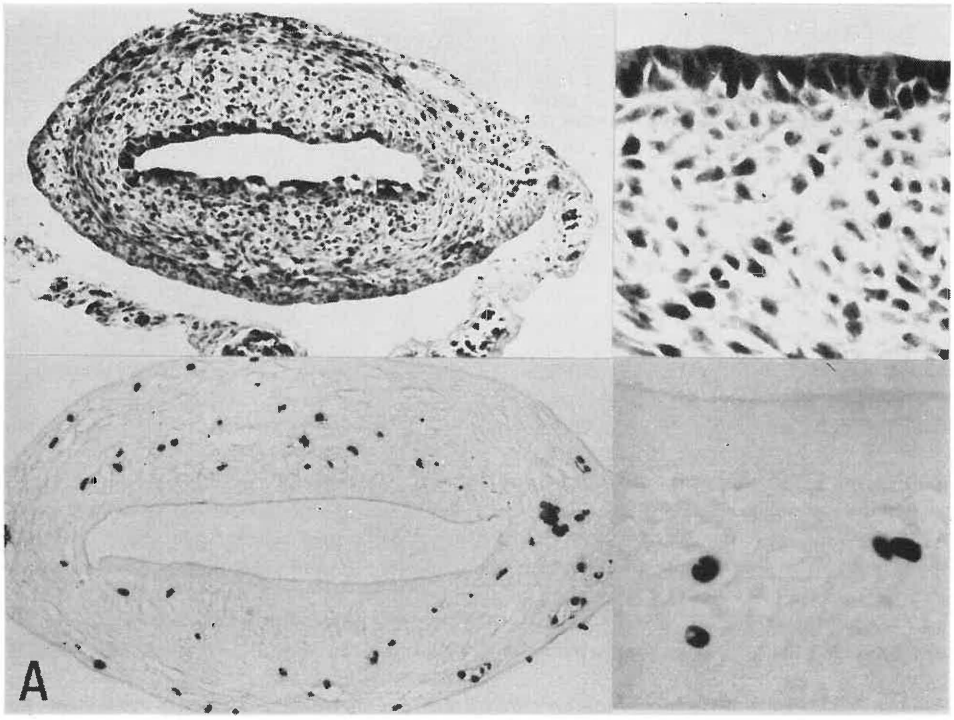
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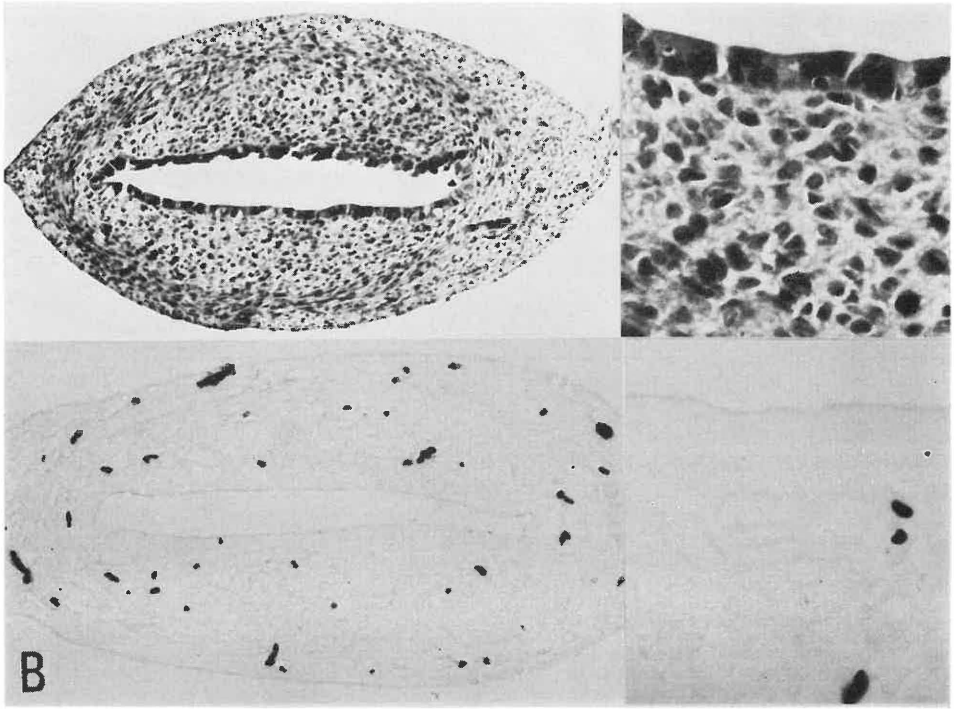
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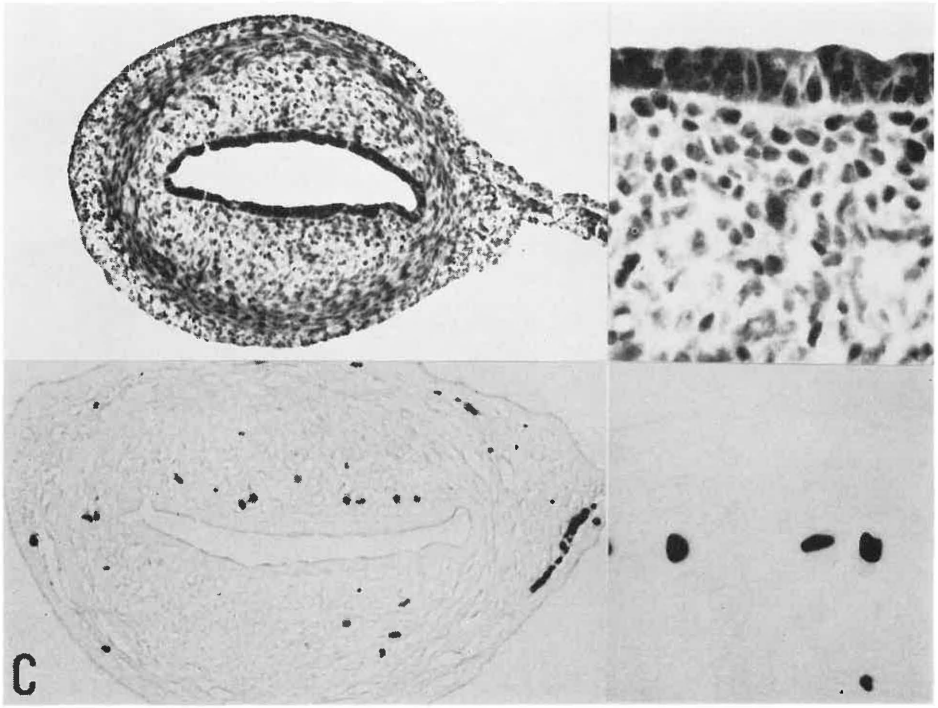
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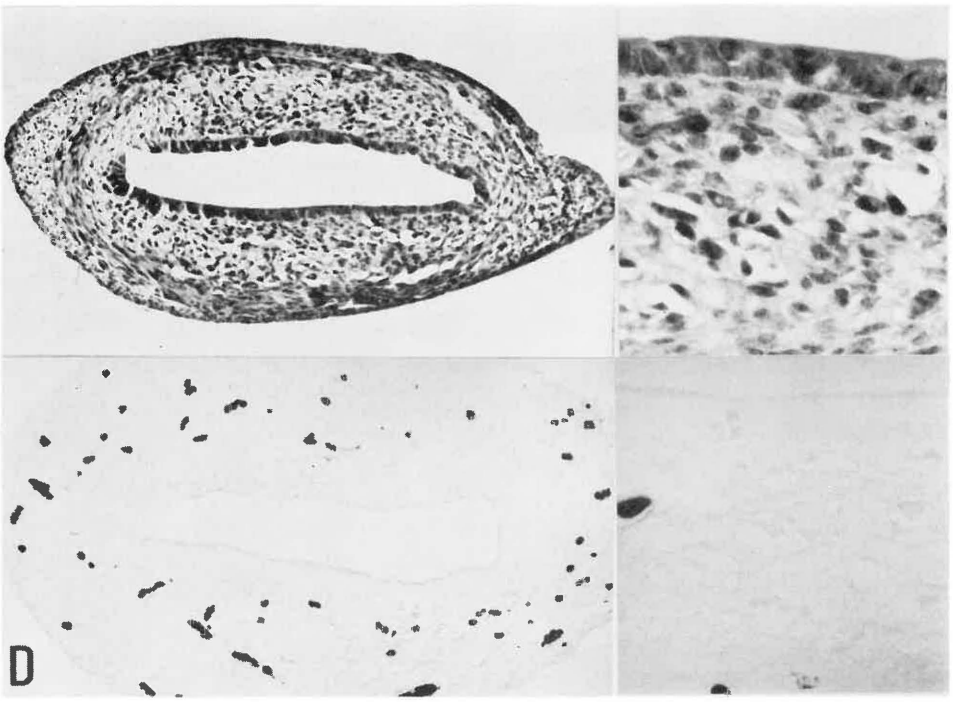
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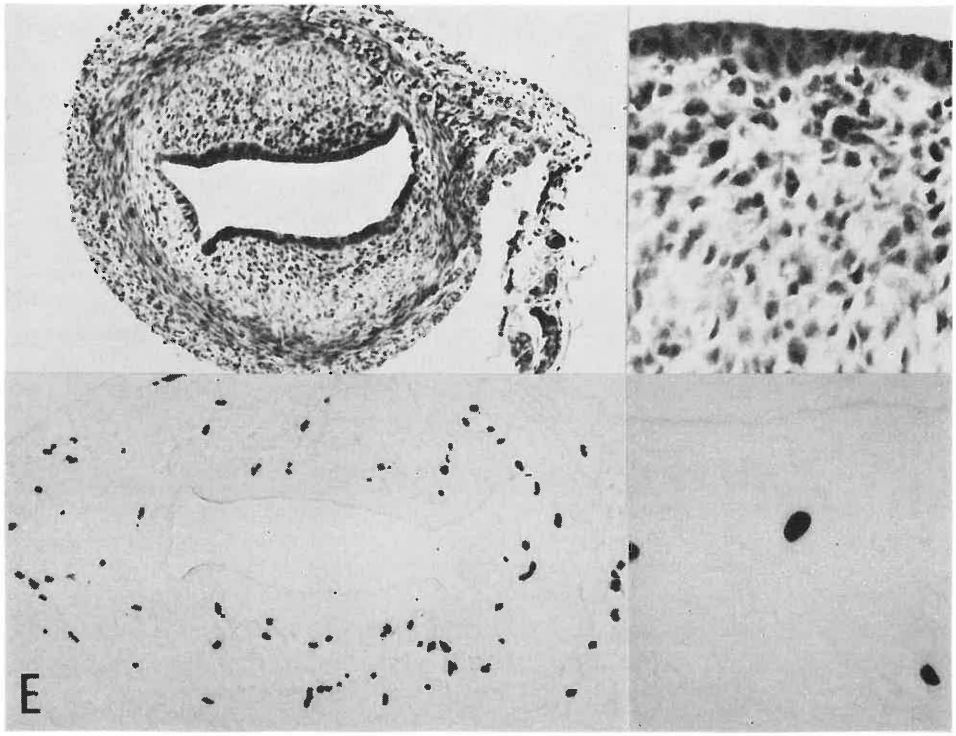
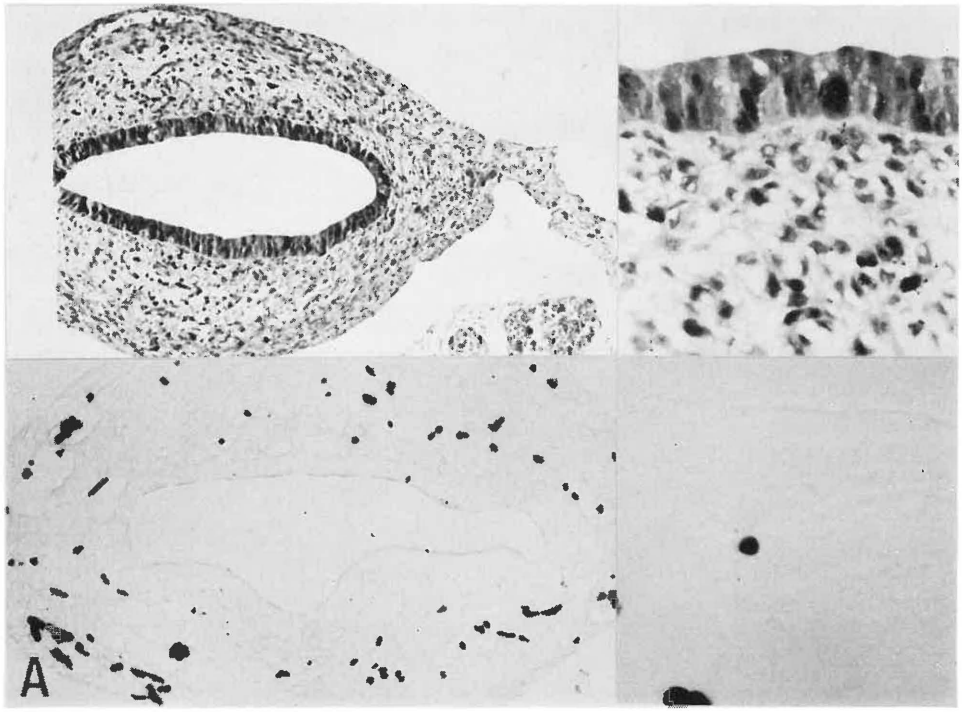


Fig. 1. Cross-cut sections of the rat uterus after each interval time following the estrogen treatment at 3 days of age. The upper left figure is the section stained with hematoxylin and eosin, and the upper right one is its higher magnification. The lower left one is the section demonstrated peroxidase activity, and the lower right one is its higher magnification.
(A) 24 hours after the treatment: The surface epithelium was low and no peroxidase activity was seen. Granulocytes in the stroma were markedly-positive for peroxidase.
(B) 36 hours after the treatment: There was no significant alteration in the surface epithelium.
(C) 48 hours after the treatment: There were almost similar findings to (B).
(D) 60 hours after the treatment: There were almost similar findings to (B).
(E) 72 hours after the treatment: There were almost similar findings to (B).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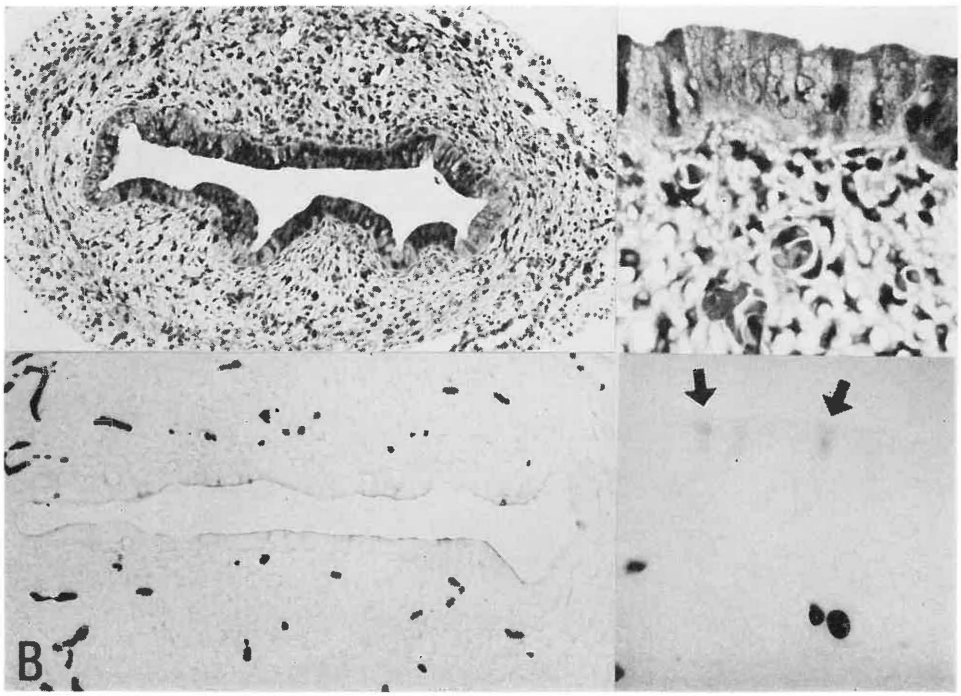
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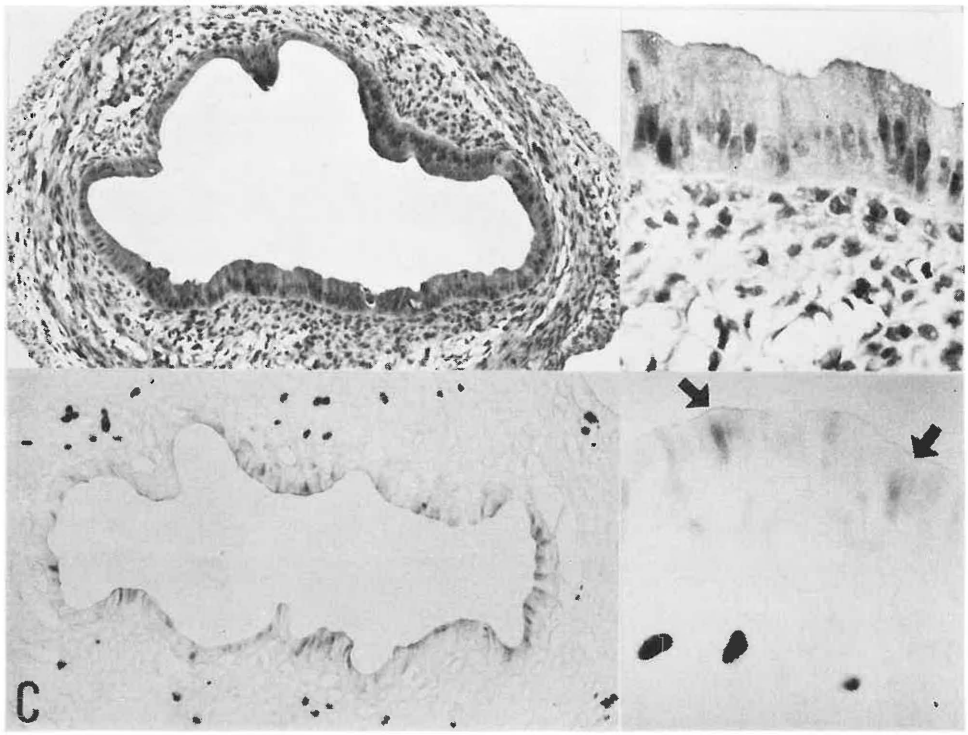
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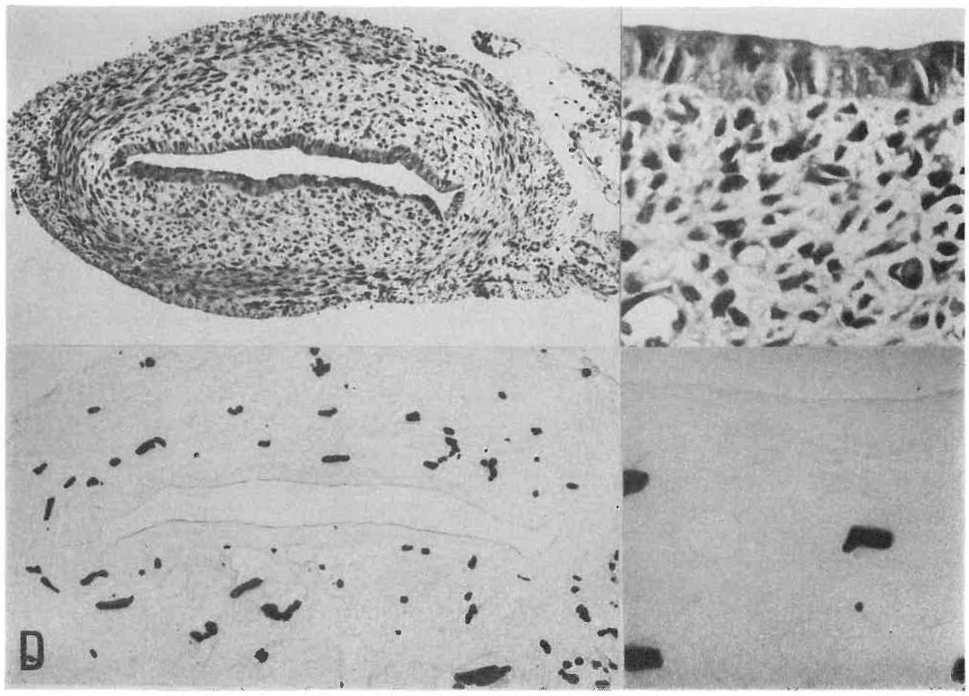
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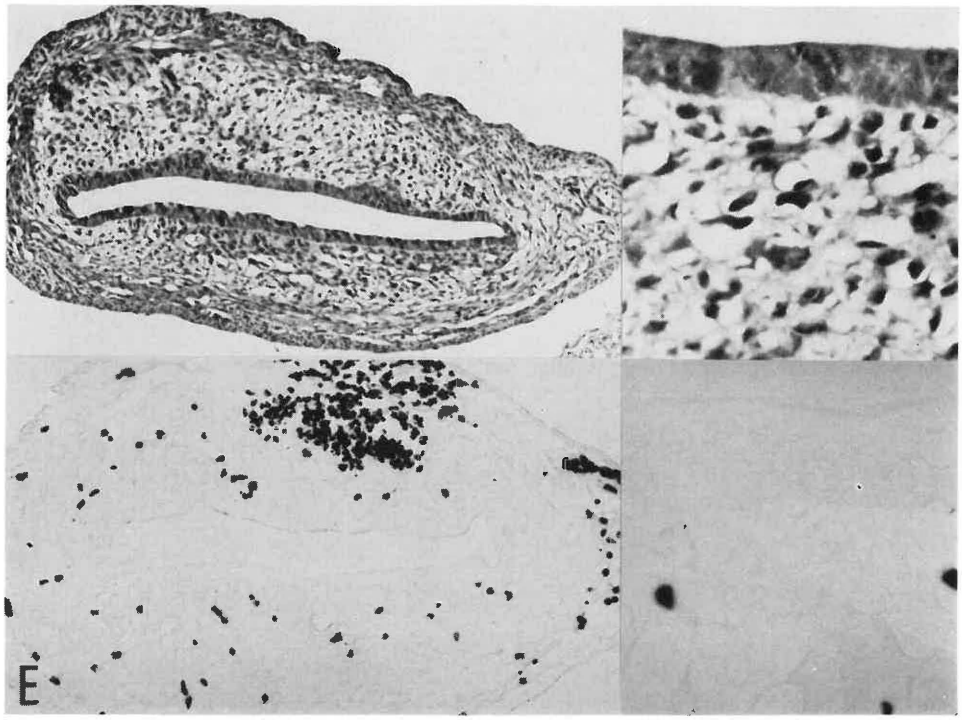


Fig. 2. Cross-cut sections of the rat uterus after each interval time following the estrogen treatment at 6 days of age.
(A) 24 hours after the treatment: The surface epithelium became tall, however, no peroxidase activity was seen.
(B) 36 hours after the treatment: The surface epithelium remained to be tall, and some of the cells showed weak peroxidase activity. (arrows: peroxidase reaction products)
(C) 48 hours after the treatment: The surface epithelial height reached the maximum, and most of the cells showed variable peroxidase activity.
(D) 60 hours after the treatment: The surface epithelial height was reduced, and peroxidase activity disappeared.
(E) 72 hours after the treatment: There were almost similar findings to (D), however in the stroma, granulocytes were prominent.

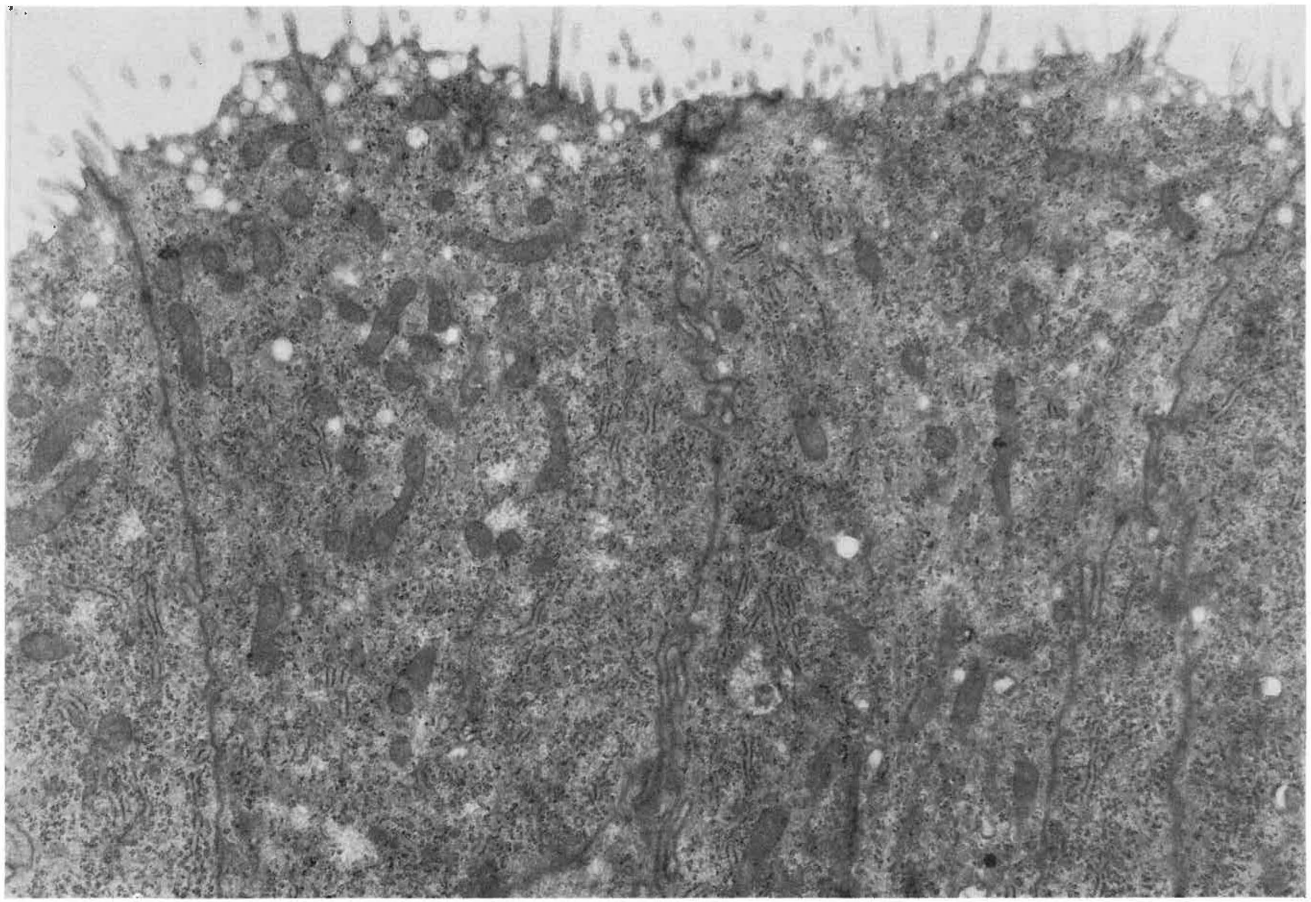


Fig. 3. Figure at 36 hours after the treatment to the rats, 6 days of age. Note long microvilli and small vesicles in the apex of cytoplasm. (×20,000)

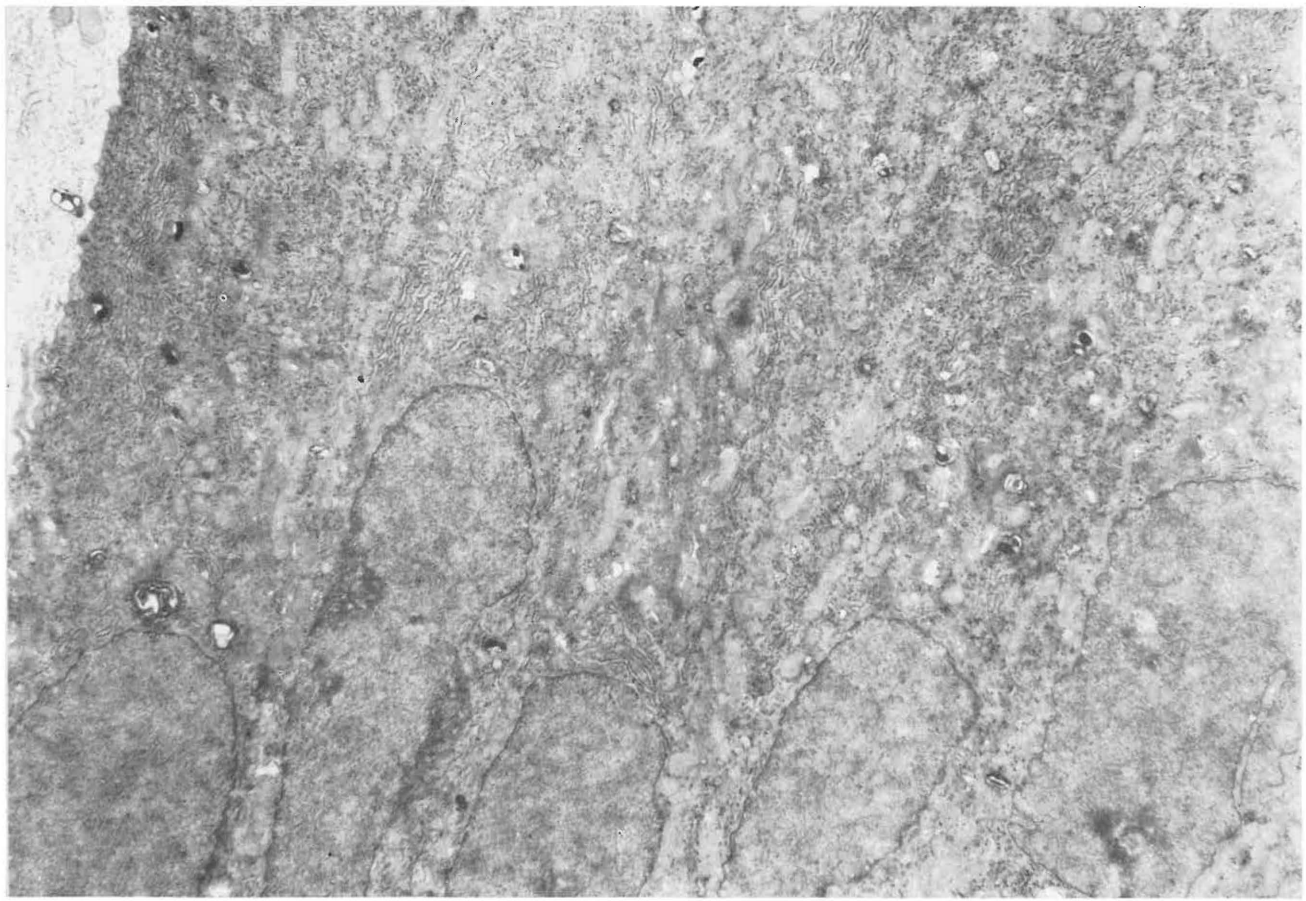


Fig. 4. Figure at 48 hours after the treatment to the rats, 6 days of age. Note marked development of the rough endoplasmic reticulum. (×13,000)

Fig. 5. Higher magnification of Fig. 4. (×17,000)

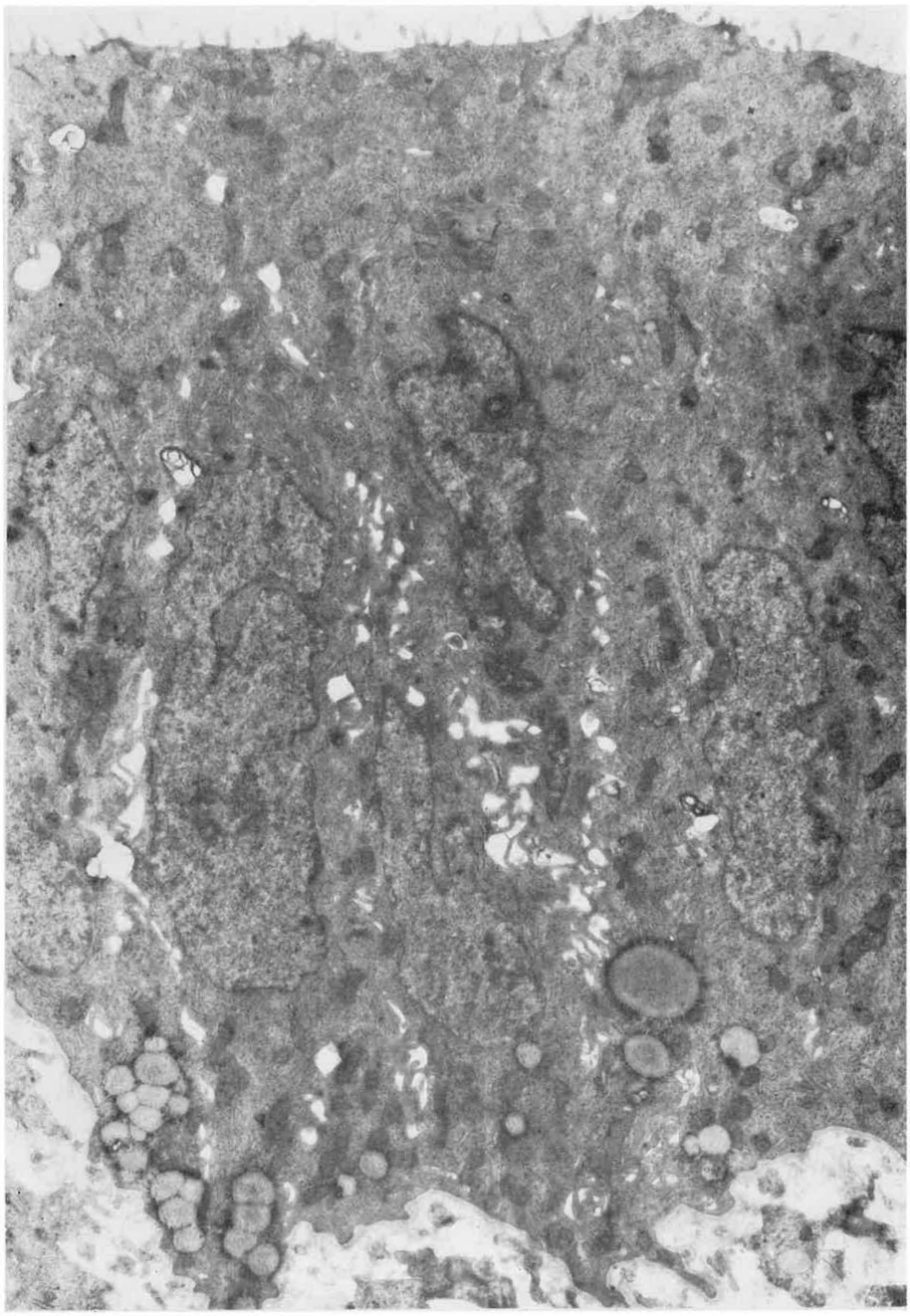


Fig. 6. Figure at 60 hours after the treatment to the rats, 6 days of age. Note low cellular height and extreme reduction of the rough endoplasmic reticulum. (×11,000)

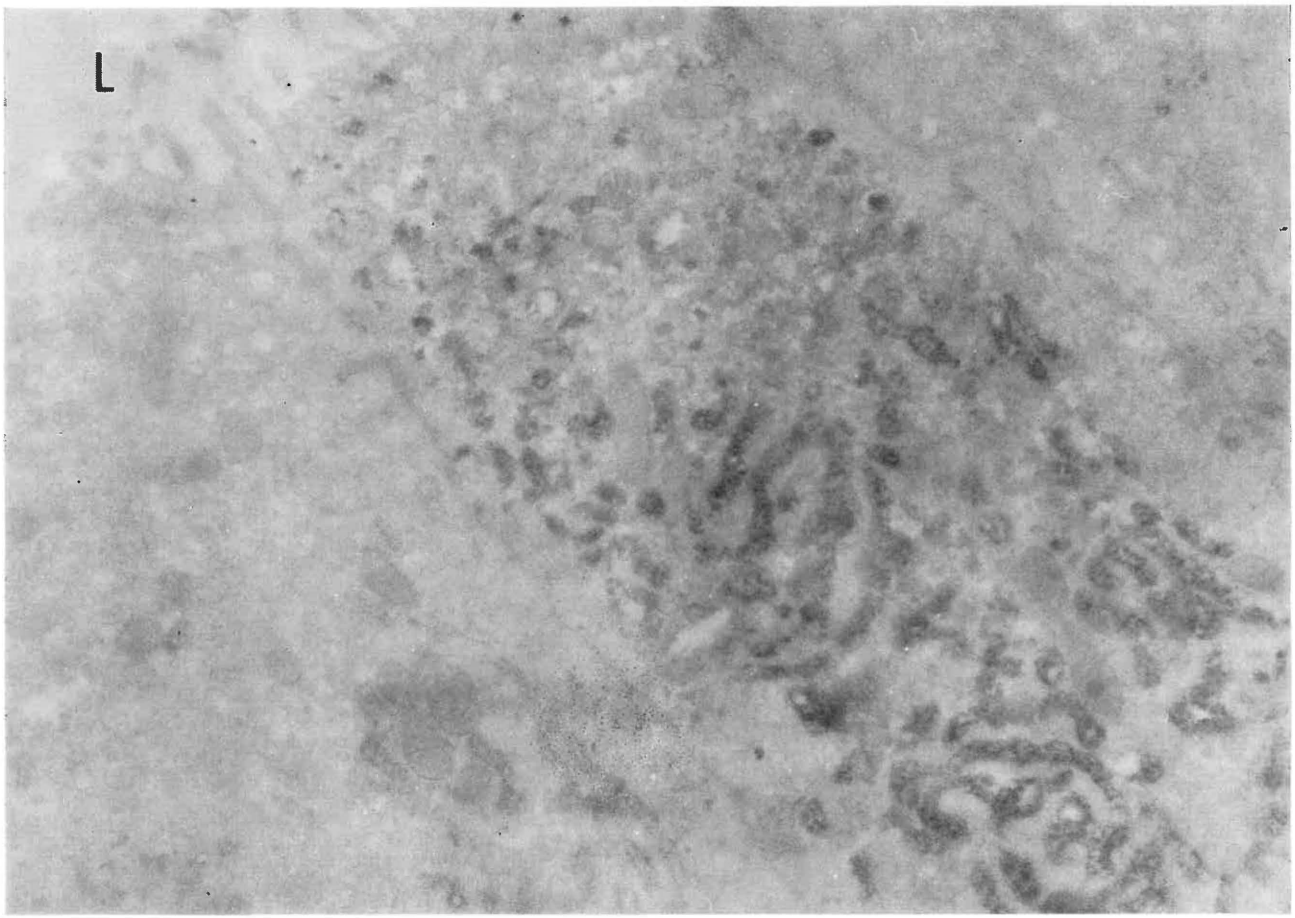


Fig. 7. Unstained ultrastructural figure of peroxidase activity at 48 hours after the treatment to the rats, 6 days of age. In the strongly-positive cell, its activity was observed within the cisterna of rough endoplasmic reticulum, the Golgi apparatus and the small vesicles (L: uterine lumen). (×24,000)

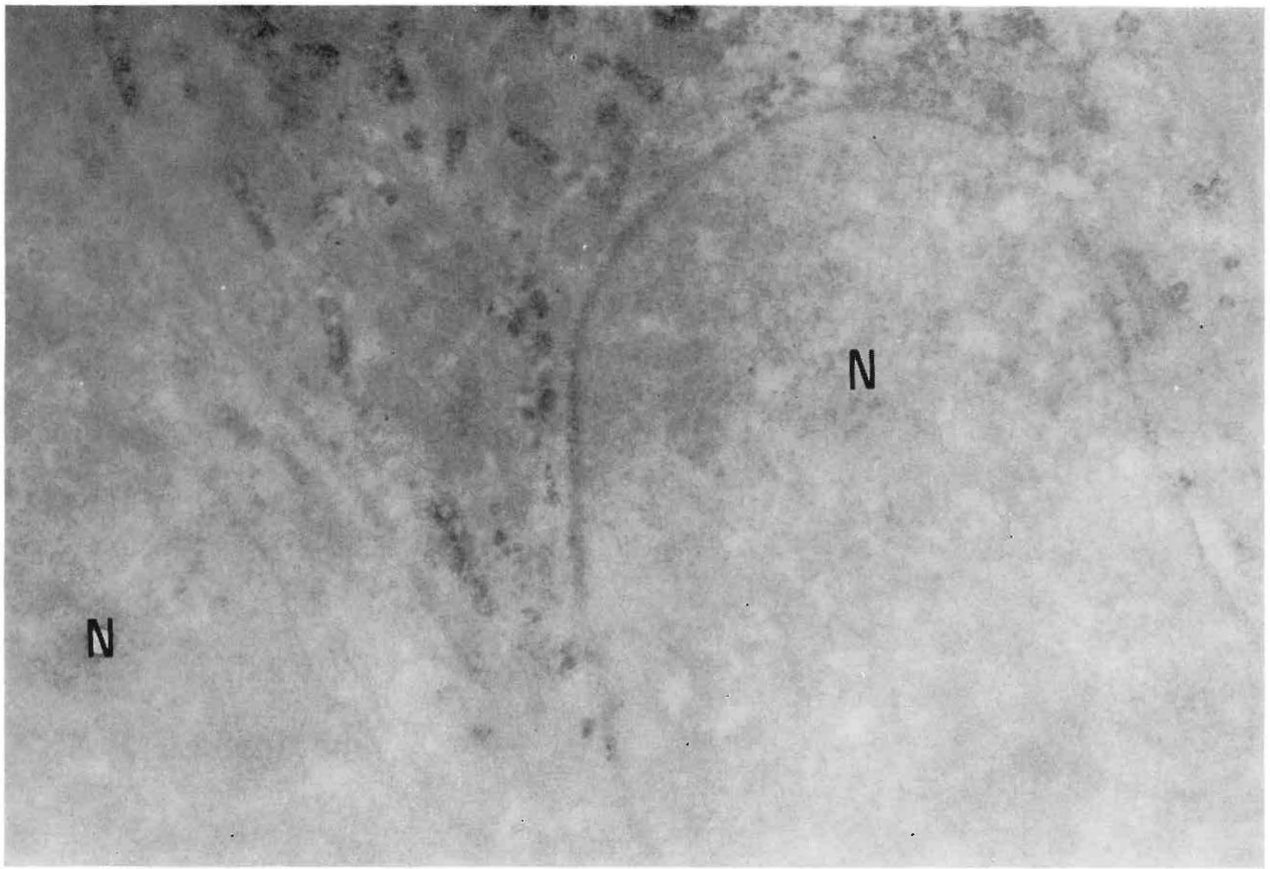
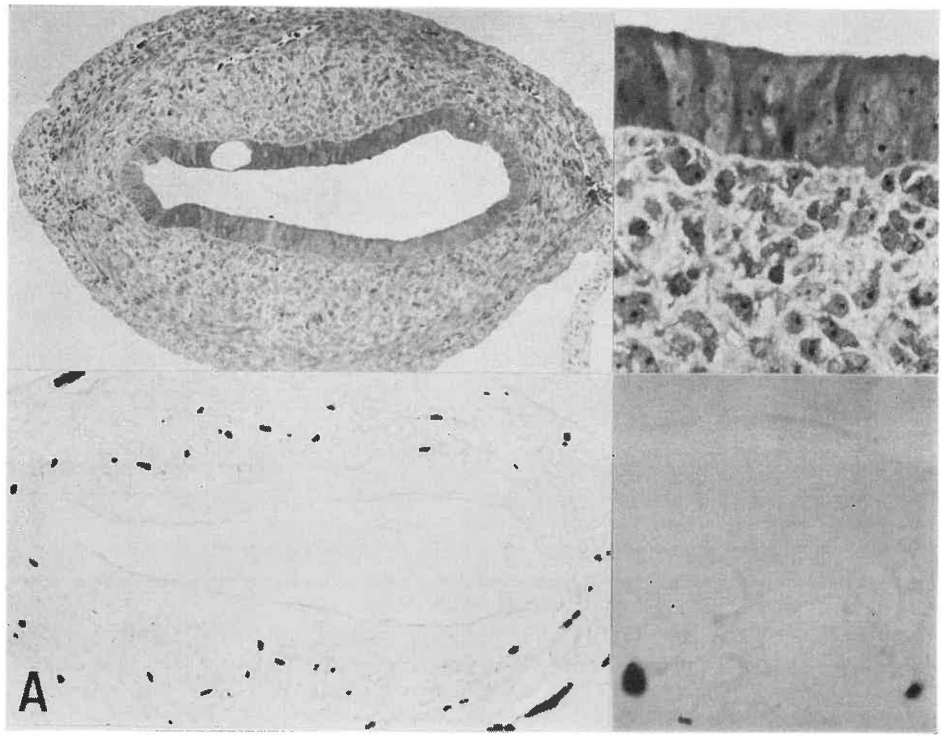


Fig. 8. The same as Fig. 7. In the weakly-positive cell, peroxidase activity was observed within the cisterna of nuclear envelope and limited rough endoplasmic reticulum near the nuclei (N: nucleus). (×26,000)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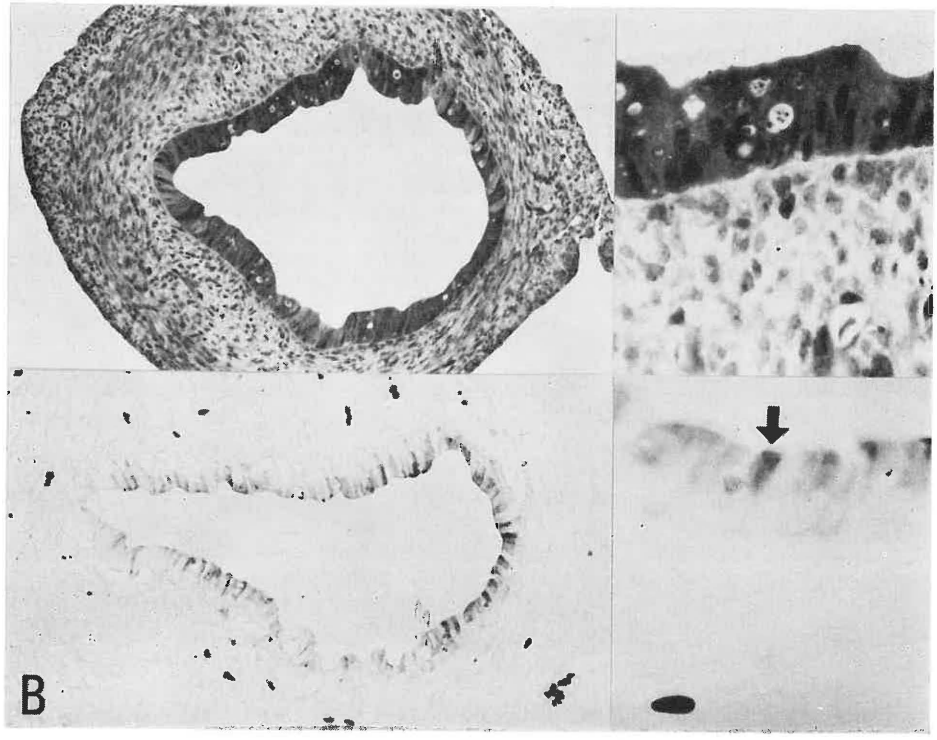
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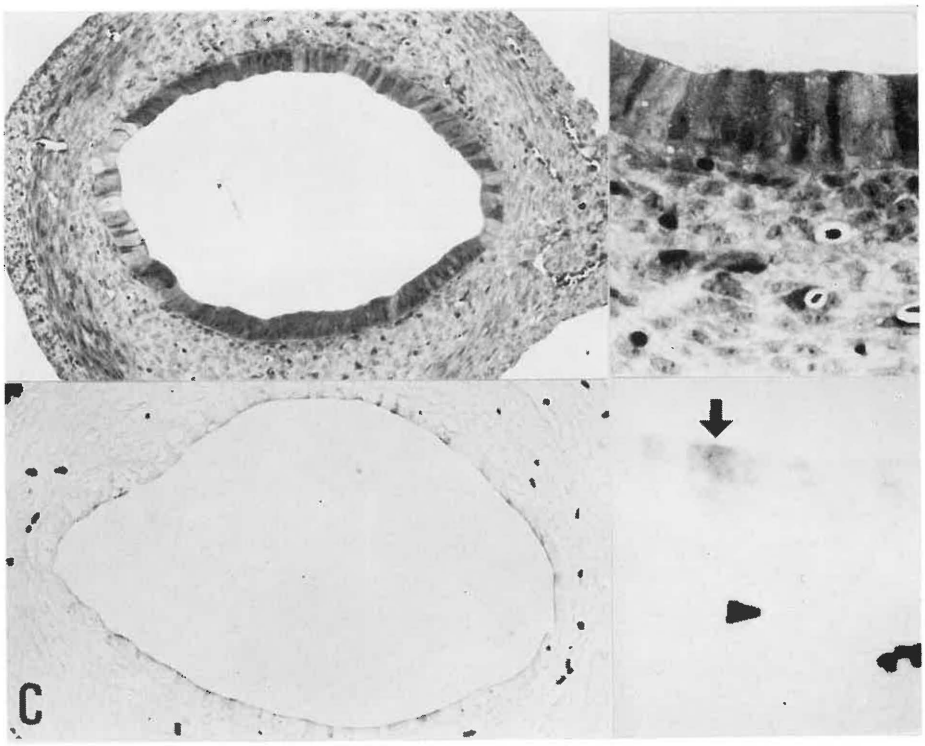
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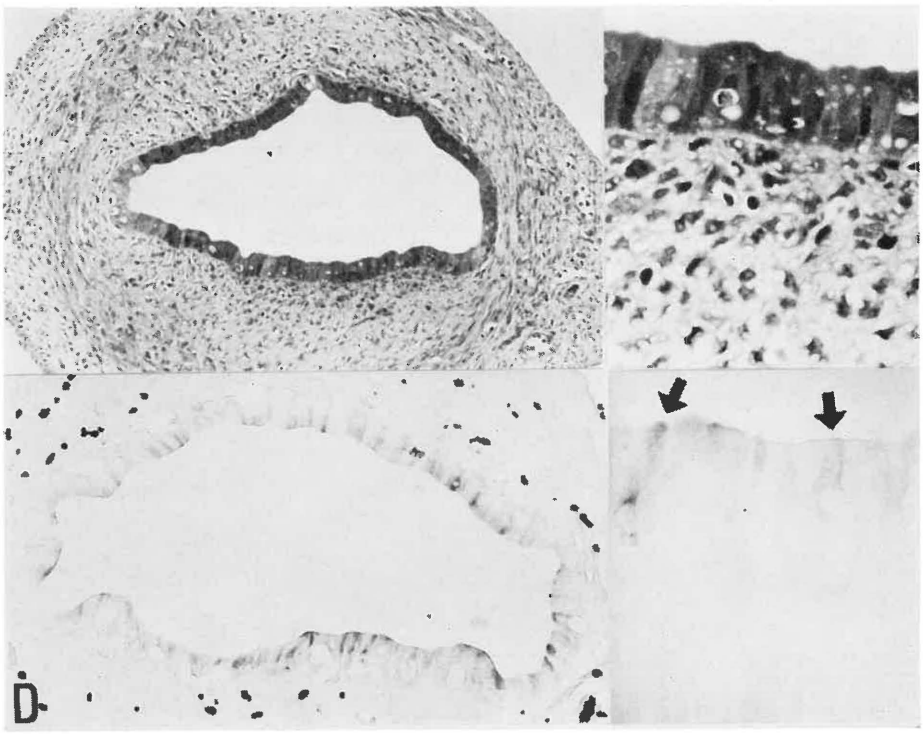
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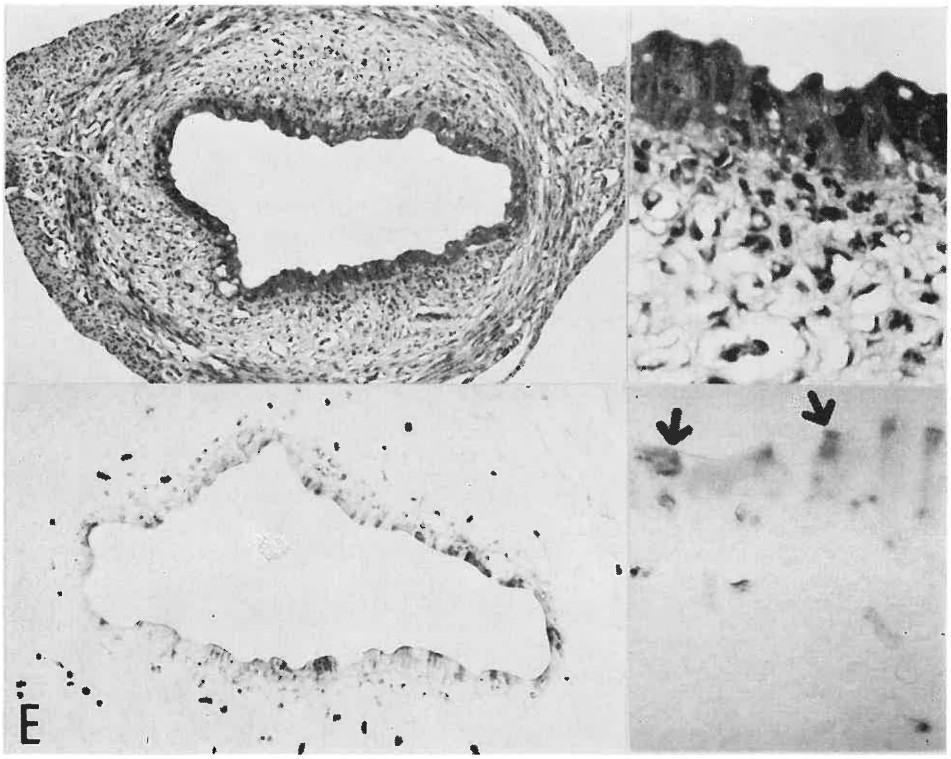

Fig. 9. Cross-cut sections of the rat uterus after each interval time following the continuous estrogen treatment at 6 days of age.
(A) 24 hours after the initial treatment: The surface epithelium was tall, however no peroxidase activity was seen.
(B) 48 hours after the initial treatment: The surface epithelium remained to be tall, and in most of the cells, peroxidase activity was prominent.
(C) 60 hours after the initial treatment: There were almost similar findings to (B).
(D) 72 hours after the initial treatment: There were almost similar findings to (B).
(E) 84 hours after the initial treatment: There were almost similar findings to (B), however in the subepithelial stroma granulocytes were prominent.

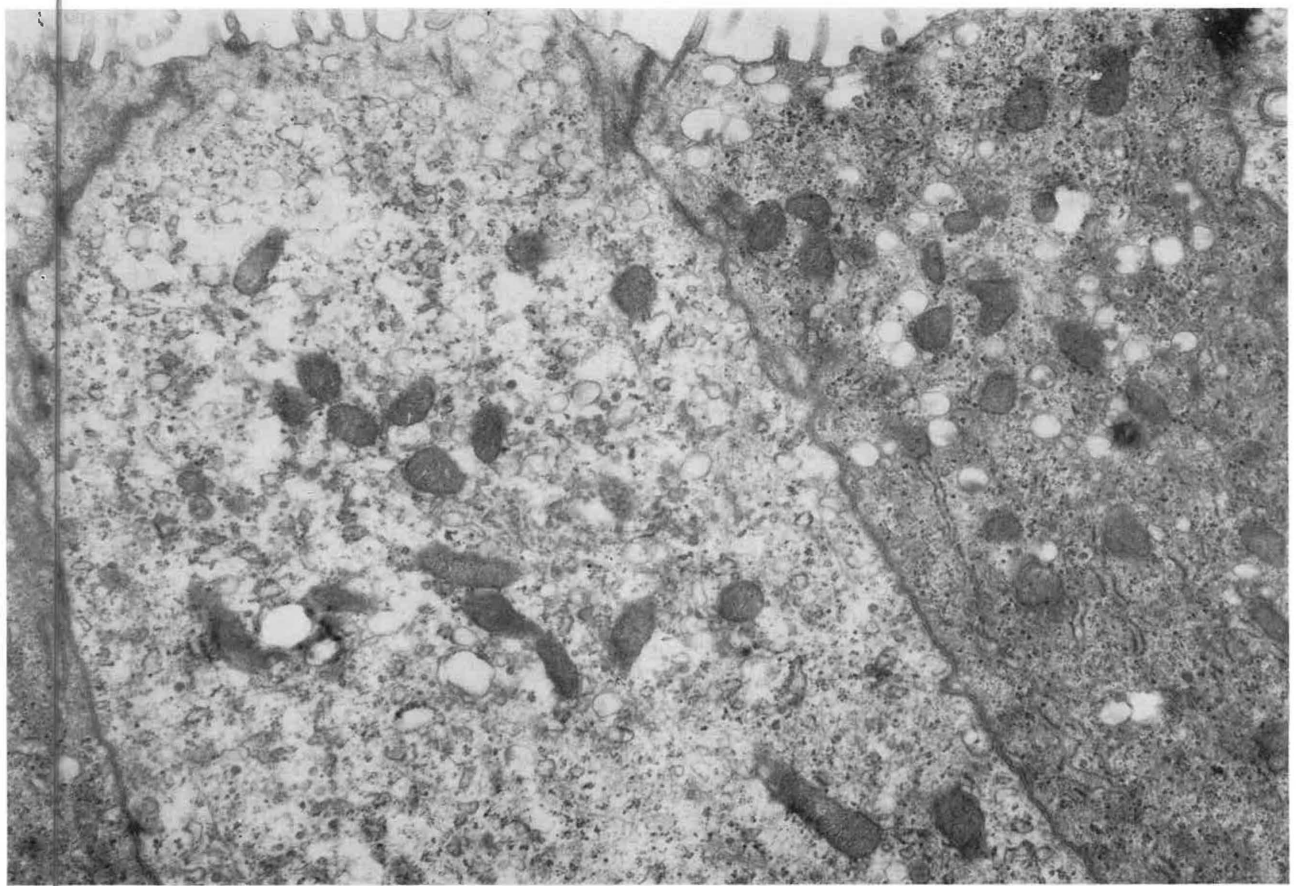

Fig. 10. Figure at 48 hours after the initial treatment to the rats at 6 days of age. Note the similar findings to the Fig. 3. (×23,000)

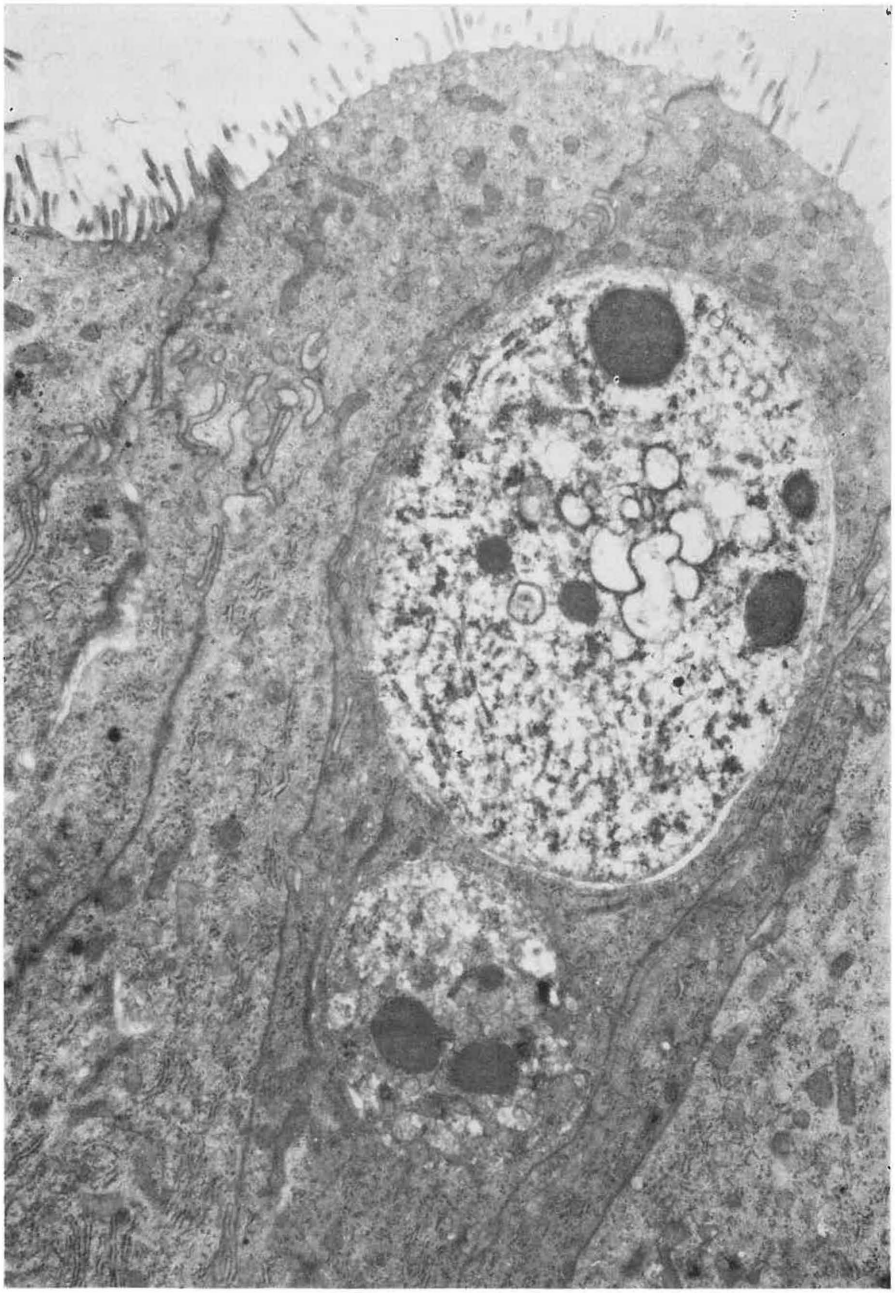

Fig. 11. Figure at 72 hours after the initial treatment to the rats at 6 days of age. Note large autophagic vacuoles containing degenerative organellaes. (×17,000)