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Upper Genital Tract Abnormalities in the Syrian Hamster as a Result of *in utero* Exposure to Diethylstilbestrol

I. Uterine Cystadenomatous Papilloma and Hypoplasia

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Summary. Prenatal exposure to diethylstilbestrol (DES) causes a significant increase in the carcinogenic response of the hamster's reproductive tract to subsequent DES treatment. Uteri from DES treated females from untreated- and DES treated mothers (C.D & D.D) have abnormal hyperplasia with characteristic finger-like structures projecting into the lumen of the uteri. Inside these papillae along with the rest of the stroma are cystic glands. We found that these glands had no openings into the uterine lumen and that they "begin" and "end" in the stroma. In addition there are two types of cells lining the cystic gland i.e., pale cells and acidophilic cells. Capillary beds surround the cystic glands. We have named these uterine structures "cystadenomatous papilloma". In addition, we found a spectrum of hyperplastic abnormalities in C.D and D.D uteri and carcinoma *in situ* in D.D uteri. Similar neoplasms have been described in human pathology. Ultrasound observations have demonstrated that *in utero* exposure to DES may result in uterine hypoplasia and because it appears to be similar to the changes seen in prenatally DES treated (D.C) hamsters, cross sectional areas of C.C (untreated control), D.C, C.D and D.D uteri were compared. Our results show that later in life uterine hypoplasia also occurs in the 100 days old D.C hamsters and since D.C uteri present hyperplasia with cystic structures, our data support the hypothesis that *in utero* DES-treated human females may later in life develop benign and malignant lesions in their reproductive tract. Much of these data corresponds to what has been found in humans, and consequently warrants further investigation into the use of Syrian hamster as a model to understand the uterine abnormal morphogenesis in regards to hypoplasia, adenocarcinoma and carcinoma development in the human.

Key words: Diethylstilbestrol – Carcinogenesis – Animal model – Hypoplasia – Hyperplasia

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Diethylstilbestrol (DES) is a synthetic compound with estrogenic properties similar to those of the natural estrogens, and was first used clinically in America and Europe in 1938 (Dodds 1962; Dodds et al. 1938, 1939). In the same year the first report on DES-induced cancer in animals appeared (Lacassagne 1938). Between 1945 and 1955 large doses of DES were widely prescribed for the prevention of miscarriage (Smith 1948; Smith GV and Smith OW 1954; Smith et al. 1946 and 1979), and it has been estimated that 500,000–2 million pregnant women in the USA alone have been treated with DES (Noller and Fish 1974). Even now, DES is still being used for a variety of purposes including estrogen replacement therapy, prevention and treatment of certain menopause related problems, treatment of advanced breast and prostate cancer, suppression of lactation in new mothers, as a postcoital contraceptive, i.e., cases of rape, (Kuchera 1974; Haspels et.al. 1978; Fink 1980), as a morning after pill (Garcia et al. 1977; Morris and VanWagenen 1973) or to reduce the height of potentially very tall girls (Wittlinger 1980). Meanwhile its therapeutic value has been questioned (Dieckmann et al. 1953; Ferguson 1953). Aside from its clinical usage, DES has been used for agricultural purposes, e.g. as an animal food additive to promote growth (Dinusson et al. 1948; Quisenberry and Krueger 1948).

In 1970, Herbst and Scully reported seven cases of vaginal adenocarcinoma in females aged 14–22 years; this a very rare form of cancer for women in this age group. The following year an epidemiological study showed that the risk of carcinoma development was relatable to intrauterine exposure to DES (Herbst et al. 1971). Further observations confirmed these preliminary findings and also related DES treatment to the incidence of clear cell adenocarcinoma of the vagina and cervix (Greenwalt et al. 1971; Fetherston 1975; Fetherston et al. 1972; Herbst et al. 1972, 1974, 1975 and 1977; Noller et al. 1972; Scully et al. 1978; Nordquist et al. 1976; Stafl and Mattingly 1976; Bibbo et al. 1975; Kaufman 1977; Burke et al. 1978; Murphy 1980). Using biopsy samples, Robboy et al. (1979) reported finding benign uterine abnormalities in 94% of 3,246 DES exposed women. In addition, it is now known that some of the DES children have other problems e.g., low sperm counts and/or abnormal growth of the reproductive tract in males, (Fink 1980; Gill et al. 1977) or increased incidence of miscarriage related to uterine hypoplasia in females (Fink 1980; Kaufman et al. 1977). Overall these studies indicated that there was a relationship between intrauterine DES exposure and genital tract abnormalities in both males and females, which often become apparent at, or shortly after, the onset of puberty (Fink 1980).

Previous studies have focused mainly on vaginal and cervical abnormalities, with few observations on abnormalities localized in the upper genital tract, e.g. the uterus-fallopian tubes, ovaries (Dallenbach-Hellweg 1980; WHO 1975). We felt that the incidence of uterine abnormalities had been understated and therefore decided to study the uterine pathology resulting from both single (*in utero*) and double (pre- and postnatal) exposures to DES. The results of such a study would not only demonstrate the pathology resulting from intrauterine exposure, but also indicate the pathological predispositions that an *in utero* exposure to DES confers to subsequent exposure to natural estrogens or to other synthetic estrogenic compounds (e.g., oral contraceptives). The latter knowledge is of special value since an increased incidence of endometrial cancer has been observed in women who are users of oral contraceptives (Herbst et al. 1972). In addition "precursor

lesions of endometrial invasive carcinoma are less well understood, partly because they are detected much less frequently and partly because the lesions are altered by endogenous and exogenous hormonal variations" (Vellios 1972). This statement is important since there now appears to be an increased frequency of endometrial carcinoma (Christopherson et al. 1971), which may be related to the aformentioned hormonal manipulations.

Recently Kaufman et al. (1977), Rennel (1979) and Viscomi et al. (1980) all reported that some of the women who had been exposed to DES *in utero* developed hypoplastic uteri in adult life. However no histological examination of human hypoplastic uterus has been reported.

The transplacental carcinogenicity of DES has also been demonstrated in 3 animal species, i.e. mice (Lamb et al. 1981; McLachlan 1977), rats (Boylan 1978) and Syrian hamsters (Rustia and Shubik 1976). We chose the Syrian hamster for our study because of its low incidence of spontaneous tumor development and because DES has a tumorigenic effect on other organs including the reproductive tract (Rustia and Shubik 1976; Leavitt et al. 1980).

Material and Methods

Thirty (30) timed pregnant Syrian hamsters (*Mesocricetus auratus* Waterh., strain lak LVG. SYR) were purchased from Charles River Co. Twenty (20) were injected subcutaneously with DES in corn oil (100 µg DES/kg·bw/day) on days 8–11 (inclusive) of pregnancy. Control animals (10) received oil only. Preliminary observations show that DES-treated mothers deliver fewer pups than the control mothers, therefore to compensate for this, the DES treated group was larger than the control group. The use of DES pellets has been described previously (Forbes 1943; Steggles and King 1968). The effectiveness of DES treatment and the metabolic fate of DES has been evaluated previously (Metzler 1975 and 1976; Gottschlich and Metzler 1980).

After weaning, the female pups were housed three to a cage. When the hamsters were 50 days old half ot the animals were given a subcutaneous implant of DES (15 mg pellet) in the interscapular dorsal region, and new pellets added every three months. The animal treatment groups are designated as follows: (See Table 1).

(i) ♀ from untreated mothers (C.C)
(ii) ♀ from DES-treated mothers (D.C)
(iii) ♀ from untreated mother then postnatally DES-treated (C.D)
(iv) ♀ from treated mothers then postnatally DES-treated (D.D).

After 100, 200 and 300 days of treatment, animals from each group were killed by cervical dislocation. The reproductive tract was removed, as well as the kidney, liver, heart, and skeletal

Table 1. Days of age and postnatal treatment

	None (C)[b]			DES (D)		
Treatment *in utero*	150	250	350	150	250	350
None (C)	6[a]	6	6	11	6	5
DES (D)	6	6	6	10	7	5

[a] Number of animals in each group
[b] C = control, D = DES

muscle. The tissues were either fixed in 4% neutral buffered formaldehyde or frozen in liquid N_2 for future morphological analyses. Each uterus was divided into two equal parts. *One part* was fixed with 4% buffered paraformaldehyde (pH 7.3; 0.1 M phosphate buffer) and examined after routine paraffin embedding and topographical histological stainings. Histological preparations were obtained using either 8 µm thick serial longitudinal parasagittal sections of one $^1/_2$ horn, serial cross-sections of the other $^1/_2$ horn, or 2 µm thick sections obtained from Epon embeddings (cut with Sorvall microtome). Serially cut histological sections of 8 µm (paraffin) were stained by H.E., Gomori trichromè, P.A.S.-Alcian blue and Perls technique and Epon embedded specimens (2 µm plastic) were stained by toluidine blue. The other part of the uterus was either frozen and prepared for further histoenzymological studies or processed for ultrastructural examination using transmission scanning electron microscopy (Gilloteaux and Steggles 1981 and 1982b in press).

Morphometrical measurements were carried out by using drawings made from microprojections and photomicrographs taken through the Zeiss photomicroscope. These data were analyzed by an Apple computer program providing perimeter-areas analysis using uterine serial histological sections.

Results

Anatomical-Pathological Aspects. In the hamster, the uterus consists of bilateral horns connected to uterine body or tube *via* an unpaired body and neck (cervix) which joins a short vaginal canal. Compared to the untreated (C.C), and to the D.C uteri, all postnatal DES-treated uteri (C.D and D.D) are always enlarged ($p<0.01$). This is a characteristic result of the combined hyperplastic and hypertrophic growth response to the DES-treatment. In contrast, 150 day D.C uteri are hypoplastic when compared to C.C uteri ($p<0.01$). The D.C uteri present a very narrow lumen: the average uterine cross-sectional areas were $4.84 \pm 1.68\ mm^2$ ($N=6$) for C.C and $2.03 \pm 0.26\ mm^2$ ($N=6$) for D.C. It is only after 300 days of treatment that the C.C and D.C uteri have similar size: D.C uteri become enlarged with an average uterine cross-section area of $10.78 \pm 2.28\ mm^2$ ($N=6$) in comparison to C.C uteri at $9.35 \pm 1.01\ mm^2$ ($N=6$) ($p>0{,}5$). In addition, 350 day D.C are morphologically similar to 250 day C.D uteri.

All the C.D and D.D uteri present many ridges in the lumen. These are extremely exagerated in the case of D.D uteri since the ridges undergo abnormal growth and changes into papillae that are of varying diameters and intertwine with each other and fill the lumenal space like a plug (Fig. 12 and Steggles and Gilloteaux 1980). Our anatomical and morphometrical observations are summarized in Figs. 1 and 2 and in Table 2.

It is clear from Table 2 that the cross-sectional area of the uterus increases with age and with the increasing degree of the DES treatment. The only exception appears in the hypoplasia in the 150 and 250-day old D.C animals.

Histological Aspects

DES-treated Hamster Uteri. (C.D and D.D) show the presence of generalized abnormal hyperplasia with finger-like polyp structures or papillae projecting into the lumen. These observations have been confirmed by recent scanning electron microscopy studies (Gilloteaux and Steggles 1981). Inside these "papillae" we find cystic glandular structures which had no apparent

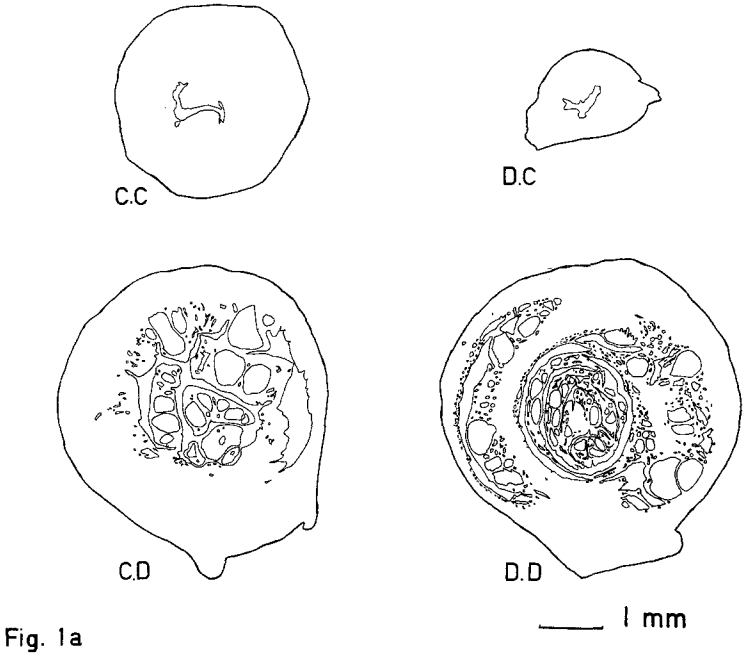


Fig. 1a

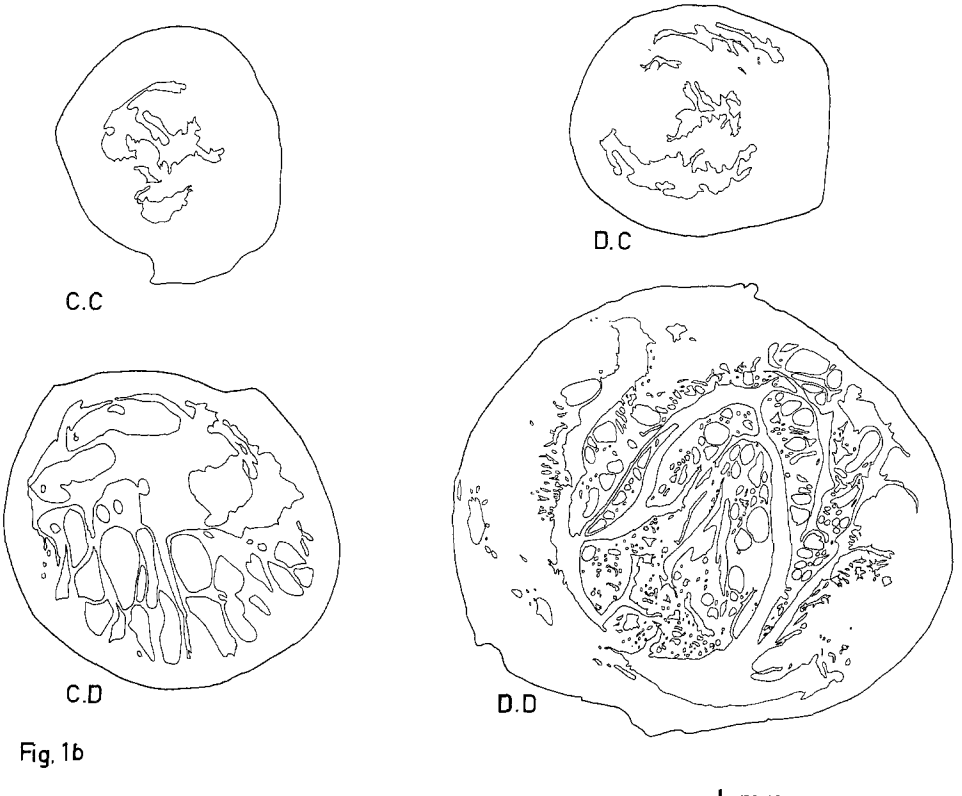


Fig. 1b

Fig. 1a and b. Cross-sectional views of *C.C, C.D, D.C* and *D.D* hamster uteri. The drawings depict the outlines of the serosa, uterus lumen and cystic glands. **a** 150 day uteri; **b** 350 day uteri

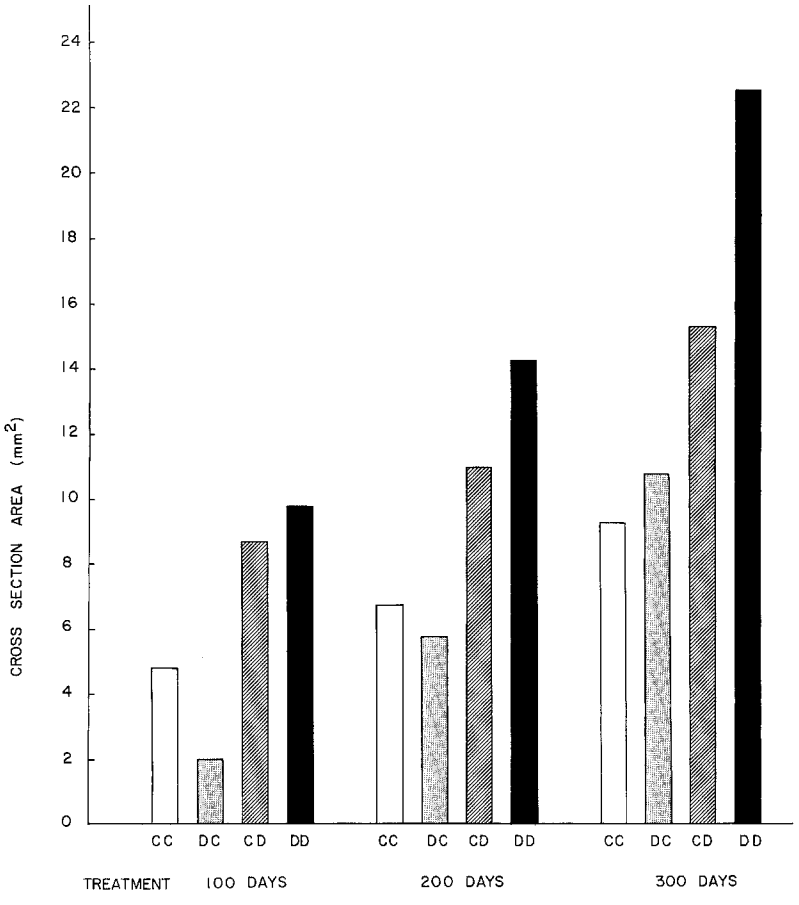


Fig. 2. Quantitation of uterine body cross-sectional areas. The histograms represent areas measured with computer graphic analysis, using drawings similar to the ones shown in Fig. 1a and b. Table 1 summarizes data collected and their significance

openings into the main luminal space of the uterine horn. In addition, the uterus is more glandular in D.D than in C.D hamsters. The glandular proliferation fills the whole uterine cavity leading to uterine swelling. Moreover, the number of cystic glands in random uterine cross-sections is increased. The number of cysts/mm^2 uterine area for C.D uteri did not alter with time of treatment whereas D.D uteri show a tremendous increase in the number cystic glands/cross section from about 125 at 150 days of age to more than 200 at 350 days of age (Table 3).

Many of these glandular structures appear to be lying back to back. In the oldest animals the cystic stroma is often hemorrhagic, invaded by inflammatory tissues and fibrous material. Parts of the uteri were hard to section because of the presence of firm fibrous or chondromatous areas (Steggles and Gilloteaux 1980). In contrast, we have *not* seen cystic glands in C.C uteri.

Table 2. Uterine cross-sectional areas after different treatment regimens

Days of age		C.C	D.C	C.D	D.D
150	lumen	0.21 ± 0.10[b]	0.07 ± 0.02	1.39 ± 0.50	2.88 ± 0.64
	body	4.84 ± 1.68[c]	2.03 ± 0.26	8.87 ± 1.36	9.80 ± 1.24
		($N=6$)	($N=6$)	($N=11$)	($N=10$)
250	lumen	0.30 ± 0.03	0.36 ± 0.29	1.87 ± 0.60	4.65 ± 1.50
	body	6.71 ± 1.21	5.86 ± 0.76	11.05 ± 0.43	14.15 ± 1.96
		($N=6$)	($N=6$)	($N=6$)	($N=7$)
350	lumen	0.61 ± 0.35	1.30 ± 0.80	4.51 ± 0.43	5.41 ± 2.46
	body	9.35 ± 1.01	10.78 ± 2.28	15.5 ± 0.84	22.66 ± 4.55
		($N=6$)	($N=6$)	($N=5$)	($N=5$)

[a] Measurements were taken from the lumen and body of the uterus
[b] Means are in $mm^2 \pm$S.D.; significance of comparisons between means is indicated in text
[c] All the measurements correspond to the average of total cross sectional area minus the average lumen cross sectional area

N = number of animals, at least 5 sections per uterus were examined

Table 3. Number of cystic glands per uterine cross-section (number $\pm$ S.D.)

	Days of age		
	150	250	350
C.C	ND[a]	ND	ND
D.C	ND	ND	15 ± 5
C.D	51 ± 18[b]	59 ± 16	61 ± 13
D.D	125 ± 30	165 ± 35	215 ± 75

[a] ND = none detected
[b] at least 5 sections from at least 5 animals were examined

In order to determine whether these cystic glands have a secretory duct reaching the luminal epithelium of the endometrium (i.e. do they represent hyperplastic acinar glands?), we obtained longitudinal and cross-sectional serial sections from intact uteri (5 from each of the C.D and D.D groups). By following several glandular structures through these serial sections, we were able to show that no apparent openings exist from these glands to the uterine lumen (Figs. 3 and 4). Recent SEM observations support these observations (Gilloteaux and Steggles 1981). Moreover, in the C.D and D.D uteri the glands are lying back to back with a "Swiss cheese" structure resembling human adenoma or adenocarcinomatous lesions and the cystic stromal atrophy (Figs. 3–5b, 11, and 12). In addition most of the C.D and D.D uteri show adenomyosis with glandular profiles appearing within smooth muscle bundles of the myometrium (Fig. 12).

The cystic structures which develop in the stroma of C.D uteri correspond to a cystic hyperplasia of the endometrium with marked dilation of many glands. In D.D uteri, stroma the cystic structures represent a type

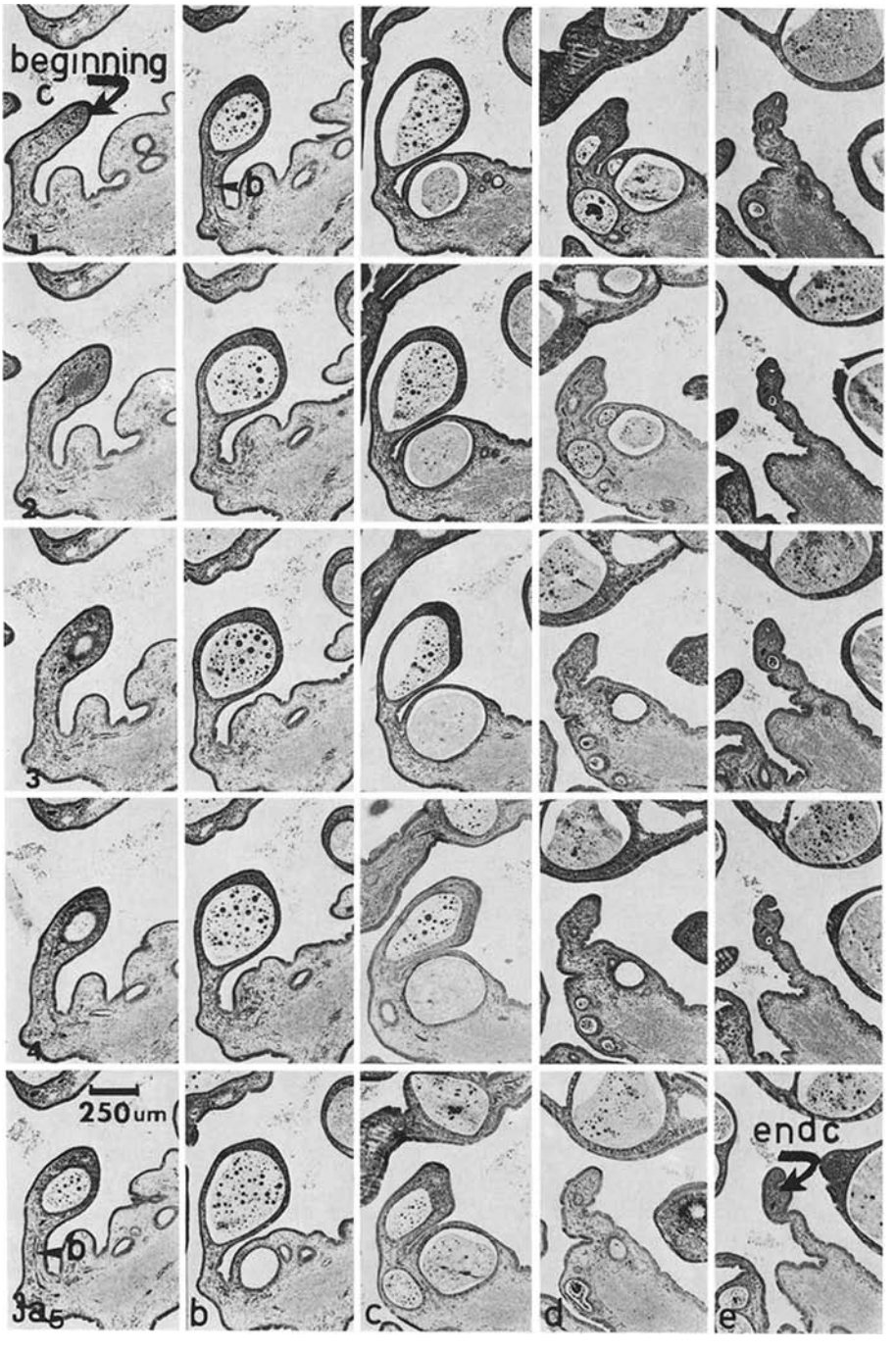


Fig. 3a–e. Micrographics obtained from longitudinal parasagittal serially cut sections from DD treated uterus showing several cystic glands "opening" and "ending" within the stroma of a uterus polyp. One of them is followed from "beginning" ($\mathbf{a}_1$ *arrow*) to "end" ($\mathbf{e}_5$ *arrow*). A straight arteriole directed towards the cystic gland and running within the stalk of the polyp is shown (*arrow b*). In addition note the heterogenous content of these cystic glands. Gomori trichrome stain. Scale of 250 μm in $\mathbf{a}_5$ is for all the illustrations

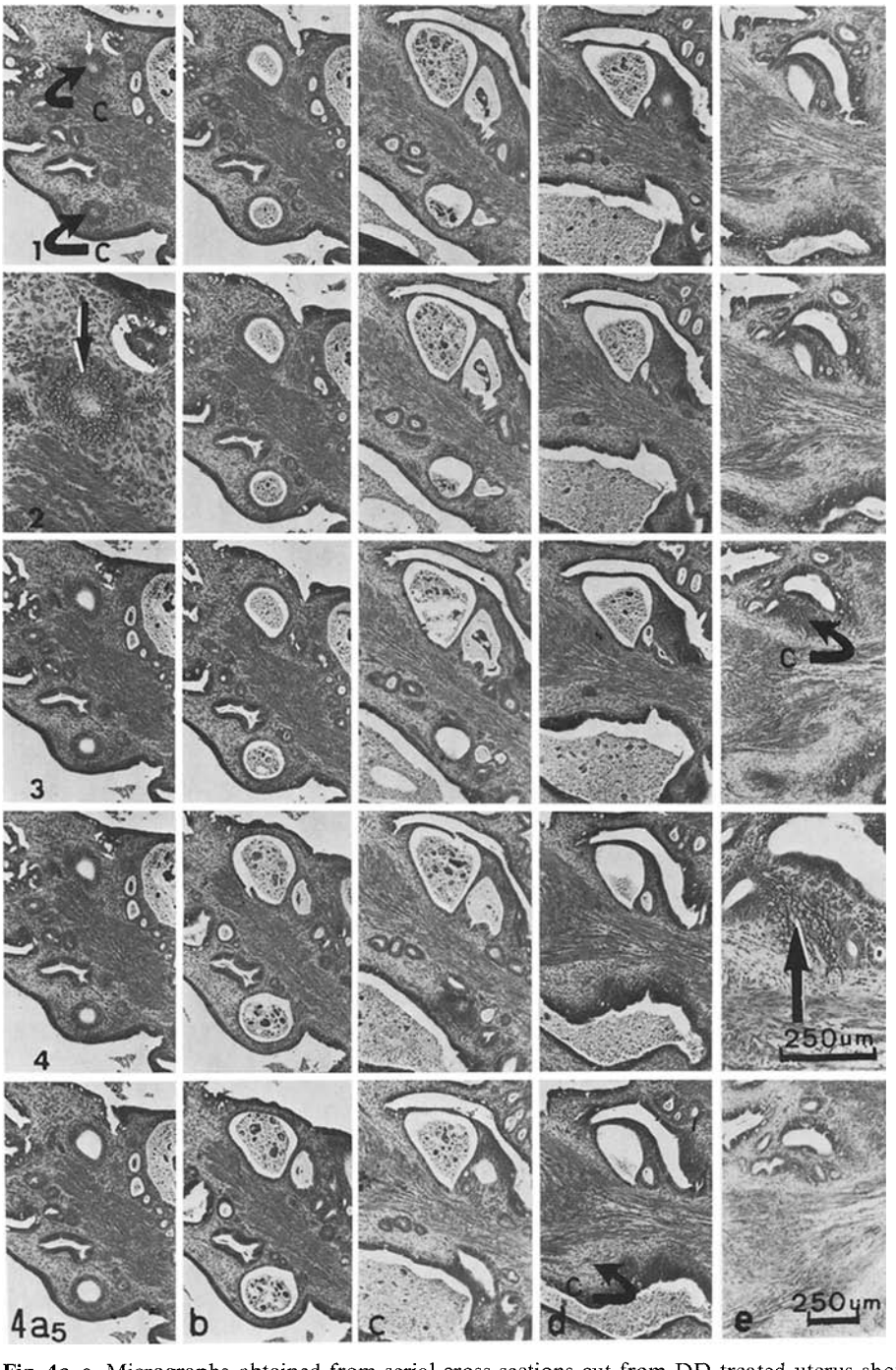


Fig. 4a–e. Micrographs obtained from serial cross-sections cut from DD treated uterus show at least two cystic glands "beginning" ($\mathbf{a}_1$) and "ending" ($\mathbf{d}_5$ and $\mathbf{e}_3$) within the stroma of a uterus polyp (*folded arrows*). Note that a capillary bed completely surrounds the cystic glands. In addition note the heterogenous cystic gland content. ($\mathbf{a}_1$ region is enlarged in $\mathbf{a}_2$ and $\mathbf{c}_3$ region is enlarged in $\mathbf{c}_4$; *straight arrows*). Gomori trichrome stain. Scale is 250 μm for all the illustrations

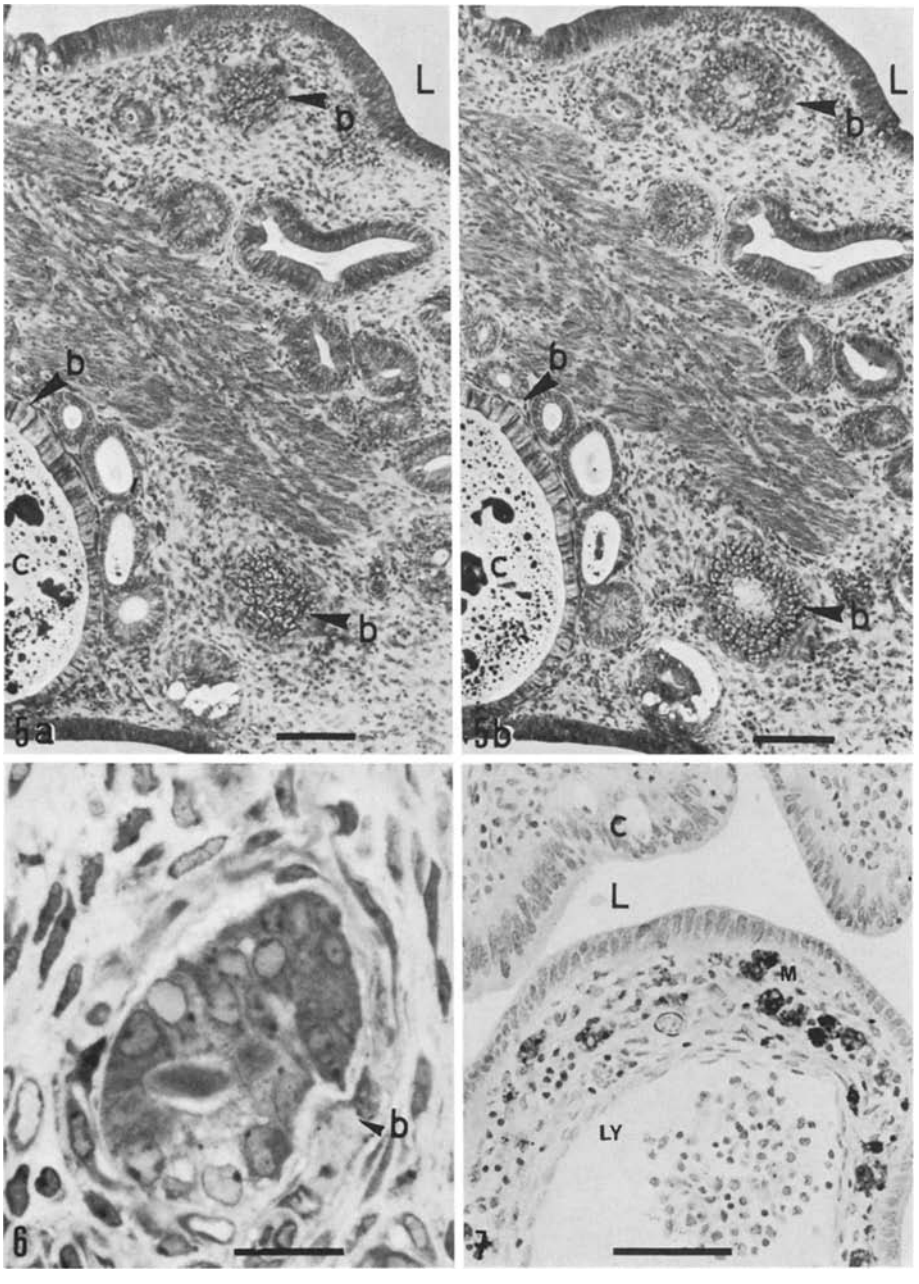


Fig. 5a–b. Serial cross-sections from the DD uterus. Note capillary beds (*b*) ensheating cystic glands (*c*) within the uterus stroma. The lining epithelium of cystic glands presents clear and eosinophilic cells. Beneath the lining epithelium a capillary bed is also observed (*b*). *L*: main uterus lumenal space. Gomori trichrome stain. Scales = 125 μm

Fig. 6. Cross-section of normal uterus glandular pit showing the closeness of a capillary (*b*) surrounding the lining epithelium basement membrane. Toluidine blue staining (2 μm thick Epon section). Scale = 25 μm

Fig. 7. The stroma of DD uterus polyp shows many macrophages (*M*). This particular polyp contains a dilated lymphatic (*LY*) lake. *L*: main uterus lumen; *c*: cystic gland. Perls-eosin staining. Scale = 100 μm

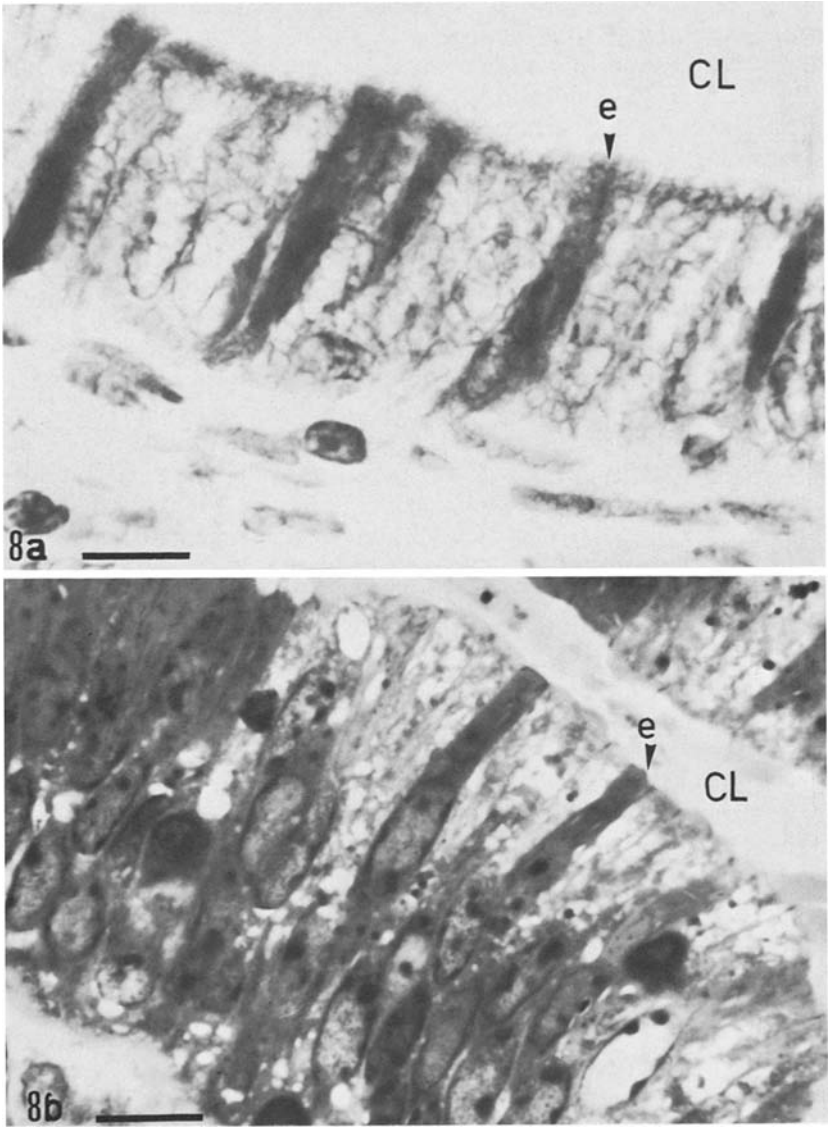
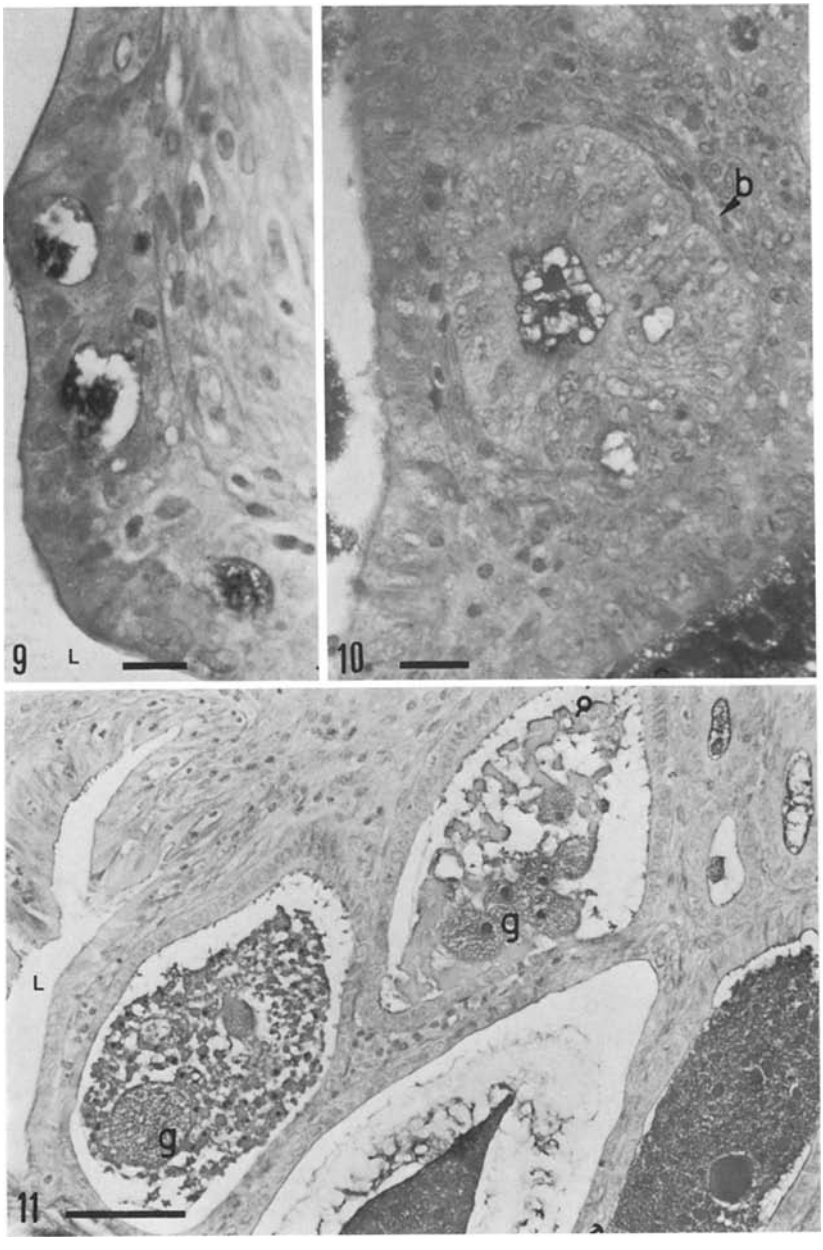


Fig. 8a–b. A simple to pseudostratified columnar epithelium with clear and eosinophilic (*e*) epithelial cells delineating the cystic lumen (*CL*) of stromal glandular structures. Note the morphological difference between a paraffin Gomori trichrome stain (**a**) with a 2 μm thick Epon toluidine blue stained preparation (**b**). In addition, (**b**) shows the convex cell apices of the epithelial lining, the pseudostratification, the secretory lipid-rich build up within cells and heterogen morphology typical for this abnormal epithelium, including pyknotic nuclei. Scale = 10 μm

of pathology which can be named "cystadenomatous papilloma"; "cystadenomatous" because of the benign epithelial neoplasms forming cystic glandular structures, and "papilloma" because many of these cystic glands are located in finger-like structures or extended folds of the uteri which project from the epithelial surface into the lumen of the uterus. The structures we observed resemble a pathological range from cystic atrophy to atypical adenomatous hyperlasia (WHO classification, 1975). Cysts are developed within the endometrium lining and/or the stroma and a second, third, fourth, etc. cystic gland generation can occur from these stromal cystic glands (Figs. 9–11).

Because many of the abnormal cystic glands have no openings into the uterine lumen, they fill up with secretions, and flatten the lining cells; therefore the epithelium lining the cystic glands ranges from columnar to cuboidal to almost squamous. This latter condition corresponds to the human cystic atrophy of endometrium pathology. In addition two different cell types are found lining the glandular spaces – a wider "normal" stained type and a narrower, highly acidophilic staining cell type. Since highly acidophilic staining usually indicates a high amount of proteinic material, unless these cells are undergoing necrosis, this is not evident from our ultrastructural observations (Gilloteaux and Steggles 1982b in press) we think this cell type is actively secreting some form of proteinaceous material and since P.A.S. demonstrate the presence of carbohydrate(s) in the constitution of this material, we conclude it should be a glycoprotein. The content of the cystic lumen is heterogenous, since Gomori trichrome and P.A.S.-Alcian blue technique demonstrate that the secretory products contain neutral and acid mucopolysaccharides (Figs. 9–11). For some cysts it includes cellular debris from granulocytes which degenerate into these spaces. Finally, all of the cystic glands are born surrounded and encased in a capillary bed taking the appearance of a dense network (Figs. 5a–b, 6). This tridimensional organization is more clearly seen when sections are examined from complete serially cut uterine horns (Figs. $3a_1$–$4e_5$). Thin plastic sections clearly show these close neighboring capillaries originating from a straight arteriole (Figs. $3a_1$–b_1). In C.D and D.D uteri subendometrial stroma, many macrophages are already apparent when trichrome is used (gold-brown pigment) and even more specifically stained for iron deposits when the Perls technique is utilized (dark blue stained) (Fig. 7).

The Prenatal Treated Hamster Uteri. (DC), young adults (150 days of age), have a characteristic uterine hypoplasia, i.e. the uteri were usually less than half the size of their age matched controls. Indeed the lumen is narrower then in the C.C uteri and the stroma adjacent to the endometrial epithelium shows an inflammatory response. In older animals, the D.C uteri show an increase in size eventually becoming larger than the control animals. In addition, cystic glands (comparable to those formed in C.D and D.D uteri) start to develop. We suspect that in time these D.C uteri will eventually present pathologic structures similar to those observed in C.D and D.D uteri.



Figs. 9–11. Sections from D.C, C.D and D.D uteri showing the formation of cystic glands within the endometrium lining (*g*, D.C) and stroma (10 C.D, 11 D.D). The staining technique shows cystic formation and the heterogeneity of their secretion (red, purple and blue staining). Fig. 10 (C.D) shows that cystic glands can also be formed within the endometrial lining of an already formed cystic gland (second generation gland) and Fig. 11 depicts small to large cystic glands containing secretory material including necrotic granulocytes (*g*). *L*: uterus lumen, *b*: capillary bed. P.A.S. – Alcian blue staining. Figs. 9 and 10, scale = 25 μm; Fig. 11 scale = 125 μm

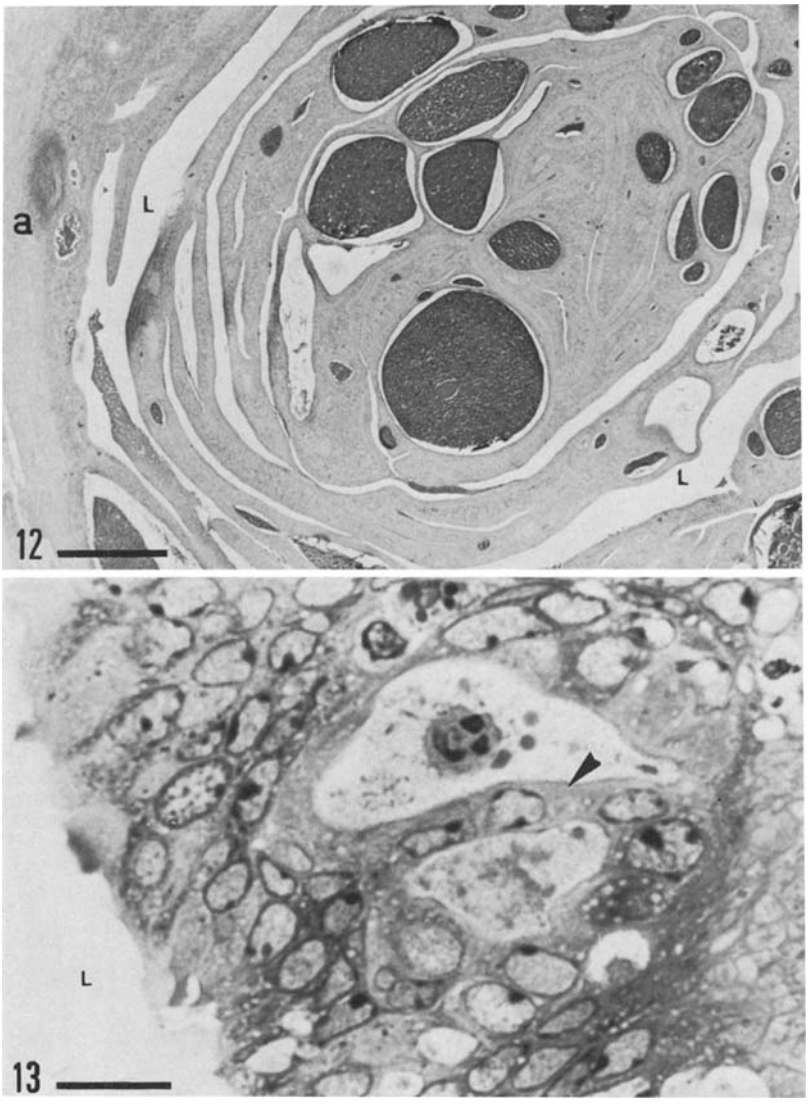


Fig. 12. Plug-like structures developed in the DD uterus lumen (*L*) as a result of papilla or polyp expansions. The atrophic stroma of these papillae depicts many cystic glands with and without secretory products. A zone of adenomyosis (*a*) is shown within the myometrium. P.A.S. – Alcian blue staining. Scale = 125 μm

Fig. 13. Atypical hyperplastic endometrium presenting glandular bridges (*arrow*) due to developing carcinoma *in situ*. Note the heterogenous nuclear sizes, pyknosis, necrosis and granulocytic invasion of the pseudostratified epithelium. Apices of lining endometrial cells appear dilacerated; *L* = main uterine lumenal space. Toluidine blue stain (2 μm thick Epon plastic section). Scale = 10 μm

In uteri from young D.C, C.D and D.D animals, the delineating atypical hyperplastic lining endometrium of some cystic glands presents crypts (Fig. 9) where the pseudostratified epithelium contains empty spaces surrounded by circularly arranged cells. In the oldest D.D uteri, these crypts are delineated by cells which have lost their polarity and are bridging the basal portion with the upper part of the endometrium (Fig. 13). These linings also present atypical cells with enlarged, pyknotic and necrotic nuclei while the cystic content is clearly made of a mucoproteinic secretory mixture resembling the one observed in the largest cysts and eventually contains migrating or decaying granulocytes. The apices of the lining endometrial cells are often irregular and appear lacerated (Fig. 13). This particular lining epithelium resembles the carcinoma *in situ* situation observed in human pathology (Hertig 1949a, b). Side by side we found cystic atrophy, adenomatous polyps, cystic hyperplasia, adenomatous hyperplasia including atypical adenomatous hyperplasia and adenocarcinoma including carcinoma *in situ*. The atypical hyperplastic endometrium also present high degree of glandular cell layering. Some of the glands become giant forms, containing secretory material, debris and granulocytes in the lumen. In the adenomatous hyperplasia outpouching of bud-like, glandular projections into the supporting endometrial stroma can be connected with the parent gland. All of these can be observed in the C.D and D.D treated hamster uteri.

In addition to these upper reproductive tract anomalies, dilatation of the cervical canal, squamous cell metaplasia with keratinization, adenosis and chondromatous areas were observed in all C.D and D.D treated animals after 180 days of treatment and adenosis was usually found in D.C animals ater 250 days of age.

Discussion

In women, endometrial hyperplasia is a potential precursor for endometrial carcinoma (Vellios 1972; Welch and Scully 1977). Similar types of hyperplasia are seen in D.C, C.D and the D.D uteri, and shows the whole spectrum of abnormalities observed in human uteri (WHO 1975). Ostör and Fortune (1980) described eleven cases of a rare human uterine neoplasm which has a strikingly similar pathology to that found in C.D and D.D hamsters. These neoplasms have both epithelial and stromal components with an architecture of "... papillary, often club-like projections, irregular clefts, and cystic spaces..." The inner portion of a D.D uteri, shown in Fig. 12, demonstrates this similarity. This observation helps support the human-hamster correlation in our study. Some of these observations (hyperplasia, cysts) were briefly presented earlier (Rustia and Shubik 1976; Leavitt et al. 1980; Steggles and Gilloteaux 1980; Gilloteaux and Steggles 1981). Some of the abnormalities described in the hamster have also been described in stilbestrol treated castrated guinea pigs (Lipschütz and Vargas 1941).

There are few studies which refer to human uterine hypoplasia resulting from prenatal exposure of a fetus to estrogens (Shapiro and Slone 1979; Kaufman et al. 1977). Viscomi et al. (1980) presented data based on the

ultrasound examination of uteri in young women who had been exposed to DES *in utero*. It is interesting that the abnormalities described in these female subjects seem to correspond to those we describe in our 150 day and 250 day old D.C. animals. In addition, the cervical abnormalities we reported are similar to those of Rustia and Shubik (1976).

The cystic structures found in the C.D and D.D uteri (both having postnatal DES treatment) are similar to the pathological structures observed in humans, (Vellios 1972; Tavassoli et al. 1978; Dallenbach-Hellweg 1980). In addition, since we found a whole spectrum of abnormalities observed in human uteri, our observations may confirm the exactitude of the theoretical pathways for the pathogenesis of endometrial carcinoma presented by Hertig et al. (1949a and b). Hence it is not surprising to find carcinoma *in situ*-like lesions in the D.D uteri.

A rather simple comparison can be made between the results from our four animal groups and various human situations: (i) C.C: untreated human females, (ii) D.C: human females exposed *in utero* to DES. (iii) C.D: human females with no *in utero* exposure, but subsequently exposed to estrogens (i.e. postnatal DES treatment, unbalanced birth control pills by progesterone, menopausal estrogenism, etc.) (iv) D.D: human females exposed to DES *in utero* and to estrogens postnatally (i.e., unbalanced birth control pills, estrogen treatment or suffering from hyperestrogenism).

This animal model is *only* comparable with the Smith type of DES prenatal treatment since the DES dose the animals received exceeds the current human contraceptive dose. Our dosage always produces changes in reproductive tract end organs (Smith 1948). Our model is adequate for chronic estrogen treatment, postcoital DES contraception, for the case of menopausal or postmenopausal hyperestrogenism when these are unopposed or complemented by a progesterone treatment where the highest dose of estrogens are present or DES used. While DES is not a constituent of birth control pills, it does have similar effects to the natural estrogens and it is possible that DES may have some particular non-estrogenic carcinogenic effect (Fink 1980; Noller and Fish 1974; WHO 1978). In terms of carcinogenicity, there is no difference between DES and extradiol (Kirkman and Bacon 1952).

Ostör and Fortune (1980), suggested that the pathologies they found in human uteri were benign and low grade variants of mixed Müllerian tumors. Also, the incidence of cancers in Müllerian-derived tissue e.g. uterine endometrium has been shown to be related to the use of exogenous estrogens (Ostör and Fortune 1980). This leads us to believe that postnatal treatment with DES has resulted in the production of benign neoplasma of Müllerian origin in the hamster uteri. We propose three hypothesis' on how the cystic glands form:

Hypothesis No. 1. The DES interacts with the estrogen receptors in the epithelial cells lining the uterine lumen. This causes some of the hyperplastic epithelial cells to divide and form a hollow structure (Fig. 9) which pinches

off from the luminal epithelium into the stroma. From these cystic linings, new cysts develop (Fig. 10).

Hypothesis No. 2. DES causes a proliferation of capillaries in the stroma. This is supported by the observations that DES causes proliferation of endometrial blood vessels and/or increases blood flow in the uterus, and that the cystic glands are increased where capillary beds are increased. These beds bring the DES into contact with the stromal cells that have estrogen receptors. Some of these cells (possibly stem cells; pericytes?) divide and differentiate after being stimulated by DES to form the stromal cystic glands.

Hypothesis No. 3. The normal uterine glandular cells either lose or somehow pinch off from the lumen due to the effects of DES and by interaction with some stem cells (including capillary pericytes?) and/or connective tissue cells of the stroma proliferate into stromal cysts.

We recognize that if we have used a lesser dose of DES we will decrease the incidence of hamster uterine abnormalities but since the classification of the human pathologies of the uterine polyps of the haperplastic and of the carcinomatous uteri is not without problems (Peterson and Novak 1956; Vellios 1972; Blaustein 1977), an animal model will be useful for understanding the etiology and evolution of endometrium carcinoma and uterine adenocarcinoma in such lesions that resemble those of the human situation. In addition, further histoenzymological (Gilloteaux et al. 1981; Gilloteaux and Steggles 1982a and b), biochemical and electron microscopical studies (Gilloteaux and Steggles 1981) are being conducted in order to further clarify the morphogenetic origin and the etiology of these abnormal glandular structures and their secretion which resemble those found in human hyperplastic endometrium. These future observations will complement this preliminary study and will further support the need for the hamster to be used as an animal model for the study of the development of human reproductive diseases like those linked to hormone-induced hypoplasia, menopausal or post-menopausal hyperestrogenism or related to a previous utilization of diethystilbestrol.

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