

PRENATAL EXPOSURE TO STILBESTROL

A Prospective Comparison of Exposed Female Offspring with Unexposed Controls

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Abstract The effects of prenatal exposure to diethylstilbestrol were studied by a prospective cohort investigation of 110 exposed and 82 unexposed females. The general health characteristics of mothers and daughters in both groups were similar. Among the exposed, there were striking benign alterations of the genital tract, which included transverse ridges (22 per cent), abnormal vaginal mucosa (56 per cent), and biopsy-proved adenosis (35 per cent). Among the unexposed there were no ridges and one case of vaginal mucosal

abnormality including adenosis ($p < 0.0001$). Abnormal cervical epithelium occurred in almost all exposed subjects but in only half the unexposed ($p < 0.0001$). The incidence of vaginal adenosis was highest when diethylstilbestrol was begun in early pregnancy. It was not detected when treatment was initiated in the 18th week or later. Oral contraceptive use and prior pregnancy were associated with less adenosis and erosion, respectively ($p < 0.05$). No cases of cancer were observed. (N Engl J Med 292:334-339, 1975)

IN 1971 a case-control epidemiologic investigation associated the recent occurrence of clear-cell adenocarcinoma of the vagina in young women with intrauterine exposure to diethylstilbestrol administered to their mothers for the therapy of high-risk pregnancies.¹ The results of this investigation were soon confirmed,² and since then, over 170 cases of vaginal and cervical clear-cell adenocarcinomas have been collected, with a definite history of maternal exposure to non-steroidal estrogens in 65 per cent of the investigated cases.³ Although these carcinomas have been encountered only rarely in females exposed to diethylstilbestrol, a variety of other abnormalities of the lower genital tract have been reported to occur with great frequency. These include vaginal adenosis (the presence of glandular epithelium in the vagina), cervical erosion, or ectropion (glandular epithelium on the portio of the cervix), and transverse fibrous ridges in the vagina and on the cervix ("cervical pseudopolyp," "cockscorn cervix," or "cervical hood").⁴⁻⁸ Although most of these abnormalities were known to occur rarely in the prestilbestrol era, their prevalence in unexposed women examined as carefully as those who have been exposed has not been established. The present study was undertaken to compare the results of epidemiologic, clinical, and pathological investigations of exposed women with those of unexposed controls, as well as to ascertain the current status of the exposed post-menarchal women whose mothers had been treated in the Boston Lying-in Hospital Diethylstilbestrol Clinic between 1947 and 1958.

MATERIALS AND METHODS

Identification of Exposed and Control Subjects

Through the generous cooperation of Drs. Olive and George van S. Smith, accurate research records of the 841 mothers treated in the Diethylstilbestrol Clinic of the Boston Lying-in Hospital between 1947 and 1958 were made available to us. These records contain both the dates of all the pregnancies and the details of

therapy. The drug was administered according to a standard schedule, with the dosage depending on the week in pregnancy in which the therapy was started. During or before the sixth week, 2.5 mg daily was given, increasing to 5 mg in the seventh week. An additional 5 mg was administered every two weeks until the 15th week, when 25 mg per day was prescribed. The daily dosage was then increased by 25 mg each month, reaching a maximum of 150 mg. All the mothers began therapy before the 23d week, and the drug was discontinued at the end of the 35th week.^{9,10} We assigned a random number to each diethylstilbestrol pregnancy, and, starting with the lowest assigned number, located the hospital birth records and then traced the parents of each female offspring. Telephone books and city directories were consulted, and the generous assistance of the Massachusetts Registry of Motor Vehicles was obtained. The correct identity of the mother was then verified by telephone or letter, and an examination of her female offspring was offered without charge if she lived within approximately 1000 km of Boston.

We selected the control group simultaneously by examining the hospital birth records and identifying an unexposed female who was born on the clinic service closest in time to (and always within five days of) each exposed female. It was further required that the mother's initial visit to the prenatal clinic occur before the 23d week of gestation. The controlled portion of the study was limited to white women 18 years of age or older, for several reasons. First of all, it appeared unreasonable to request healthy, unexposed girls under the age of 18 years to be examined. Secondly, since all but five exposed subjects were white, it was decided to limit the controlled portion of the study to whites. Finally, it was not possible within the hospital data base to match maternal gravidity and parity and still find an adequate number of unexposed subjects. Therefore, these factors were not controlled. The same technics used to find exposed subjects were used to locate controls. The results of the examination and tests with appropriate recommendations for follow-up observation were sent by letter to the subject and her physician. In addition to those included in the controlled study, all exposed post-menarchal subjects who could be located were offered appointments, to be certain that they would be examined by a physician. This precaution also permitted analysis of a larger number of cases in the second, uncontrolled portion of this investigation.

Investigation of the Subjects

Special clinics at each of which six to eight subjects were examined were conducted over an 18-month period. The two research analysts who identified and communicated with the mothers and their daughters knew which of the latter had a history of exposure to diethylstilbestrol, but this information was not available to those who performed the physical, pathological, or cytologic examinations. The maternal health histories were obtained by personal or telephonic interview by the two research analysts. Each subject's medical history was obtained by the examining physician, and a thorough general physical examination was performed, followed by a pelvic examination that included

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both a scrape of the vaginal mucosa and a pool aspiration for cytologic testing and careful inspection, palpation, and iodine staining of the vagina and cervix. Areas that appeared red or otherwise abnormal or that failed to stain with iodine solution were recorded and biopsied. Rectangular pieces of tissue up to 3 mm in diameter were obtained for microscopical examination with the use of either the Younge or the Kervorkian punch.

Pathological Evaluation of Tissue

Biopsies from the fornices of the vagina or areas distal to it were classified as vaginal. Those from the cervix or from fibrous ridges on or in apposition to the cervix (cervical rim or hood) were classified as cervical. At least five slides were prepared from each specimen; two were stained with hematoxylin and eosin, and one each with mucicarmine and periodic-acid-Schiff with and without diastase.

Analysis of Data

In the controlled portion of the study, the findings in the exposed and unexposed subjects were compared statistically by chi-square analysis. In the second portion of the study, the variation in the incidence of vaginal adenosis as a function of the time of initiation of diethylstilbestrol therapy during pregnancy was examined by testing for a linear trend in proportions.¹¹ The findings among the exposed subjects were analyzed further by the use of a multiple logistic model¹² to provide estimates of the effect of several independent variables (log of diethylstilbestrol dosage, week during pregnancy of initiation of therapy, age, history of intercourse, use of oral contraceptives, and pregnancy history of the subject) on three dependent variables (vaginal adenosis, failure of iodine staining of the vagina, and cervical erosion). This method enabled the testing of the effect of one or more independent variables after adjustment for effects of a different independent variable — e.g., the effect of use of oral contraceptives on the presence of vaginal adenosis, with adjustment for the week in pregnancy during which diethylstilbestrol therapy was begun.

RESULTS

Investigation of 110 Exposed and 82 Control Subjects 18 to 25 Years of Age

Epidemiology (subjects and parents). The 841 pregnancies of mothers treated in the Clinic yielded 731 viable births (407 male and 324 female). Among the 324 female subjects, there were 267 white women 18 years of age or older, who were compared to 267 unexposed women for the control portion of the study. As shown in Table 1, although 192 of the exposed and 203 of the unexposed were located, only 110 exposed and 82 unexposed women agreed to be examined. Among these, 66 per cent were 24 to 25 years and the remainder were 18 to 23 years of age. Because only those who were willing to participate in the study could be examined, it is possible that bias was introduced by the greater reluctance of the unexposed women to accept an examination. However, a comparison of the characteristics of the exposed and the unexposed subjects and those of their parents revealed a surprisingly close correspondence (Table 2). Sociologic factors such as maternal education and family social class were similar.

Table 1. Results of Search for White Subjects 18 Years of Age or Older.

GROUP	NO. OF MOTHERS WITH FEMALE CHILDREN	NO. FOUND	NO. OF DAUGHTERS EXAMINED
Exposed	267	192	110
Controls	267	203	82

There was no difference in the maternal histories of serious illnesses, including cancer. Likewise, no differences were noted in the health histories of the exposed and the control daughters, none of whom had cancer; both groups were also similar in age at onset and frequency of menstruation. The reproductive histories of both groups of daughters were also similar.

Physical examination. Between the two groups of subjects several differences that did not achieve statistical significance were noted on general physical examination. Abnormalities detected included scoliosis (three exposed), Charcot—Marie—Tooth disease (one exposed), and cardiac murmurs that were interpreted as organic (nine exposed and seven control). There was a difference in the height (160.5 cm exposed vs. 158.2 cm control, $p < 0.05$) but not in the weight (60.0 kg vs. 56.9 kg respectively) or in the ponderal index, which integrates weight as a factor of height (12.6 vs. 12.7 respectively). Otherwise, the general physical characteristics of the exposed and control subjects were similar.

Pelvic examination. No differences were detected in the appearance of the external genitalia, or in the estimated sizes of the uterus or the ovaries. Vaginal examination, however, revealed numerous differences between the two groups that were highly significant statistically (Table 3). Transverse fibrous ridges of the vagina and cervix were seen in 22 per cent of the exposed females, but in none of the controls. These ridges usually appeared as transverse bands in the upper vagina or near the cervix. They occasionally obliterated the vaginal fornices and often obscured the boundary between the vagina and cervix. Failure of portions of the vaginal mucosa to stain with iodine solution was observed in over half (56 per cent) the exposed subjects but in only 1 per cent of the controls. Some of these abnormal areas appeared red in the exposed subjects before iodine staining, but red areas in the vagina were not observed in the controls. Also, failure of portions of the cervix to stain with iodine was noted almost twice as often in the exposed (95 per cent) as in the control subjects

Table 2. Characteristics of Exposed and Control Populations That Were Not Significantly Different.*

CHARACTERISTIC
Parents:
Education of mother (11.8 vs 11.9 yr)
Maternal smoking
Paternal smoking
Social class
Maternal history of surgery
Maternal history of cancer or serious illness
Daughters:
Intrauterine exposure to x-rays
Length of gestation
Birth weight (3120 vs 3200 g)
Breast feeding
Smoking
Onset of menses (12.7 vs 12.5 yr)
Frequency of menses
Use of oral contraceptives (37 vs 38%)
No. who had been pregnant (46 vs 52%)
No. who produced viable births (23 vs 28%)
History of surgery
History of cancer or serious illness

*At 0.05 level or less.

Table 3. Results of Pelvic Examinations.

GROUP	NUMBER EXAMINED	FAILURE OF PART OF VAGINA TO STAIN WITH IODINE (%)	VAGINAL ADENOSIS IDENTIFIED IN BIOPSY SPECIMEN (%)*	FAILURE OF PART OF CERVIX TO STAIN WITH IODINE (%)	CERVICAL EROSION IDENTIFIED IN BIOPSY SPECIMEN (%)*	VAGINAL OR CERVICAL FIBROUS RIDGES (%)
Exposed	110	56	35	95	85	22
Control	82	1	1	49	38	0
chi-square		64.8	33.4	52.5	47	20.4
p value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

*Biopsies taken from areas that appeared red or failed to stain with iodine solution.

(49 per cent). Red areas on the cervix were seen before iodine staining in both groups.

Pathology. Vaginal adenosis was detected by biopsy in 35 per cent of the exposed but only 1 per cent of the control subjects. In the former the adenosis was usually multifocal. On microscopical examination it was characterized by the presence of a single layer of mucinous columnar cells of endocervical type or a single or pseudostratified layer of cells that resembled endometrial and tubal epithelial cells. The latter cells were often ciliated; most of them had dark, deeply eosinophilic cytoplasm, but some had clear cytoplasm. In occasional cases, minor degrees of atypicality or loss of polarity of the nuclei were observed. The columnar epithelium lined the surface of the vagina or formed glands in the lamina propria (Fig. 1). Often, the glands were largely or totally replaced by solid masses of squamous epithelium (squamous pegs), which were continuous with the surface squamous epithelium (Fig. 1); the epithelium both in the pegs and on the surface was almost always composed of glycogen-poor or glycogen-free basal and prickle cells. This characteristic of the squamous cells or in some cases the replacement of the surface squamous epithelium by a layer of glandular epithelium accounted for the lack of staining with iodine. Two histologic variants of adenosis that were observed in the squamous pegs or in the surface squamous epithelium were droplets of mucin within individual squamous cells



Figure 1. Vaginal Adenosis (Hematoxylin and Eosin Stain). Glands with ciliated dark cells that resemble the endometrial or tubal lining lie in the lamina propria and merge in areas with squamous pegs (arrow). Both the squamous epithelium that lines the surface of the vagina and the squamous pegs continuous with it are composed of glycogen-free basal and prickle cells.

(Fig. 2) and small pools of mucin surrounded by tall to inconspicuously flat cells with mucinous cytoplasm (Fig. 3). The mucin droplets and pools, which sometimes became apparent only after mucin staining, were present in 74 per cent of the subjects with adenosis and were the only evidence of it in 29 per cent of these cases (8 per cent of all the exposed subjects). Squamous pegs were observed in 76 per cent of the exposed subjects with biopsy-proved adenosis. Although pegs were observed in the vaginal biopsies of 16 per cent of the exposed subjects without adenosis, they were not observed in the unexposed.

Adenosis was detected in only one control, and it differed from the typical form of the disorder in the exposed subjects. In that case a single cystic gland lined by mucinous epithelium lay in the lamina propria; the overlying squamous epithelium was normally glycogenated, and no squamous pegs were seen.

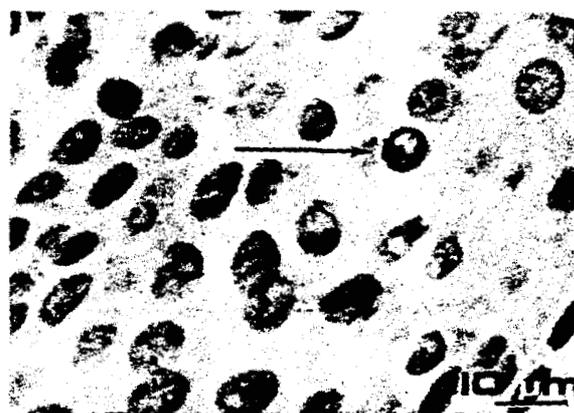


Figure 2. Vaginal Adenosis (Mucicarmine Stain).

In the tissue slide the mucin droplet (arrow) is bright red, whereas the nuclei of the cells are brown-black, and the cytoplasm pale yellow.

In contrast to vaginal adenosis, the cervical erosions were morphologically similar in the exposed and the control subjects, being characterized by an epithelium that was almost always mucinous; cells of tubal or endometrial type were only occasionally seen. The glandular epithelium lay on the surface, sometimes covering micropapillae, or lined glands in the lamina propria. There were, however, quantitative differences in the erosions of the two groups of subjects. Squamous pegs were more frequent in the cervical biopsies of the exposed (43 per cent) than in the control series (6 per cent). Also, mucinous droplets and pools were more commonly encountered in the former.

Cytology. Quantitative differences also existed in the cytologic specimens of the two groups. In the vaginal scraping, columnar cells, metaplastic squamous cells, squamous cells containing mucous droplets, or dysplastic cells were found in 9 per cent of the exposed subjects (11 per cent in those with adenosis and 7 per cent in those without), in contrast to 1 per cent of the controls. In the aspirates of the vaginal pool, these abnormalities were also observed more frequently in the exposed (19 per cent) than in the control subjects (9 per cent), being present

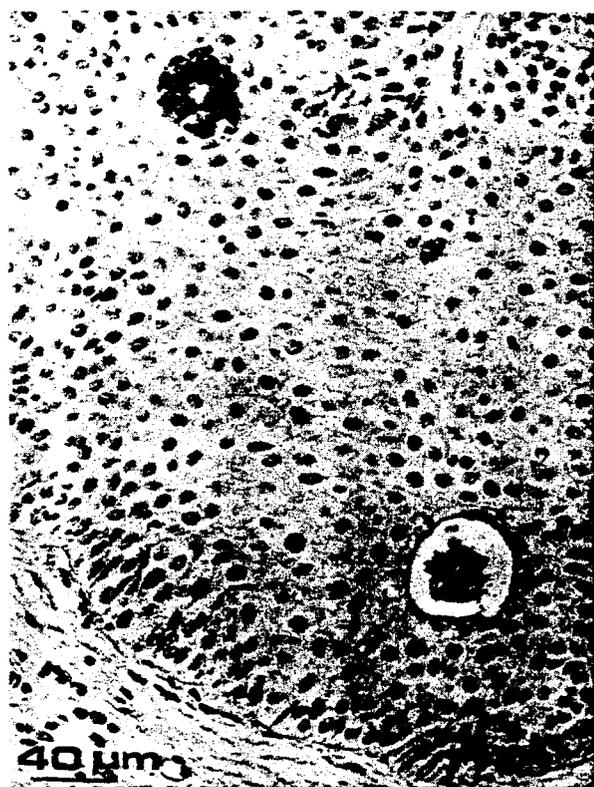


Figure 3. Vaginal Adenosis (Mucicarmine Stain).

One pool of mucin is surrounded by flat cells with mucinous cytoplasm that were obvious only with mucin stains (upper left), whereas a second pool is surrounded by cells with foamy cytoplasm obvious both with hematoxylin and eosin and with mucicarmine stains (lower right).

in 27 per cent of the exposed girls with biopsy-proved adenosis. Although mild squamous-cell dysplasia was observed in the vaginal scrapings from three exposed subjects and in the vaginal aspirate from a fourth, this change was not confirmed in any by multiple punch biopsies.

Other factors. The increased occurrence of adenosis, erosion, non-iodine-staining areas, and ridges in the exposed in comparison to the unexposed subjects was investigated further to determine whether other features (e.g., intrauterine x-ray exposure, smoking by parents and daughters, or maternal medications other than diethylstilbestrol) could have also had a role. No association with any factor other than intrauterine diethylstilbestrol could be identified.

Investigation of the Total Exposed Population of 133 Subjects (13 to 25 Years of Age)

Eighteen exposed white female subjects 13 to 17 and five non-white subjects 19 to 24 years of age were also examined, and the findings were combined with those of the 110 exposed subjects from the controlled study to provide the results of the investigation of all the post-menarchal females from the Diethylstilbestrol Clinic who agreed to be examined. Non-iodine-staining areas of the vagina were detected in 80 (60 per cent), and biopsy-proved adenosis in 49 (37 per cent). Non-iodine-staining epithelium was found on the cervix in 126 (95 per cent),

and biopsy-proved erosion or ectropion in 112 (84 per cent). Fibrous ridges of the vagina and cervix were found in 28 (21 per cent) of the subjects. No cases of cancer were detected. Only seven (5 per cent) of the subjects had pelvic examinations that were normal on inspection and after staining with iodine solution.

Table 4 examines the relation between the week of initiation of diethylstilbestrol therapy and the proportion of subjects having abnormalities of the vagina or cervix. Vaginal adenosis was more frequent in those daughters whose mothers began diethylstilbestrol in early pregnancy. It was observed in 73 per cent of subjects initially exposed during the first two months of pregnancy, but in only 7 per cent of the 30 initially exposed in the 17th week or later (Table 4). None of 19 subjects whose mothers began therapy in the 18th week or later were found to have adenosis. The linear regression of the proportion of those found to have adenosis with respect to the month of initiation of therapy was highly significant ($p < 0.001$).¹¹ Similarly, cervical erosion was more frequent when therapy was begun in early pregnancy, but the regression ($p < 0.01$) was not as striking as for vaginal adenosis (Table 4). This result can be explained in part by the fact that cervical erosion often occurs in the absence of diethylstilbestrol exposure (Table 3). No association was demonstrated between the time of initiation of therapy and the presence of vaginal or cervical fibrous ridges, and one ridge was detected in a subject whose mother began treatment as late as the 21st week. The location and appearance of the ridges, however, varied to some extent with the time therapy was initiated. Nine of the 10 daughters with ridges who were exposed before the 12th week had a concentric ridge surrounding the external os of the cervix, with the formation of a pseudopolyp; the 10th had a transverse narrowing in the upper vagina. Both pseudopolyps and cervical ridges that were only partly circumferential were observed in subjects exposed between the 13th and 16th weeks of gestation. None of the four with ridges whose mothers started treatment in the 17th week of pregnancy or later had a cervical pseudopolyp, but all had partial ridges on the cervix or in the vagina. Because the mothers who were treated in the Diethylstilbestrol Clinic maintained a strict and identical dosage schedule, the total dosage of maternal medication was directly related to the time in pregnancy diethylstilbestrol was begun, so that it was impossible in this study to separate the effect of dosage from that of the onset of medication.

The analysis by the use of a multiple logistic model¹² revealed no correlation of vaginal or cervical abnormalities with the age of the subjects at the time of examination or with their sexual histories. As individual factors, the

Table 4. Frequency of Abnormalities in 133 Exposed Subjects in Relation to Week of Initiation of Diethylstilbestrol Therapy During Pregnancy.

Wk	TOTAL NUMBER	VAGINAL ADENOSIS (%)	CERVICAL EROSION (%)	RIDGE (%)
≤8	22	73	100	23
9-12	39	49	92	28
13-16	42	29	81	19
>17	30	7	70	13

age of the subjects at the time of examination, as well as the week in pregnancy in which diethylstilbestrol was begun, did affect the incidence of adenosis. However, the mothers of the younger subjects began diethylstilbestrol earlier in pregnancy, resulting in a high correlation between subject age and the week of initiation of maternal medication. After adjustment of the incidence of adenosis for the week in which diethylstilbestrol was started, there was no residual effect owing to the age of the subjects. Vaginal adenosis, when adjusted for the week in which the estrogen was started, was detected less frequently in patients who had used oral contraceptives (chi-square = 5.05, $p < 0.05$), but the use of oral contraceptives had no effect on the frequency of non-iodine-staining areas in the vagina. Cervical erosion, adjusted for the week in which diethylstilbestrol was started, was less frequent among those who had been pregnant (chi-square = 5.44, $p < 0.05$). Pregnancy did not affect the incidence of vaginal adenosis or of non-iodine-staining areas in the vagina.

DISCUSSION

The results of this investigation of diethylstilbestrol-exposed and unexposed female subjects are consistent with previous uncontrolled observations that vaginal adenosis as well as transverse vaginal and cervical ridges are very rare in young females in the absence of intrauterine exposure to diethylstilbestrol.⁴⁻⁶ Although cervical abnormalities (failure of the portio mucosa to stain with iodine and erosion) were encountered in almost all the exposed subjects in this study, these findings were less specific, being present in about half the controls. There were no significant differences between the exposed and control groups in postnatal medical histories, including the onset and frequency of menstrual bleeding. These limited data suggest that ovarian function is not altered, at least in the first three decades of life, as a result of prenatal exposure to diethylstilbestrol. Similarly, the fact that there was no significant difference in health histories of the mothers in each group up to the present time, including the development of cancer after the ingestion of diethylstilbestrol, suggests that the medication did not affect their health. This interpretation, however, is based only on interview histories and not on physical or laboratory examinations.

The gynecologic and pathological findings of the present study were based on inspection, iodine staining, cytologic sampling and biopsies of the vagina and cervix. In view of the fact that scraping the vagina yielded cells compatible with vaginal adenosis (columnar cells or mucin droplets within squamous cells) in only 11 per cent of the cases of adenosis, and aspiration of the vaginal pool in only 27 per cent, cytology must be considered unreliable as a screening test for this disorder. Nevertheless, the identification of these cell types in a routine vaginal-pool aspiration, as well as the failure of the vagina to stain with iodine during a pelvic examination, should raise a high index of suspicion of diethylstilbestrol exposure in a young woman, who may be unaware of her prenatal history. The additional performance of mucin staining of biopsy specimens of the vagina facilitates the detection of mucin

droplets and pools in squamous epithelium, permitting the pathologist to recognize adenosis more easily than with the use of routine hematoxylin and eosin stains alone.

The failure to detect vaginal adenosis in any subject whose mother began therapy during the 18th week of pregnancy or later suggests that this disorder is congenital, reflecting a derangement of vaginal development related to the action of diethylstilbestrol before that time.¹³ This observation correlates well with data from the Registry of Clear-Cell Adenocarcinoma of the Genital Tract in Young Females, in which no cancers have yet been uncovered in female subjects exposed to diethylstilbestrol or similar drugs later than the 17th week of gestation.³ Although it was not possible from the current study to separate the effects of the total dosage of diethylstilbestrol from the time of its initiation during pregnancy, previous uncontrolled studies^{4,5} as well as data from the Registry³ have provided evidence that neither adenosis nor carcinoma is dose related, within the wide range of the amounts of diethylstilbestrol that are known to have been prescribed.

The incidence of vaginal adenosis that has been reported in diethylstilbestrol-exposed female subjects has varied. For example, Staff and his associates⁶ found it in 57 of 63 exposed subjects on colposcopic and biopsy examination. Their results are in contrast with those of Townsend, who also used the colposcope but demonstrated adenosis in only 30 per cent of 286 exposed females; he identified a metaplastic squamous transformation zone corresponding to non-iodine-staining areas of the mucosa in an additional 30 per cent (personal communication). These results are similar to those of the present study, in which the method of examination was palpation and iodine staining followed by biopsy of abnormal areas. Some of the reported variations in incidence of adenosis may be related to differing estimates by the examiners of the boundary between the vagina and the cervix, which may be difficult to define in the presence of ridges, as well as differences in histologic terminology, the time of initiation of diethylstilbestrol therapy, and the population examined (e.g., unselected, asymptomatic, exposed subjects vs. symptomatic patients or those referred because of abnormalities noted by other physicians).

A proposed mechanism by which intrauterine exposure to diethylstilbestrol acts to result in vaginal adenosis is inhibition of the normal replacement of the embryonic müllerian epithelium of the vagina by the upgrowing squamous epithelium of the vaginal plate, which may be of either wolffian or urogenital-sinus origin.^{4,8} Normally, in the unexposed mature female, the transformation zone between the two types of epithelium is at or near the external os of the cervix. It has been suggested that cervical erosion and vaginal adenosis in exposed subjects represent simply an increasingly more distal positioning of the transformation zone onto the cervical portio and vagina, respectively.⁶ If this hypothesis were true, one might expect that cervical erosion and vaginal adenosis would be identical on histologic examination, but differences do exist. For example, in the biopsy specimens of cervical erosion in the present investigation, almost all the glan-

dular epithelial cells had the appearance of mucinous cells of the endocervix, whereas in vaginal adenosis, many of them resembled those of the endometrium or fallopian tube. These findings suggest that the pathogenesis of cervical erosion and vaginal adenosis may not be identical, and that the two processes may differ in biologic behavior on long-term investigation.

The data from the current study indicate a decreased frequency of vaginal adenosis in exposed subjects who have used oral contraceptives. This apparent healing might be related to the progestational component of these agents, in view of the suggestive evidence provided by a recent pilot study that healing of vaginal adenosis is promoted by the use of progesterone-theobroma oil suppositories.⁷ However, in spite of these preliminary findings, we have not advised the routine use of these agents because of the uncertainty regarding their long-term effects. The decreased frequency of cervical erosions observed in the exposed subjects in this study who had been pregnant might have been due to cervical cauterization after delivery, rather than to the effects of the pregnancy itself, but the inaccurate histories of that procedure did not allow testing of this hypothesis.

It has been suggested that replacement of glands by squamous epithelium may be a mechanism by which vaginal adenosis and cervical erosion heal,^{6,7,14,15} and because metaplastic squamous epithelium was observed frequently, it was anticipated that these disorders might be less frequent in older subjects as a consequence of spontaneous healing. Although the data afforded no evidence in support of this expectation, all the subjects in this study were younger than 26 years of age at the time of examination, and follow-up observation as they age further may reveal spontaneous healing.

Because the presence of vaginal adenosis has been demonstrated in almost all the cases of clear-cell adenocarcinoma of the vagina in young women when adequate tissue has been available for examination, and because cervical erosion is a consistent finding in association with clear-cell adenocarcinoma of the cervix,³ it is logical to assume that the glandular tissue characterizing adenosis and erosion may provide the base from which these adenocarcinomas develop. Although glandular atypicality has been reported in the vicinity of one clear-cell adenocarcinoma of the vagina,¹⁶ to our knowledge no case has yet been recorded in which a direct temporal or spatial transition from adenosis to adenocarcinoma has been documented in a diethylstilbestrol-exposed woman. In the present study, only minor degrees of glandular atypicality were observed in the biopsy specimens of the cervix and vagina. The results of many studies continue to show that non-neoplastic cervical and vaginal abnormalities are common in the exposed population, whereas clear-cell adenocarcinomas and glandular abnormalities that can be recognized morphologically as precancerous are very rare.

An additional hazard to diethylstilbestrol-exposed female subjects has been suggested by some authors¹⁷ who have proposed that squamous-cell carcinomas also may occur with increasing frequency as these patients age, in

view of the recent rare findings of squamous dysplasia and carcinoma-in-situ of the cervix and vagina in them (Staff and Mattingly,¹⁷ Lanier et al.,¹⁸ Robboy, S.J., Scully, R.E., and Herbst, A.L.: unpublished data) and the knowledge that the peak incidence of such alterations in the general population is at an older age than that of exposed women at present. In this study, only four of the 110 exposed subjects (4 per cent) had squamous dysplasia on cytologic examination, and it was slight in all four. With only sporadic reports of these precancerous or possibly precancerous squamous changes, it is impossible to be certain how often they will either progress to invasive cancer or prove to be only a transitory phenomenon related to active squamous metaplasia.

Obviously, many years may be required to determine the risk of development of genital cancer in diethylstilbestrol-exposed female subjects. During this time, thorough periodic examination is clearly indicated.

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