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15. Approved as TA 13001 by the director of the Texas Agricultural Experiment Station. Supported in part by the Texas Department of Agriculture Interagency Agreement IAC (74–75)–0448. We thank R. H. Crozier, G. E. Hart, and P. Sroka for discussion; and W. L. Brown, W. F. Bruen, and E. O. Wilson for comments on the manuscript.

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Steroid Contraceptive Use and Cervical Dysplasia: Increased Risk of Progression

Abstract. *In a prospective study of women with dysplasia of the cervix, there was an increase in severity of dysplasia and of conversion to cancer in situ in users of the contraceptive pill compared with users of other contraceptive methods. There was a delay in this adverse response. Nonreversal of dysplasia within the first 6 months of pill use is predictive of progression after prolonged exposure.*

We investigated the possible association of steroid contraceptive use and carcinogenesis of the cervix by longitudinal observation of women with the cancer precursor, dysplasia of the cervix. All of the women in the study were using either the contraceptive pill or another method of contraception. The objective was to look for a differential effect of the pill on progression from dysplasia to cancer in situ. In this case, the possible carcinogenic effect of steroid contraception on dysplasia could be of a promoting rather than initiating nature.

According to the natural history model of stepwise progression in carcinogenesis of the cervix (*1*), dysplasia is an intermediate step from which progression to cancer or reversal toward normal may occur as in the following scheme:

normal ⇄ dysplasia → cancer in situ → invasive cancer → death

By limiting the scope of our study to progression from dysplasia to cancer in situ (*2*), we circumvented the problem of obtaining the large sample sizes that would be required in studying differential cancer incidence in normal young women.

Studies of the possible carcinogenic effect of the contraceptive pill on the cervix have yielded conflicting results (*3*). A greater prevalence of cancer in situ in women using oral contraceptives than in women using other contraceptive methods was reported (*4*), but an association of pill use with the development of cervical cancer was not substantiated after further follow-up by the same investigators (*5*). The higher prevalence may have been attributable to a self-selection factor, since women who chose the pill had a higher prevalence of dysplasia before ever using it than women who chose other contraceptive methods (*6*). Random allocation of a contraceptive method is unacceptable to women, and even though known differences between study and control groups have been taken into account in our analyses, the problem of self-selection in a nonrandomized study is never completely resolved.

Our study design enabled us to monitor other possible confounding factors such as changes to other methods or cessation of contraception, or subjects becoming pregnant or dropping out of the study. Confounding effects resulting from pregnancy or changes in method of contraception were avoided by excluding the data on such women from the time such an event occurred. Dropout rates or reasons for dropping out did not differ according to the contraceptive method used. The question of possible bias due to differences in the characteristics of those subjects who continued in the study and those who dropped out was an important consideration, and our analysis of subjects by dropout status and contraceptive method revealed no evidence that dropping out of the study was related to disease outcome (*7*). Another important source of artifact specific to studies of cervical neoplasia and pill use is the Papanicolaou (Pap) test that is given preferentially to users. We avoided this problem by baseline assessment of the cervical mucosa of all subjects before the steroid contraceptive was ever used, and by equal application of test procedures to pill users and nonusers over the duration of the study.

The subjects were selected from approximately 11,000 women enrolling sequentially in Los Angeles County family planning clinics from 1967 through 1971, the cutoff for entry. Even at their first visit almost 50 percent of the women had already used the contraceptive pill and were thus ineligible for the study; others were excluded because of a history of illness that could bias the free choice of contraceptive method. From almost 6000 eligible women (*8*), Pap screening identified 300 women with dysplasia. A sample of 300 women (5 percent) with negative smears was selected concurrently and followed in the same way as the women with dysplasia. Subjects were examined again at 2 months, 6 months, and then every 6 months. The status of the cervical mucosa was monitored by Pap smear, and this information was supplemented in the women with dysplasia by annual biopsy limited to superficial samples of the mucosa (*9*). All cytologic and histologic evaluations were performed blind. Women using the steroid contraceptive pill (users) were compared to women using all other contraceptive methods combined (nonusers) (*10*). Over 90 percent of the pill nonusers used an intrauterine device (IUD). The pill was limited to one progestin-estrogen compound at one dose level throughout the study (*10*). Both study and control women used contraceptives and were thus presumably sexually active.

In this study of carcinogenesis in young women, average age 23 years, using contraception, we found that conversion to cancer in situ was slow even in women who already had dysplasia and were thus at high risk. We therefore also examined transitions over the range of dysplasia, assuming that conversion to cancer involves an ordered progression through increasing degrees of severity of dysplasia (*11, 12*). As an aid to measuring degrees of progression, we constructed a numerical scale to cover the entire gradient of abnormality in the cervical mucosa (*12*). It was then possible to relate oral contraceptive exposure to change in diagnostic score over time (*13*) (see Fig. 1).

In the sample of normal women there is as yet no evidence of a differential effect of the pill (Fig. 1A). However, in the dysplasia sample, there was a differential effect over time, and after 2 years the pill users maintained higher scores than the nonusers for the duration of the study. The differential effect is enhanced after scores are adjusted for concomitant variables (*7*). The decline in scores after 2 and 6 months is also of interest. The early drop is greater, though not significantly so, in the pill users than in the

nonusers. This is suggestive of an initial beneficial effect of the pill (Fig. 1B). The biphasic difference in response to the pill over time, beneficial over the short term and adverse over the long term, was further examined in subsets of women with and without early reversal. Differences in mean scores over time between pill users and nonusers are more evident when early reverters are excluded (Fig. 1C). Within the group using the pill (Fig. 1D), women who do not revert to normal after a short time appear to become more susceptible to progression as pill use is prolonged, and they may represent a group that is at higher risk than the early reverters. In that sense, the diagnostic scores during the first 6 months of pill use may be predictive of dysplasia outcome after long-term use of the pill. In the group not using the pill, the early drop in scores had no predictive value.

In most of these young women, dysplasia was no more than minimal at the time they entered the study, and there were few conversions to cancer in situ in the first years of follow-up. As the study continued, we observed more conversions to cancer in situ with the typical case progressing through increasing degrees of severity of dysplasia. Seven years after the study began it was possible to estimate differential progression from dysplasia to cancer in situ.

The probability of progressing from dysplasia to cancer in situ was calculated for pill users and nonusers by means of life-table analysis (*14*) (Table 1). A difference in the cumulative probability of progressing from dysplasia to cancer in situ first becomes evident after 5 years of study. The difference increases and becomes statistically significant at a marginal level ($P < .05$) at 6½ years. The probability of progressing from dysplasia to cancer in situ in pill users is .30, compared to .05 for nonusers at 7 years, and the risk of conversion to cancer in situ is six times greater.

Although the number of women studied for up to 7 years is small, we find a significant difference in the cumulative probability of progressing to cancer in situ. Thus, the results of the life-table analysis and the analysis of mean scores over time are consistent in showing a differential effect of the contraceptive pill.

A clinical implication of the study is that women with dysplasia who take the pill are at increased risk for developing cancer in situ of the cervix. We do not think the IUD exerts a protective effect, since the cancer in situ rate in the pill nonusers approximates the expected rate for this population. A differential effect is not evident in the sample of normal women. These findings apply to a defined population base and a particular protocol. Whether the results are applicable to the general population will require additional research. In any case, the results of this study suggest that curtailment of use of steroid contraceptives in women with dysplasia of the cervix should be considered.

The augmented progression from dysplasia to cancer in situ with prolonged use of a hormonal contraceptive is con-

Table 1. A life-table analysis showing the probability of progression from dysplasia to cancer in situ (CIS) in pill users and nonusers.

Length of follow-up		Number of women at start of interval		Number progressing to CIS during interval		Cumulative probability of progression	
Interval (months)	Midpoint (years)	Users	Nonusers	Users	Nonusers	Users	Nonusers
0 to 6		203	97	0	0	0.00	0.00
7 to 14	1	128	65	2	2	0.02	0.03
15 to 20		103	50	1	1	0.03	0.05
21 to 26	2	84	42	1	0	0.04	0.05
27 to 32		72	35	0	0	0.04	0.05
33 to 38	3	64	33	0	0	0.04	0.05
39 to 44		53	31	0	0	0.04	0.05
45 to 50	4	50	27	0	0	0.04	0.05
51 to 56		42	23	0	0	0.04	0.05
57 to 62	5	37	19	0	0	0.04	0.05
63 to 68		32	12	3	0	0.13	0.05
69 to 74	6	27	9	0	0	0.13	0.05
75 to 80		17	6	2	0	0.23*	0.05
81 to 86	7	15	4	1	0	0.30*	0.05

*The difference in probability of progression between pill users and nonusers is significant at $P < .05$.

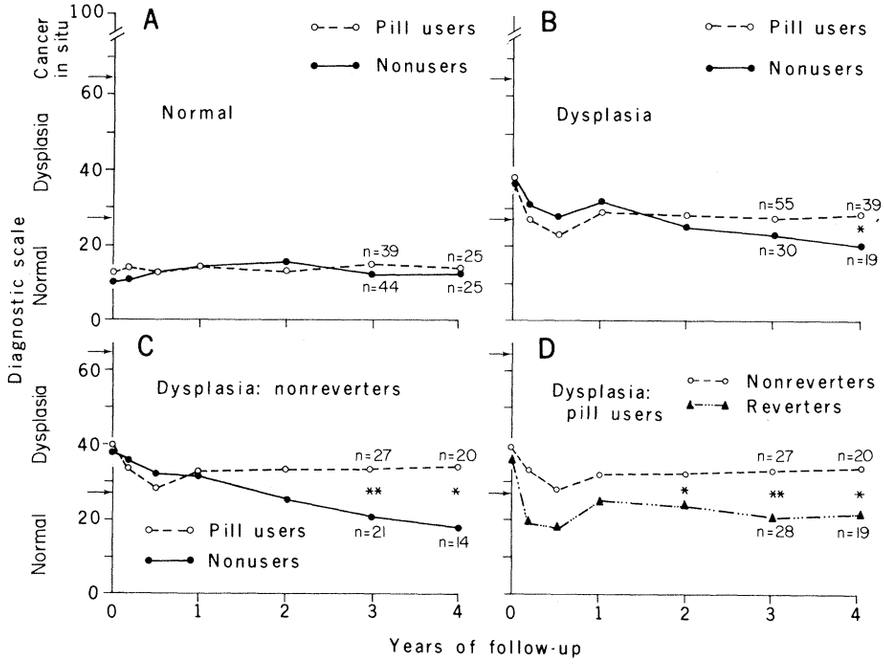

Fig. 1. Mean diagnostic scores over 4 years of follow-up. (A) In the sample of normal women (without dysplasia) there is no evidence of a differential effect of the pill. (B) For women with dysplasia, there is no difference in mean scores between pill users and nonusers at entry into the study. After an initial drop the curves level off, then begin to diverge at 2 years, and the mean score for users is significantly higher than for nonusers at 4 years. (C) Differences in mean scores over time between pill users and nonusers are more striking when early reverters are excluded (early reversal is defined as a drop in diagnostic score to normal levels after 2 and 6 months of study). (D) Among women using the pill, those who do not show an early reversal in score continue to have significantly higher scores after long-term use than those showing an early reversal. In the dysplasia group, standard deviations for the group of pill users tend to increase over time. In the (C) sample, the difference in standard deviations between pill users and nonusers is significant, $P < .01$, after 3 years of study. Sample sizes are for subjects followed a minimum of 3 years and are large enough to provide 90 percent probability of detecting a difference of 10 points in the score, as defined in (*12*) when a 5 percent level of significance is used in the test. Single asterisks denote $P < .05$; double asterisks, $P < .01$ (Student's t-test, unequal variances being assumed).

sistent with the hypothesis of a hormonal factor in the etiology of cervical cancer. This concept is of scientific interest since the uterine cervix is an endocrine target organ—the structure and function of the mucosa is responsive to the internal hormonal environment, and specific estrogen-binding proteins have been found in the human cervix (*15*). Some insight into the biological mechanism by which the contraceptive pill as a synthetic steroidal progestin-estrogen compound could exert an adverse effect on cervical mucosa is provided from experimental work on competition by synthetic hormones for specific protein receptor sites in cervicovaginal epithelium (*16*). Further investigation of the cervix as a target organ may provide a rationale for considering hormone dependency as a factor in the management of advanced cervical cancer.

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   To ensure against bias by failure to include in the set of covariates any factor related to differential dropout between pill users and nonusers, we analyzed the characteristics of subjects continuing in the study and those dropping out. Covariance analysis was applied at each of 12 time intervals to three contrasts: method (pill users versus nonusers), follow-up status (dropping out versus staying in), and method by follow-up interaction. The demographic and biomedical variables examined were adjusted by the above set of identified risk factors. We found no evidence that subjects dropping out of the study introduced bias into the results on the basis of the variables considered. In particular, there was no significant difference in the dysplasia score at each time interval between subjects dropping out and subjects staying in, nor was there a significant interaction between dropout status and contraceptive method. However, we cannot rule out the possible effect of unmeasured or unmeasurable variables.
   Covariate adjustment did not change the results shown in the sample of normal subjects. In dysplasia, differences between pill users and nonusers are increased with covariate adjustment, becoming statistically significant at 2, 3, and 4 years of follow-up. Covariate adjustment did not alter the significant differences observed between pill users and nonusers in nonreverters. The adjusted analyses indicate that it is unlikely that the substantive results of this study are a consequence of the subjects' initial self-selection of the pill or other method of contraception or of differential dropout.
8. Demographic features of the base population included a racial composition of 47 percent black, 38 percent Mexican-American, 13 percent white, and 2 percent others. Ages ranged from 14 to 49; approximately 5 percent were over 35, and the median age was about 21. The number of births ranged from 0 to 12; 13 percent had not yet borne a child and 34 percent had only one birth. The median monthly income was about $400 per household.
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Rapid Response to Selection for a Nondiapausing Gypsy Moth

Abstract. *By genetic selection a gypsy moth strain was obtained within eight generations that could be reared continuously in the insectary without its normal "obligatory" diapause. This strain should be useful for making physiological and genetical comparisons with normal diapausing gypsy moths. It could also be used in a genetic control program. However, caution should be exercised, for its release could create a new environmental hazard.*

Selection and inbreeding over eight generations have produced a new nondiapausing strain of the gypsy moth, *Lymantria dispar* L. This colony might prove useful in the genetic control of gypsy moths, and shows that it is possible to rear healthy gypsy moths throughout the year for laboratory experiments.

Laboratory research with the gypsy moth is hampered by the fact that the eggs require a minimum of 90 days exposure to a low temperature for termination of the "obligatory" egg diapause (*1, 2*). Consequently, eggs collected in the field for laboratory experiments or insectary production will not hatch for several months each year. Furthermore, the contamination of field-collected eggs with nuclear polyhedrosis virus (NPV) is a problem that surface disinfection does not adequately solve. Thus a virus-free nondiapausing strain would be useful for the mass-rearing of gypsy moth parasites or NPV, or for various experiments including future comparative physiological analyses of diapause and diapause genetics.

It is well known that the duration of diapause in the gypsy moth is variable (*3–5*), but attempts to obtain a nondiapausing strain by genetic selection have not been reported.

Selection for the nondiapausing strain reported here began in October 1973, when 230,000 eggs were collected in the field (in northern Connecticut), disinfected, and pooled. Half of the eggs were held under ambient conditions (22°C, natural daylength) and half were chilled (5°C) for 2 weeks. Larvae were reared on a synthetic diet under controlled constant temperature (22°C) and a long day (18 hours), although previous authors (*2, 6*) reported the gypsy moth to be one of the few insect species that is photoperiodically neutral. The rarity of truly neutral species led me to control the photoperiod. During the F_2 through F_4 generations, "token" chills were given half of the eggs for 2 weeks, to increase the level of hatch. Virus incidence was so low that disinfection of the eggs was not required after the F_1 generation because care was taken to prevent contamination.

The response to selection for rapid