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Meeting Report

Epidemiology of Ovarian Cancer

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A workshop on the epidemiology of ovarian cancer was held at the National Institutes of Health, Bethesda, Maryland, on September 30, 1991. The goals of the workshop were to address: (*a*) reproductive factors; (*b*) exogenous hormone use; (*c*) diet, life style, and health practices; (*d*) tumors of low malignant potential and nonepithelial germ cell ovarian tumors; (*e*) prospective studies; (*f*) determinants of genetic susceptibility; and (*g*) directions for future research in ovarian cancer.

Session 1: Reproductive Factors

Alice Whittemore (Stanford University School of Medicine, Stanford, CA) reported preliminary results of the analysis of combined data from 12 U.S. case-control studies on ovarian cancer (1). The objective was to assess the influence on ovarian cancer risk of events affecting ovulation, such as timing of menarche and menopause, timing and outcome of pregnancies, duration of breast-feeding, and duration of OC[2] use. These data were examined in light of two currently prevailing hypotheses about ovarian cancer risk. The "ovulation" hypothesis was proposed by Fathalla in 1971 (2). It states that repeated minor trauma to the epithelial surface of the ovary, caused by incessant ovulation, is a major risk factor for ovarian cancer. The "gonadotropin" hypothesis (3), proposed by Stadel a few years later, suggests that exposure of the ovarian epithelium to high levels of circulating pituitary gonadotropins enhances ovarian cancer risk.

The combined data showed strong trends of decreasing ovarian cancer risk with increasing parity, duration of breast-feeding, and duration of OC use. Failed pregnancies (i.e., spontaneous abortion, ectopic pregnancy, and miscarriage) were also protective, but less so than full-term pregnancies. There was a weak trend of cancer risk with age at menarche, no clear trend with age at menopause, and a greater risk reduction associated with each incremental month of pregnancy than with each month of OC use. The trends of decreasing ovarian cancer risk with increasing parity and duration of OC use are consistent with both the ovulation and gonadotropin hypotheses.

A month of breast feeding 6 or more months after delivery was found to be less protective than a month within the first 6 months of delivery. This supports the ovulation hypothesis because the longer a women breast-feeds, the less effective lactation is at suppressing ovulation. The risk reduction associated with breast-feeding conflicts with the gonadotropin hypothesis; in lactating women, follicle stimulating hormone levels remain elevated until the return of ovarian estrogenic function. The gonadotropin hypothesis predicts that breast feeding would increase the risk of ovarian cancer.

The relationship between infertility and increased ovarian cancer risk was also evaluated. Among nulliparous women, ovarian cancer risk did not vary by marital status or gravidity. There was a weak association with duration of longest attempt at pregnancy, total duration of unprotected intercourse before pregnancy, or history of clinically diagnosed infertility not attributed to fertility problems of the male. The risk was elevated among women whose infertility was attributed to inadequate ovulation and among women who had used fertility drugs.

Epithelial tumors of low malignant potential, frequently called borderline tumors, have various features of malignant epithelial ovarian cancers, but they do not invade the ovarian stroma. Women with these tumors are usually younger when diagnosed and have a better prognosis than women with malignant tumors. Oral contraceptives are generally less protective against borderline tumors than against malignant invasive tumors.

David Rose (American Health Foundation, Valhalla, NY) indicated that two widely discussed hypotheses of ovarian cancer causation (2, 4) may relate to dietary effects on endogenous hormones. Both hypotheses are compatible with the known international and regional associations between ovarian cancer risk and dietary fat consumption. Although such associations do not imply causality, the increase in incidence of ovarian, breast, and prostate cancers as fat consumption has increased in Japan is particularly striking.

The plasma estradiol and estrone levels of both premenopausal and postmenopausal women were found to decrease when they were switched from a typical high-fat (35–40% total calories from fat) diet to one providing only 20% or less of calories from fat. Switching from a low fiber to a high fiber diet had a similar effect. A low fat diet also caused some reduction in midluteal phase plasma LH levels.

Rose stated that there are several studies of which epidemiologists should be particularly aware when thinking about ovarian cancer. For example, a study published by Lloyd *et al.* (4) merits confirmation and elaboration. This was a case control study to examine the effect of nutritional factors on menstrual function and bone density in collegiate women. Three groups, matched with

Received 9/23/92

[1] To whom requests for reprints should be addressed.

[2] The abbreviations used are: OC, oral contraceptive; LH, luteinizing hormone; PG, prostaglandin; RR, relative risk.

respect to age, height, and weight were studied: sedentary women with regular menses, athletic women with regular cycles, and a small group of athletic women who had become oligomenorrheic. Average age at menarche was greater in normal (13.1 years) and oligomenorrheic (14.3 years) athletes compared with the sedentary women (12.2 years). Average bone density was lower in the oligomenorrheic athletes compared with normal athletes and sedentary women. Dietary fiber intake was significantly higher in the oligomenorrheic women compared with the other two groups of women. It was concluded that increased dietary fiber intake was associated with menstrual dysfunction in collegiate athletes. Plasma hormones were not measured in this study. In another study (5), menstrual irregularities were reported among vegetarian women compared to omnivorous women whose diets were somewhat higher in fat but much lower in fiber. The significance of these findings in the context of ovarian cancer risk needs further consideration.

Pirke *et al.* (6) studied groups of premenopausal women who were placed on weight-reducing diets, one group receiving a vegetarian diet and the other a mixed diet. Weight losses were similar in both groups, but after 6 weeks the women eating vegetarian diets exhibited a shortening of the menstrual cycle and some became anovulatory. These changes were associated with a significant reduction in the height of the ovulatory plasma LH peak and in the luteal phase LH concentrations. Midluteal plasma estradiol levels were also reduced in women on vegetarian diets.

Hill *et al.* (7) carried out a dietary intervention study on 4 volunteer nurses. Two women who were switched from a mixed diet to a vegetarian diet reported shortening of the menstrual cycle by 1–2 days, and their serum LH peak occurred earlier in the menstrual cycle and was of reduced amplitude.

From these data, it seems that fat and fiber influence the menstrual cycle, the hypothalamopituitary regulation of ovulation, and the levels of circulating estrogens. Moreover, it appears that a low fat/high fiber diet may result in fewer ovulatory cycles, which could favorably influence ovarian cancer risk.

PGs of the E and F series accumulate rapidly in the preovulatory follicular fluid and reach a peak at ovulation, the stimulus being provided by gonadotropins. Both LH and estradiol accelerate PG production, with PGE_2 contributing to vasodilation and progesterone production, and $PGF_{2\alpha}$ stimulating contraction of the myoid cells involved in the extrusion of the oocyte follicle. Inhibition of PG synthesis prevents rupture of the follicles, and suppression of ovulation can be induced in women by treatment with a PG synthesis inhibitor, indomethacin. It is not yet known whether long term treatment with drugs such as indomethacin reduces ovarian cancer risk.

Session 2: Exogenous Hormones

Malcolm Pike (University of Southern California, Los Angeles, CA) began his presentation by pointing out that the age-specific incidence of most non-hormone-dependent epithelial cancers shows a linear increase (on a log-log plot). However, there is a distinct reduction in the rate of increase around age 50 (8). This protective effect of menopause is the most fundamental epidemiological fact about ovarian cancer. Since gonadotropin levels are high in postmenopausal women, it seems that high levels of gonadotropins per se may not be important in the etiology of later onset ovarian cancer. High levels of gonadotropins may be important in premenopausal women, however, when they have a "substrate" to work on, namely oocytes.

Pregnancies have a greater protective effect (expressed as reduction in risk per month) than breastfeeding or OC use, but the latter two variables are subject to much greater measurement error. Three aspects of the protective effects of OC use need further research. First, how does this effect change with time and with increasing age since stopping use? The Cancer and Steroid Hormone Study data, for example, stop at age 54. Second, Whittemore's metaanalysis suggests that the use of OCs for longer than 6 years confers no additional protection. This is contrary to other data (9, 10), and further information on long-term use is needed either to refute this finding or to try to explain it in terms of, for example, the time to return of ovulation after cessation of OC use. Third, do modern low-dose pills have the same protective effect on ovarian cancer risk as older higher-dose pills? The answers to these questions are very important, since they have a profound effect on the risk-benefit equation for OC use.

The apparent lack of effect of age at menopause on ovarian cancer risk described by Whittemore and colleagues is likely to shed light on the etiology of ovarian cancer. Women who have had their last menstrual period after age 53 have undoubtedly ovulated more times than women whose last menstrual period was before age 45. This observation may be evidence against the "incessant ovulation" hypothesis.

Pregnancy, variables related to menstruation, and OC use cannot explain the international differences in ovarian cancer rates. It is conceivable that diet may explain part of the geographic variation in ovarian cancer rates.

Noel Weiss (University of Washington, Seattle, WA) reported that although estrogens used for hormone replacement therapy may reduce gonadotropins to a level between pre- and postmenopausal values, and hence perhaps reduce risk of ovarian cancer, no evidence is available to support a decreased risk of ovarian cancer associated with the use of postmenopausal hormones. Whittemore and colleagues have evaluated the relationship of menopausal estrogens to the risk of epithelial ovarian cancer using data from five hospital-based and five population-based case-control studies.[1] No clear trends in risk were observed with increasing duration of estrogen use or with increasing time since last estrogen use after adjustment for duration of use. Overall, menopausal estrogen use was unrelated to risk for epithelial ovarian cancers of the endometrioid cell type. Weiss observed a slightly increased risk in his study of menopausal estrogen use and ovarian cancer risk (11).

Carlo La Vecchia (Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy) reported results of the metaanalysis of three hospital-based case-control studies

[1] A. S. Whittemore *et al.* Characteristics relating to ovarian cancer risk: Malignant epithelial cancers versus menopausal estrogen use; submitted for publication.

of ovarian cancer conducted in Greece, Italy, and the United Kingdom (12–14). In assessment of the roles of parity and age at first birth in parous women, both factors had a weak, nonsignificant influence on cancer risk. When parity and age at first birth were considered separately, there was an inverse trend in risk with increasing number of births. However, a significant trend emerged only from the British study and was largely restricted to women who reported four or more births. Compared to women who bore their first child at age 24 or less, the increase in risk for those who bore their first child at age 35 or more (RR 1.4) was of borderline statistical significance. The pooled analysis also indicated that abortions confer limited protection of 10–40% against ovarian cancer in women who reported two or more spontaneous or induced abortions.

Unlike the Whittemore analysis, there was no evidence of an association between ovarian cancer and age at menarche, and there was a consistent trend of increased risk with late age at menopause. The strength of the association was relatively weak, with a relative risk of less than 2 with menopause after age 52 compared with earlier menopause. The effect of age at menopause seemed to be long-lasting and to increase with age at diagnosis of ovarian cancer.

Compared with never users, the RR for OC users was 0.6. The RR estimates for use of OCs were even lower in women reporting their first use before age 25. The risk of disease decreased with the duration of use, being 0.7 in women reporting OC use for less than 2 years and 0.4 for OC use for 5 years or more. The protection persisted even after discontinuing OC use, the RR being 0.5 in women reporting their last OC use 15 years or more before diagnosis of ovarian cancer. The protective effect of OC use on ovarian cancer emerged consistently in all age and parity strata.

Session 3: Diet, Life Style, and Health Practices

James Marshall (State University of New York, Buffalo, NY) reported on preliminary findings from an ongoing case-control study of diet and ovarian cancer. There was a slight excess of caloric intake among cases relative to controls. Crude dietary fiber appeared to exert some protective effect. Total vitamin A intake had a marginally significant protective effect, which seems to be due to β-carotene, not retinol. There was no relationship between obesity or alcohol intake and ovarian cancer.

Daniel Cramer (Brigham and Women's Hospital, Boston, MA) pointed out that the "incessant ovulation" hypothesis for the etiology of ovarian cancer does not have supporting animal models and does not fully explain risk factors such as early menopause, radiation, infertility, and heredity, or the protective role of pregnancy and oral contraceptive use. In 1948, Gardner (15) proposed that ovarian cancer was caused by hypergonadotropic hypogonadism, i.e., high secretion of gonadotropins due to ovarian failure or lack of feedback control on the pituitary. The theory was based on animal experiments, which demonstrated that if you caused oocyte death by gonadal irradiation or use of oocyte toxins such as polycyclic hydrocarbons, thereby raising gonadotropin levels, ovarian cancer resulted. Congenital deficiency of oocytes (germ cells) also increased ovarian cancer risk. That gonadotropin stimulation was a necessary component was inferred from the ability of pituitary ablation or deficiency of gonadotropin releasing hormone to block tumor development. Relevance of animal models to human disease models has been questioned, since animal tumors are primarily stromal, whereas human tumors are primarily epithelial. Cramer and Welch (16), however, proposed that a stimulus that causes stromal tumors in rodents may also promote different types of ovarian tumors in women. According to their theory, the first stage of tumorigenesis involves formation of inclusion cysts (islands of ovarian surface mesothelium) by entrapment of ovarian surface epithelium into the ovarian stroma. In the second stage, differentiation, proliferation, and eventual malignant transformation of the epithelium lining the inclusion cysts occur due either to direct stimulation by gonadotropins or to indirect stimulation by steroids induced by gonadotropins. In addition, Cramer *et al.* (17) speculate that talc may ascend the genital tract and become incorporated into the inclusion cysts, contributing to the risk of ovarian cancer, which is consistent with some epidemiological data.

Experimental and clinical studies have reported an association of galactose consumption and metabolism with hypergonadotropic hypogonadism. In a case-control study of ovarian cancer, Cramer *et al.* (18) found that women with ovarian cancer consumed dairy products with a higher content of prehydrolyzed lactose (yogurt and cottage cheese) and had lower concentrations of galactose-1-phosphate uridyltransferase, an enzyme that converts galactose to glucose, than did control women. Risk for ovarian cancer was related to the ratio of lactose consumption to transferase activity. Cases had a mean lactose consumption:transferase activity ratio of 1.17 compared with 0.98 for controls. There was a highly significant trend for increasing ovarian cancer risk with increasing lactose consumption:transferase activity ratio.

Biological specimens are needed to further investigate the possible interactions of various exposures with biochemical or molecular genetic factors, such as transferase activity. Cramer recommended expansion of familial ovarian cancer clinics, which will permit the identification and formation of pedigrees for linkage analysis, identification of phenotypic markers for ovarian cancer, collection of biological specimens, and identification of markers that precede tumor development. Such clinics encourage development of clinical strategies for primary and secondary disease prevention.

Mitsuru Mori (Kurume University, Kurume City, Japan) reported the results of two meta-analyses of published case-control studies on ovarian cancer. Tubal sterilization was found to be significantly associated with reduced risk of ovarian cancer when nulliparous women were excluded from the analysis. Since subfertility is related to both an increased risk of ovarian cancer and a decreased frequency of tubal sterilization the observed relationship could be indirect. Among Asian women, induced abortion had a slight protective effect.

It has been hypothesized that a potential carcinogenic agent enters the peritoneal cavity through the fallopian tube, irritates the pelvic peritoneum, produces proliferation, and, with additional factors, results in the development of cancer. If this hypothesis is correct, ligation of the fallopian tube may protect against ovarian cancer by preventing the cancer inducing or promoting agents from entering the peritoneal cavity.

Session 4: Borderline and Nonepithelial Tumors

Lawrence McGowan (George Washington University, Washington, DC) emphasized the need for obtaining a more detailed clinical history, greater involvement of a gynecologic pathologist, and closer interaction of a pathologist with an operating surgeon in studies of ovarian cancer. He stated that primary peritoneal carcinoma exhibits many symptoms similar to those of primary epithelial ovarian cancer and that therefore there is a possibility of misdiagnosis.

Currently, gynecological pathologists prefer the term "low malignant potential tumor" over "borderline tumor." Women with low malignant potential tumors are usually younger when diagnosed and have a better prognosis than women with malignant tumors. Analysis of combined data from nine case-control studies of ovarian cancer in the United States revealed that the risk profile for tumors of low malignant potential was similar to that for malignant ovarian tumors with two exceptions.[*] The risk for low malignant potential tumors was less clearly reduced among women who had used OCs. There was clearly an elevated risk of low malignant potential tumors among women with a history of infertility. This was the greatest difference in risk factors between low malignant potential tumors and invasive carcinoma.

Carolyn Westhoff (Columbia University, New York, NY) stated that ovarian tumors of low malignant potential are almost entirely serous and mucinous epithelial tumors. When diagnosed, they tend to be localized to the ovary, which accounts for their good prognosis. There are very few data regarding their incidence. Frequent misclassification of these tumors reduces the value of data from clinical series.

Benign ovarian neoplasms can be of epithelial, stromal, or germ cell origin. Their occurrence is not recorded by tumor registries, and misclassification of the histological diagnosis is relatively common. The tumors of germ cell origin, the teratomas, are most common and have a unimodal age distribution with a peak near age 30, the shape of this age incidence curve resembles that of testicular tumors. The benign epithelial tumors occur somewhat less frequently than teratomas, are more subject to diagnostic misclassification, and occur about equally among women from their teens through the 70s. No study has found any evidence that OCs protect against either the teratomas or the benign epithelial tumors; nulliparity and infertility may increase the risk of these tumors. The stromal tumors are rare and occur in postmenopausal women. There have not been any reported epidemiological studies of the stromal tumors.

Session 5: Prospective Studies

Graham Colditz (Channing Laboratory, Boston, MA) reported preliminary findings from an ongoing prospective study of over 121,000 registered U.S. nurses who were aged 30 to 55 when recruited in 1976. The original aim of the study was to look at exogenous and endogenous hormones and the risk of breast, uterine, and ovarian cancers. The questionnaire obtained details on the following reproductive factors: age at menarche; age at first birth; parity; weight and height; menopause, including type of menopause; if postmenopausal, use of replacement hormones; OC use, including details of interval of use, but not details of actual preparation used. Data were collected on different types of contraception, including tubal ligation and vasectomy. The cohort was followed with a biennial questionnaire, which allowed the women to update their exposure information.

Two hundred forty-seven ovarian cancers were reported in the nurses' cohort by the end of 1988. There was an inverse association, adjusted for parity, with the use of OCs. Women with five or more years of OC use had a relative risk of 0.6 for ovarian cancer. There was a decreasing risk with increasing parity. There was no clear relationship of risk with age at first birth, even after adjustment for parity. Long duration of postmenopausal hormone use appeared to increase risk slightly. The relative risk associated with tubal ligation, adjusted for age and parity, was 0.37.

A food frequency questionnaire was administered to the cohort in 1980. There was no suggestion of increasing risk with increasing saturated fat intake among the cases diagnosed during 8 years of follow-up. For lactose intake, only the top quintile had a slight excess of cases. To date, there is no relationship with alcohol intake, cigarette smoking, or various other nutrients examined.

Session 6: Family History and Genetic Events

Bruce Ponder (University of Cambridge, Cambridge, England) reviewed preliminary data from a large population-based study carried out using public health records in the United Kingdom. Information on cancer mortality by site in first-degree relatives (2106 parents and 1949 siblings) of 1183 index cases diagnosed with ovarian cancer before age 60 (who were under age 17 in 1939) was obtained. There was a substantially increased relative risk of death from ovarian cancer in first-degree relatives of cases. The risk appeared to be greatest when the index case was diagnosed before age 50. Despite the substantial relative risks, the absolute risks are still small, with a 1 in 40 chance of death from ovarian cancer by age 70 in a sister or mother of an index case diagnosed below age 50. However, there was heterogeneity in the data; women with two affected relatives were at very substantial risk. In these families, segregation analysis was consistent with the inheritance of a single autosomal dominant gene with high penetrance. This hypothesis can only be proved by demonstration of linkage between ovarian cancer and a known genetic marker, which appears to be a marker on the long arm of chromosome 17.

Ponder suggested the establishment of registries of women with ovarian cancers at different ages, sibling pairs, and pairs of closely related women with ovarian and breast cancer, so that studies can be initiated to determine the risk attributable to specific mutations. These families will be useful for pathology studies, since so little is known about the early stages of ovarian cancer development. There is a need for studies to assess the protective effect of prophylactic oophorectomy for women at high risk for ovarian cancer. Ponder concluded his remarks by briefly describing a U.K. National Study recently initiated to identify all families with two or more ovarian cancers.

[*] R. Harris et al. Characteristics relating to ovarian cancer risk: Epithelial cancers of low malignant potential. Submitted for publication.

Henry Lynch (Creighton University, Omaha, NE) emphasized the need for the establishment of familial cancer registries and the collection of information on cancer of all anatomic sites among family members. He also recommended the establishment of specimen banks to store rapidly frozen tumor, normal ovarian tissue, and DNA for distribution to interested investigators.

Session 7: Research Directions

David Schottenfeld (University of Michigan, Ann Arbor, MI) summarized three key areas for future research on ovarian cancer:

1. Family registry resources should be further developed to address linkage markers of susceptibility, assess the interactions of inherited risk with environmental exposures, and assess the value of possibly protective interventions such as the use of combination OCs or other chemopreventive agents.
2. Epidemiological studies should further address the relationship of variations in reproductive patterns to ovarian cancer, the potential risks associated with endogenous gonadotropin levels, and the use of exogenous hormones, such as estrogen hormone replacement therapy and fertility promoting agents.
3. Studies of ovarian cancer should use precise pathologic classification.

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Macmillan Press Ltd., 1989

Dietary factors and epithelial ovarian cancer

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Summary Dietary data from a population based case-control study of 172 epithelial ovarian cancer cases and 1[illegible] controls were analysed. A significant ($P<0.01$) dose response relationship was found between intake of fat from animal sources and risk of ovarian cancer, but plant fat was not associated. Although the effect of animal fat was confounded by education, an adjusted odds ratio of 1.8 persisted for those in the upper quartile compared to the lower quartile of consumption (P for trend = 0.03). After adjustment for animal fat intake calories and protein intake had minimal effects on risk. Total vegetables were found to be somewhat protective, but the mechanism of action was unclear. Weight, height and relative weight (weight/height^2) were not related to risk of ovarian cancer.

Substantial evidence indicates that diet is a major factor in the cause of some of the most important and prevalent human cancers, especially cancers of the digestive tract and hormone dependent cancers (Williams & Wynder, 1986). An involvement of dietary fat in the aetiology of ovarian cancer has been suggested by some epidemiological studies (Armstrong & Doll, 1975; Cramer *et al.*, 1984; Rose *et al.*, 1986; La Vecchia *et al.*, 1987). Experimental studies have shown that dietary fat is related to endogenous hormone levels, providing a plausible mechanism for the association (Wilken & MacMahon, 1984*b*). A population-based case-control study in Shanghai which obtained extensive dietary intake data offered the opportunity to study the effects of dietary fat, calories and other nutrients on the risk of ovarian cancer.

Materials and methods

The Shanghai population-based cancer registry enabled rapid identification of ovarian cancer patients for this case-control study. Identified for study were all women aged 18–70 years with ovarian cancer newly diagnosed in the Shanghai urban area between 1 September 1984 and 30 June 1986. Women with borderline (uncertain malignancy) tumours and non-permanent residents were excluded from study. Of 288 eligible cases, 229 (88.9%) remained after 21 (8.1%) deceased and eight (3.1%) untraceable cases were excluded. Clinical and histopathological data at diagnosis, along with information on treatment and survival were abstracted from hospital records. Nearly all (93%) of the cases were histologically confirmed, with the remainder being diagnosed through either ultrasound (3.1%) or clinical examination (2.6%). A total of 172 women (75%) who were diagnosed with epithelial tumours are the focus of this investigation.

One control was selected from the Shanghai general population by a stratified random procedure to match each case. For each control one household committee (a residential unit of approximately 4,000 individuals) was randomly selected from the 1,487 household committees in the Shanghai urban area from which one household group (consisting of approximately 18–20 households) was selected. Subsequently, two women within five years of age of the index case were randomly selected from all eligible women. One served as the initial control and the other as an alternate. Women with a history of a bilateral oophorectomy were replaced with alternates. All selected control women agreed to participate. Information was collected through face-to-face interviews by trained interviewers. The standard questionnaire covered demographic characteristics, reproductive history, medical history, familial cancer history, personal habits, occupation and diet.

Information on usual adult consumption of 63 common foods in Shanghai was obtained. Study subjects were first asked about how often they ate each food (daily, weekly, monthly, yearly, seldom or never), followed by questions to derive the grams of food eaten per unit time. The women, who generally were responsible for buying and preparing the meals for their households, adjusted the quantities purchased by the fraction they consumed. The food composition table published by the Chinese Academy of Medical Sciences (1981) was employed to convert these foods into nutrients. The majority of nutrient values were based on data derived from the Shanghai area; when this information was missing, values from Jiangsu province, and occasionally from Beijing, were utilised. Data were not available on saturated and unsaturated fat in Chinese foods so it was not possible to examine these two variables. Multiplying the reported daily consumption (in grams) of each individual food by the nutrient content per gram in that food, and then summing over all foods, generated for each individual the total daily ingestion of each nutrient. In addition, food groups were formed based on dietary or botanical similarities. For example, meats included pork, pork chops, spareribs, pigs' feet, salted pork, pork liver, organ meats, beef, lamb, chicken and duck; the cruciferous vegetables included greens, cabbage, Chinese cabbage and cauliflower; and allium consisted of foods in the onion family (see Table VI for further details).

The odds ratio (OR, estimated relative risk) was employed in measuring the association between diet and ovarian cancer. Based upon the distribution among the controls, quartile cuts were used to create categorical variables, and the lowest 25% was chosen as the referent group. To assess and control for sources of confounding, analyses utilised conditional logistic regression techniques (Lubin, 1981). Trend tests were performed by treating categorical variables as continuous in the models.

Results

Cases and controls were well matched on age, with the mean age being 48.9 years for cases and 48.8 years for controls. Cases tended to be better educated and have higher average household incomes than controls (Table I); however, the effects of income at three separate time periods (present and approximately 10 and 20 years before diagnosis) could be explained by the effects of education. Cases tended more often than controls to be nulliparous and to be smokers. The mean number of live births was 2.0 for cases and 2.9 for controls. Cases more frequently reported histories of ovarian

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Received 15 June 1988; and in revised form 17 August 1988.

Table I Distribution of selected demographic characteristics and risk factors

	Cases	Controls
Age (%)		
≤29	10.5	11.0
30–39	17.4	15.1
40–49	17.4	17.4
50–59	29.7	32.6
>60	25.0	23.8
Education (%)		
College	14.5	2.9[b]
Senior high school	20.4	14.5
Junior high school	29.1	33.1
Primary school	18.6	23.3
No formal education	17.4	26.2
Income/month/capita in 1982 (mean Yuan)	46.5	40.2[b]
Nulliparity (%)	20.9	12.2[a]
Number of live births (mean)	2.0	2.9[b]
Ovarian cysts (%)	9.9	1.2[b]
Smoker (%)	11.0	7.6
Tubosterilisation (%)	12.2	23.3[b]
Oral contraceptive usage (%)	13.4	7.0[a]
IUD usage (%)	7.0	15.7[b]
Medroxyprogesterone usage (%)	14.0	3.5[b]

Comparisons of cases to controls by t test: [a]$P<0.05$; [b]$P<0.01$

cysts and usage of medroxyprogesterone or oral contraceptives (although the excess was exclusively for short-term use of the pill), while controls had higher rates of tubosterilisation and use of intrauterine devices (IUDs).

The relationship between various nutrients and risk of ovarian cancer is presented in Table II. High protein and fat intake were significantly related to an increased risk of ovarian cancer. Compared to the lowest quartile, the highest quartiles were associated with ORs of 1.7 (95% CI = 0.9–3.4) and 2.3 (95% CI = 1.2–4.4) for protein and fat, respectively. There was a remarkable difference between the effects of fat from plant and animal sources, with the ORs of the highest quartile being 0.8 (95% CI = 0.4–1.4) for plant fat and 2.2 (95% CI = 1.2–4.2) for animal fat. ORs increased with high animal protein, with plant protein having no apparent effect. Although those in the third quartile of calorific intake were at increased risk, the trend in risk across all quartiles was not statistically significant. However, there was a significant trend ($P<0.01$) in risk when calories from animal food alone were considered. High riboflavin consumption was also related to a slightly increased risk, but neither the point estimates nor the trend test was statistically significant. Carbohydrate intake, on the other hand, was associated with a non-significant reduction in risk.

The potential confounding effects of other risk factors on nutrient intake were considered, including education, income, number of live births, ovarian cysts, smoking, oral contraceptive and medroxyprogesterone use, tubosterilisation and IUD usage. It was found that education substantially altered a number of effects. After adjustment for education, trends in risk across quartiles of consumption remained only for total fat ($P=0.03$), animal fat ($P=0.07$) and animal calories ($P=0.06$). Thus, those in the highest quartiles of intake showed ORs of 1.7 (95% CI = 1.0–3.2) and 1.5 (95% CI = 0.9–2.9) compared to those in the lowest quartiles of animal fat and animal calories intake, respectively. Further adjustment for income or other risk factors did not affect either these point estimates or the trends in risk with these nutrients. The trend with total protein became non-significant ($P=0.12$) after adjustment for education. In addition, the association with carbohydrate intake disappeared after adjustment.

Effects of foods grouped by dietary and botanical similarity are shown in Table III. High intakes of meat, red meat, and dairy products and eggs were associated with elevated risks. In contrast, intake of vegetables and legumes appeared to reduce risk, although neither trend was statistically significant. Selected subgroups of vegetables, e.g. yellow-orange, dark-green and cruciferous vegetables, were not associated with reduced risk. Again, education exerted major confounding influences, with associations for meat, red meat and dairy product intake no longer remaining significant after appropriate adjustment. However, the decreasing risks with vegetable and legume intake persisted after adjustment for education and animal fat, although the trend tests were not significant.

Since animal fat and total calories were correlated, and both appeared to increase risk, attempts were made to identify the major determinant. By cross-tabulating these two variables, it appeared that only fat intake exerted an effect on risk. The ORs of animal fat adjusted for calories and education were 1.4, 1.9 and 1.7 for the second, third and fourth quartiles, while the corresponding ORs of calories adjusted for animal fat and education were 1.0, 1.2 and 0.8. Attempts to disentangle the effects of animal fat and animal calories were unsuccessful because of the high correlation of these measures (Spearman's correlation coefficient, $r=0.97$).

Similar analyses to disentangle the effects of total protein and animal fat also revealed that animal fat intake was more important than protein intake. After adjustment for animal

Table II Risk of ovarian cancer by selected nutrients

	Crude OR					*OR adjusted for education*				
	Q_1	Q_2	Q_3	Q_4	*Test for trend P value*	Q_1	Q_2	Q_3	Q_4	*Test for trend P value*
Protein	1.0	1.1	1.8	1.7[a]	0.03	1.0	1.0	1.8	1.4	0.12
Plant	1.0	0.8	0.8	0.9	0.80	1.0	0.9	1.1	1.2	0.48
Animal	1.0	0.9	1.7	1.6	0.03	1.0	0.8	1.5	1.2	0.36
Fat	1.0	0.9	2.0[a]	2.3[a]	<0.01	1.0	1.1	1.8	1.9	0.03
Plant	1.0	0.9	1.0	0.8	0.51	1.0	0.9	1.0	0.8	0.58
Animal	1.0	1.4	2.1[a]	2.2[a]	<0.01	1.0	1.4	1.9	1.7	0.07
Calories	1.0	1.0	1.6	1.3	0.29	1.0	1.2	1.6	1.2	0.40
Plant	1.0	0.8	0.8	0.7	0.22	1.0	1.0	1.2	0.9	0.99
Animal	1.0	1.0	2.1[a]	2.2[a]	<0.01	1.0	0.9	1.9[a]	1.5	0.06
Carbohydrate	1.0	0.5[a]	0.8	0.5[a]	0.09	1.0	0.6	1.0	0.6	0.39
Crude fibre	1.0	1.5	1.2	1.1	0.78	1.0	1.4	1.4	1.1	0.91
Vitamin A	1.0	1.1	0.8	1.0	0.66	1.0	1.0	0.8	0.9	0.71
Carotene	1.0	1.1	0.9	1.0	0.70	1.0	1.3	1.0	1.1	0.97
Ascorbic acid (C)	1.0	0.9	0.7	0.9	0.59	1.0	1.0	0.7	0.9	0.58
Thiamin (B_1)	1.0	0.7	0.9	1.1	0.59	1.0	0.8	1.2	1.3	0.30
Riboflavin (B_2)	1.0	0.9	1.4	1.4	0.19	1.0	1.0	1.5	1.0	0.64
Retinol	1.0	1.2	1.2	1.4	0.36	1.0	1.2	1.0	1.0	0.77

Q_1: lowest 25%; Q_2: lower middle 25%; Q_3: higher middle 25%; Q_4: highest 25%; [a]$P<0.05$; [b]$P<0.01$

Table III Risk of ovarian cancer by selected food groups

	Crude OR					*OR adjusted for education*				
	Q_1	Q_2	Q_3	Q_4	*Test for trend P value*	Q_1	Q_2	Q_3	Q_4	*Test for trend P value*
Meat	1.0	0.8	1.3	1.6	0.05	1.0	0.6	1.1	1.2	0.30
Red meat	1.0	0.9	1.2	1.8	0.03	1.0	0.8	1.0	1.4	0.19
Poultry	1.0	1.5	0.8	1.5	0.62	1.0	0.9	0.8	1.1	0.78
Fish	1.0	0.7	0.7	1.2	0.84	1.0	0.7	0.7	0.9	0.70
Dairy and eggs	1.0	1.0	1.1	1.4	0.13	1.0	1.0	1.1	0.4	0.98
Eggs	1.0	1.0	1.3	1.3	0.29	1.0	1.0	0.9	1.1	0.62
Vegetables	1.0	0.7	0.5	0.8	0.45	1.0	0.7	0.5	0.8	0.45
Dark green vegetables	1.0	1.1	1.0	1.1	0.76	1.0	1.2	1.1	1.3	0.53
Yellow orange vegetables	1.0	1.2	1.1		0.55	1.0	1.1	1.0		0.92
Cruciferous vegetables	1.0	0.8	1.0	1.0	0.83	1.0	1.0	1.1	1.2	0.55
Allium	1.0	1.0	1.6	1.1	0.83	1.0	1.1	0.6	1.4	0.54
Legumes	1.0	0.9	0.5*	0.8	0.19	1.0	0.8	0.4*	0.8	0.22
Fruits	1.0	0.6	0.9	1.3	0.23	1.0	0.4	0.6	0.9	0.68
Complex carbohydrates	1.0	0.5*	0.8	0.6	0.16	1.0	0.6	1.0	0.7	0.61
Vegetable oils	1.0	1.1	0.0	0.9	0.52	1.0	1.1	0.0	0.9	0.58

Q_1 lowest 25%; Q_2 lower middle 25%; Q_3 higher middle 25%; Q_4 highest 25%; *$P < 0.05$

Table IV Risk of ovarian cancer by relative weight

	Cases	*Controls*	*Crude OR*	*Adjusted OR*	*95% CI (for adjusted OR)*
Relative weight (weight/height^2)					
15.86 (*Q*$_1$)	35	43	1.0	1.0	
15.87–20.82 (*Q*$_2$)	56	43	1.6	2.1	1.0–4.2
20.83–22.31 (*Q*$_3$)	35	42	1.0	1.2	0.6–2.6
22.32– (*Q*$_4$)	46	44	1.3	1.6	0.8–3.3

ORs is adjusted for education and animal fat intake.
Q_1 lowest 25%; Q_2 lower middle 25%; Q_3 higher middle 25%; Q_4 highest 25%

fat and education), the ORs associated with protein intake were reduced to 0.9, 1.4 and 1.0 for the second, third and fourth quartiles of consumption. The ORs for progressive quartiles of animal fat intake after adjustment for protein intake and education were 1.3, 1.7 and 1.4.

Table IV presents the association between relative weight (weight/height^2) and ovarian cancer. Neither the ORs for those with increased body mass nor the trend tests were significant. Adjustment for education and animal fat intake did not substantially alter the relationship of risk to relative weight. Other anthropometric measures, such as height, average adult weight, maximum adult weight or weight/height^2 were unrelated to risk.

Only one case and no controls reported regular alcohol consumption, limiting the ability to further assess this exposure as an aetiological factor.

Discussion

International comparisons indicate that ovarian cancer incidence rates correlate with per capita fat availability (Armstrong & Doll, 1975; Rose *et al.*, 1986). The increased incidence of ovarian cancer among Japanese migrants to the USA has been viewed as further support for an aetiological role of dietary fat (Haenszel & Kurihara, 1968). However, such descriptive studies, concerned with total population characteristics rather than those of individuals, do not account for potential confounders such as parity and socio-economic status.

Follow-up studies provide some support for an association of ovarian cancer risk with fat intake, although the results are not consistent. A follow-up study of Seventh Day Adventists, many of whom are ovo-lacto vegetarians, showed a standardised mortality ratio for ovarian cancer of about 0.6 compared to the general California population (Phillips *et al.*, 1980). Reporting the preliminary results for a 20-year follow-up study among 16,190 white Seventh Day Adventists, Snowdon (1985) found that women who consumed high amounts of eggs or fried food were at a three-fold excess risk. It was suggested that the use of fat in the process of frying, especially animal fat, was more important than the eggs (Rose & Boyar, 1985). However, Mormons in Utah showed a standardised incidence ratio of 1.7 for ovarian cancer compared to the US population, although their diet is not unusually low in animal fat (Lyon *et al.*, 1980). Kinlen (1982) also found no reduction in risk among nuns who either completely or partially abstained from meat as compared to other nulliparous women.

Case-control studies are somewhat more provocative. In one case-control study in the United States, cases were found to consume more whole milk, butter, animal fat and saturated fat and less skimmed milk, margarine, vegetable fat and unsaturated fat (Cramer *et al.*, 1984). Supporting these findings, La Vecchia *et al.* (1987) found significantly elevated risks among Italian women who reported frequent consumption of meat, ham and fats, especially butter. In addition, low risks were associated with consumption of fish, green vegetables, carrots and wholemeal bread or pasta. In another US study no effect of fat was found but a protective effect of vitamin A intake among women aged 30–49 years was noted (Byers *et al.*, 1984). However, all of the previous studies included only a limited number of food items, and no data about portion size were available.

The present study is the first to obtain sufficiently detailed dietary data to allow for an assessment of ovarian cancer risk in relation to such nutrients as fat, protein, calories, vitamins, etc. The broad range of nutrient intake was an obvious asset (see Table VII for details). For example, between the 25th and 75th percentiles of intake there were approximately 100, 300 and 225% increases for total fat, animal fat and ascorbic acid, respectively. The study indicated that high animal fat intake was significantly related to the risk of ovarian cancer, an effect that was not found for

fat from plant sources. This effect was not explained by calorific intake, relative weight or non-dietary risk factors although adjustment for education resulted in a diminution of the observed risks. This could represent true confounding by true lifestyle risk factors or a systematic overadjustment. The effect of calories, which only existed for calories from animal sources, appeared to be explained by high animal fat intake. In addition, total protein intake was not related to risk of ovarian cancer after adjustment for education and animal fat.

The mechanism whereby animal fat might increase the risk of ovarian cancer is not clear. An effect of diet through a hormonal mechanism is consistent with findings of oestrogen receptors in epithelial ovarian tumours (Friberg *et al.*, 1978; Holt *et al.*, 1979; Galli *et al.*, 1981). It has been suggested that a diet high in animal fats can produce extragenital oestrogen via gut bacteria (Hill *et al.*, 1971) and that oestrogen bioavailability is altered in vegetarian women (Armstrong *et al.*, 1981; Goldin *et al.*, 1981). On the other hand, it has been suggested that diet may operate directly, since animal fat could contain carcinogenic contaminants, such as polycyclic hydrocarbons, which are recognised carcinogens of the ovary in certain animal species (Cramer *et al.*, 1984). An alteration of the immune response of the host by dietary factors is yet another plausible mechanism (Carroll, 1981).

Our study did not show a protective effect of vitamin A, from either plant (carotene) or animal (retinol) sources, and failed to support Byers's previous finding (Byers *et al.*, 1983). However, a slight protective effect was observed with high intake of total vegetables, an effect consistent with that reported by La Vecchia *et al.* (1987). The mechanism of a possible protective effect is not clear, although vitamin C has been suggested to be involved in maintaining the integrity of the intercellular matrix, enhancing the immune response, promoting tumour encapsulation and preventing oxidative degradation (Willett & MacMahon, 1984*b*). Nevertheless, the possibility that our observed association arose solely by chance cannot be eliminated.

Interpretations other than causal relationships should be considered because of the limitations of case-control studies in assessing the effects of diet. However, results with food frequency questionnaires have been found to be reproducible and correlate with intake determined by more detailed dietary methods (Block, 1982; Willett & MacMahon, 1984*a*). In addition, the estimate of consumption among our controls for most nutrients appeared quite reasonable when compared to data from a representative Shanghai population (5-day weighed food records from 2,000 people, conducted in Shanghai, 1981) (Table V). We attempted to minimise recall bias by asking about usual adult diet; however, if we were in fact obtaining information on diet affected by disease status, it would be difficult to know how such a bias would operate. In addition, both the interviewer and study subjects were generally unaware of the hypotheses about diet and ovarian cancer. Although nutritional status might have influenced the survival time of cases, this should not have affected our results since only 8.1% of eligible cases were excluded because of death. Finally, of concern was the possibility of residual confounding, particularly whether the animal fat association merely reflected as yet undetermined lifestyle patterns. The fact that the association persisted after adjustment for education and income lent support to a true effect, but some caution in interpretation is in order.

The calculation from this population-based study of an attributable risk for animal fat intake (adjusted for education and ascorbic acid intake) indicates that, if real, the relationship could explain as much as 34% of the incidence of ovarian cancer in China. High fat intake thus might partially explain the different incidence of ovarian cancer between women in China and Western countries, as well as the increasing incidence among Chinese immigrants to America (Waterhouse *et al.*, 1982), although the findings are not conclusive. Further well-designed studies of diet on ovarian cancer risk are obviously warranted.

Table V Comparison of mean intake of nutrients between controls in the present study and a representative sample of Shanghai residents

	Controls of present study	*Sample of Shanghai residents*[a]
Calories (kcal)	2,365.2	2,437.0
Protein (g)	70.1	74.9
Fat (g)	68.1	65.5
Carbohydrate (g)	367.9	382.3
Crude fibre (g)	4.1	6.7
Retinol (RE)	129.5	156.3
Carotene (RE)	542.7	633.3
Thiamin (mg)	1.0	2.1
Riboflavin (mg)	0.7	1.0
Ascorbic acid (mg)	121.4	117.9

[a]Based on 5-day food records involving 7,959 person days, conducted in September 1982 in Shanghai. Both sexes combined.

Table VI Food items included in questionnaire

1. Rice	22. Fresh shrimp	43. Cabbage
2. Noodles	23. Fresh crab	44. Chinese cabbage
3. Steamed buns	24. Yellow eel	45. Cauliflower
4. Pork (fat and lean)	25. Salted fish and dried fish	46. Celery
5. Pork (fat only)	26. Cows' milk	47. Beansprouts
6. Pork (lean only)	27. Soya bean milk	48. Aubergine
7. Pork chops	28. Powdered cows' milk	49. Wild rice stem
8. Pork spareribs	29. Cakes, biscuits, pastries	50. Snow peas
9. Pigs' feet	30. Candy*	51. String beans, asparagus beans
10. Salted pork	31. Sugar*	52. Lettuce
11. Pork liver	32. Ice milk bars*	53. Chinese waxgourd
12. Other pork organ meats	33. Ice cream*	54. Cucumbers
13. Beef	34. Soya bean	55. Carrots
14. Lamb	35. Textured soya products	56. White radish
15. Chicken	36. Wheat gluten	57. Mushrooms
16. Duck	37. Dried beans or peas	58. Sweet green, red peppers
17. Pork and cereal sausage*	38. Peanuts	59. Tomatoes
18. Eggs	39. All vegetables*	60. Apples
19. Vegetable oil (rapeseed, soya bean, sesame)	40. Greens	61. Pears
20. Lard	41. Spinach	62. Oranges, tangerines
21. Fresh fish	42. Chinese chives	63. Watermelon

*Not included in analysis (items 17, 29, 30, 31, 32 and 33 were rarely eaten, and item 39 was repetitive). Food items included in food groups: meat, 4–16; red meat, 4–11, 13–14; poultry, 15–16; fish, 21–25; dairy and eggs, 18, 26, 28; eggs, 18; vegetables, 40–46, 48–58; dark green vegetables, 40–42; yellow orange vegetables, 55; cruciferous vegetables, 40, 43–45; allium, 42; legumes, 34, 35, 37, 38, 47, 50, 51; fruits, 60–63; complex carbohydrates, 1–3, 36; vegetable oils, 19.

Table VII. 25th and 75th percentiles (g day^{-1}) for daily intake of selected nutrients and food groups among controls

Nutrients	*25%*	*75%*	*Food groups*	*25%*	*75%*
Protein	49.0	81.1	Meat	36.4	106.4
Plant protein	36.8	52.0	Red meat	29.9	83.9
Animal protein	9.1	33.0	Poultry	2.7	16.4
Fat	39.4	77.0	Fish	15.1	58.4
Plant fat	23.7	38.1	Dairy and eggs	8.2	55.9
Animal fat	13.0	46.6	Eggs	8.2	32.9
Calories	1,901.6	2,674.9	Vegetables	241.9	528.0
Plant calories	1,617.9	2,155.5	Dark-green vegetables	70.2	174.2
Animal calories	168.3	529.5	Yellow-orange vegetables	0.0	0.5
Carbohydrate	313.8	416.5	Cruciferous vegetables	98.4	234.0
Crude fibre	2.5	5.0	Fruits	22.8	164.4
Vitamin A (RE)	349.4	863.5	Complex carbohydrates	363.1	513.2
Carotene (RE)	283.3	650.0	Vegetable oils	13.2	24.7
Retinol (RE)	4.8	208.4	Legumes	46.3	95.8
Thiamin (B_1) (mg)	0.8	1.1	Allium	0.0	2.2
Riboflavin (B_2) (mg)	0.4	0.9			
Ascorbic acid (mg)	68.2	144.8			

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Br. J. Cancer (1989), 59, 97-99

Zoladex: Endocrine and therapeutic effects in post-menopausal breast cancer

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Summary The endocrine and therapeutic effects of the LHRH agonist Zoladex have been assessed in 28 post-menopausal women with advanced breast cancer. Fourteen had responded to previous hormone therapy and 14 had no previous hormone therapy. There were two partial responses and two patients with stable disease for more than 6 months in the former group, and one partial response and two with stable disease for more than 6 months in the latter group. Toxicity was minimal. All responses occurred in soft tissue. Six out of seven patients who received tamoxifen after progression of disease on Zoladex showed a response. Peripheral oestradiol levels were measured, and they fell after 1 month from 33 pmol l^{-1} ($\pm$20, s.d.) to 22 pmol l^{-1} ($\pm$11, s.d.) ($P < 0.005$). Responders and non-responders showed similar changes in oestradiol. Oestrone levels did not change significantly. These results suggest that Zoladex acts indirectly via changes in peripheral hormones, rather than directly on LHRH receptors on the tumour.

Recently luteinising hormone releasing hormone (LHRH) agonists have been shown to have direct inhibitory effects on human breast cancer cell lines *in vitro* and low affinity binding sites were demonstrated (Miller *et al.*, 1985; Blankenstein *et al.*, 1985). LHRH binding sites have also been demonstrated in primary breast tumours (Eidne *et al.*, 1985). Therefore, LHRH agonists such as Zoladex (Goserelin) have been assessed for potential efficacy in post-menopausal breast cancer, on the assumption that a therapeutic effect may indicate a direct antitumour effect (Plowman *et al.*, 1986; Waxman *et al.*, 1985), whereas in pre-menopausal women the major effect would be via ovarian suppression (Nicholson *et al.*, 1985). However, other endocrine effects may occur in post-menopausal women.

In the post-menopausal woman, adrenal and ovarian androgens are the main source of oestrogens (Judd *et al.*, 1982, 1974; Grodin *et al.*, 1973). They are converted peripherally by aromatase to oestrone and oestradiol. Inhibitors of aromatase (e.g. aminoglutethimide) are effective endocrine therapies in the treatment of post-menopausal breast cancer (Harris *et al.*, 1983*c*) and occasionally pre-menopausal breast cancer (Bezwoda *et al.*, 1987, Wander *et al.*, 1986). In the latter case, it has been suggested that intratumour conversion of androgens to oestrogens may be important and direct inhibition may be important, without any detectable lowering of peripheral oestrogens (Miller *et al.*, 1982; Bezwoda *et al.*, 1987).

Hydrocortisone alone can suppress adrenal androgen production (Harris *et al.*, 1984) and hence lower peripheral oestrogen levels (Harris *et al.*, 1984). We have recently shown that the androgens produced by the post-menopausal ovary are under pituitary FSH and LH control (Dowsett *et al.*, 1988). Zoladex may therefore have indirect endocrine effects on post-menopausal breast cancer. We have evaluated in this study the therapeutic effects of Zoladex and the correlation of response with peripheral endocrine changes in post-menopausal breast cancer patients.

Materials and methods

Twenty-eight post-menopausal patients with locally advanced or progressive breast cancer were studied. Fourteen had no previous endocrine therapy and 14 had previously shown either stable disease for more than 6 months or partial response to other endocrine therapies.

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Received 8 July 1988; and in revised form, 13 August 1988

Twelve patients had received Tamoxifen and three had a complete response, seven had a partial response and one stable disease. Eight had been given aminoglutethimide and four had a partial response. No patient had received chemotherapy. Other pre-treatment characteristics are shown in Table I.

Patients received monthly subcutaneous Zoladex, 3.6 mg, after infiltration of the local site with lignocaine. Response was assessed by UICC criteria (Hayward *et al.*, 1977). After Zoladex failure, patients who had received no previous endocrine therapy were treated with Tamoxifen 20 mg daily when appropriate.

Oestrone and oestradiol were measured by radio-immunoassays which we have previously described (Harris *et al.*, 1983*a*; Dowsett *et al.*, 1987). These analyses have been specifically developed for the analysis of post-menopausal plasma oestrogen levels and have sensitivity limits of 30 and 3 pmol l^{-1} respectively.

Results

Responses to endocrine therapy

Three patients showed partial responses for durations of 21 weeks and 141 weeks (continuing) and one patient died after 6 weeks from other causes. Four patients showed stable disease for more than 6 months (range 29-66 weeks). All responses were in soft tissues. Twenty-one patients had progressive disease.

Previous hormone therapy did not affect the likelihood of response to Zoladex. Of the 14 patients with previous endocrine therapy, two showed partial responses and two stable disease, while of the 14 with no previous endocrine treatment, one showed a partial response and two stable disease.

Toxicity was minimal, with no complaints of problems

Table I Pre-treatment characteristics

	Mean	*Median*	*Range*
Age (years)	67	70	32-83
Time from LMP (years)	21	22	3-39
Disease-free interval (weeks)	103	45	0-471
Pre-treatment weight (kg)	62	65	42-102
Time from first relapse to start of Zoladex (weeks)	73	2	0-508

Previous endocrine therapy, 14
Sites of disease: Soft tissue 23, lung 1, liver 1, nodes 9, bone 7.

ANTICANCER RESEARCH 8: 417-434 (1988)

Ovarian Cancer (*Review*)

Etiology, Diagnosis, Prognosis, Surgery, Radiotherapy, Chemotherapy and Endocrine Therapy

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Abstract. *The increased incidence of ovarian cancer in women whose ovulations were not suppressed by pregnancy or oral contraceptives and the increase in the incidence of the disease with the onset of climacterium support the hypothesis that ovarian cancer is an endocrine-related disease. Animal experimental results further support this contention. However, identification of a population at risk, prevention, and early detection is difficult. At present, tumor markers are useful in monitoring the disease, though cannot be used for screening. Most of the diagnosed cases are of advanced stages. Besides staging, tumor histology, and residual tumor load are of prognostic importance.*

The need for accurate initial surgical staging with optimal tumor cytoreduction and the importance of second-look surgery to confirm the response to therapy are emphasized. The precise role for radiotherapy in the treatment of ovarian cancer is still to be established. Presently, response rates of 80% are achieved using cisplatin based combination chemotherapy. In spite of this, long term survival has not improved.

Endocrine therapy has hitherto been used empirically and mainly as a last resort in the treatment of advanced ovarian cancer. Response rates of about 10% have been reported. Information on tumor predisposition related to hormonal control should be a key parameter in selecting the appropriate therapy. Tumor analysis has shown that androgen receptors predominate in ovarian cancer, compared to estrogen and progestin receptors. A clinical trial based on endocrine parameters is warranted.

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Key Words: Ovarian cancer, etiology, diagnosis, prognosis, surgery, radiotherapy, chemotherapy, endocrine therapy.

Contents

Introduction

Cancer of the ovary is the leading cause of death among women with gynecologic malignancies (1). In spite of the improvement in the treatment of several forms of cancer in the last decades, the survival rates for patients with ovarian cancer are still poor. Only 5 to 15% of the patients with advanced disease (FIGO stage III and IV: Table I) survive 5 years or longer (2). The 5-year survival rates for other stages vary between 45% for stage II to 72% for stage Ia (2). Since most of the patients are in stage III or IV at the time of

diagnosis, the overall survival rate for ovarian cancer is low.

Ovarian neoplasms can originate from the surface epithelium, the germ cells and the ovarian stroma (3) (Table II). The vast majority of the malignant ovarian tumors are of the common epithelial type, and include serous, mucinous, endometrioid, clear-cell and undifferentiated adenocarcinomas, as well as the rare malignant Brenner tumor. These tumors are derived from the surface epithelium of the ovary, which is the adult equivalent of the mesothelium of the embryonic gonad (Mullerian epithelium). There is a close relationship between epithelial tumors and the Mullerian epithelium. Serous tumors resemble the epithelium of the Fallopian tube, endometrioid and clear cell tumors the endometrium and mucinous tumors the endocervical epithelium. The papillary serous cystadenocarcinoma is the most common type of malignant epithelial ovarian tumor, constituting about 40 (4) to 68% (5). The malignant mucinous tumors constitute 3 (6) to 21% (5), whereas 5 (5) to 20% (4) of the malignant epithelial tumors are of the endometrioid type, and 5 (7) to 10% (4) of the clear cell type. Other epithelial tumors are less common.

The spread of epithelial ovarian cancer occurs mainly by continuity to the peritoneum and paracolic gutters, omentum, diaphragm, and serosa of the liver. The proximity of omentum, terminal ileum, cecum and sigmoid colon to the pelvis makes these organs frequent sites of metastatic implantation. Obstruction of the lymphatics of the diaphragm by tumor cells may lead to the formation of ascites. Furthermore, direct growth of tumor to the surrounding tissues and lymphatic dissemination may take place. Lymphatic drainage from the ovary is mainly to the paraaortal nodes, but occasionally also to the pelvic or inguinal nodes. However, extra-abdominal dissemination by the lymphatic route is slow. Haematological spread is usually late and infrequent (8).

Etiology

Understanding the epidemiology and etiology of ovarian cancer may contribute to prevention and earlier diagnosis. However, in spite of extensive research on endocrinological, environmental, and genetic factors, the causal analysis of ovarian cancer still escapes comprehension.

Endocrine factors. Chronic administration of estrogens, progestins and androgens has been shown to result in ovarian cancer in animal studies (9-11). High levels of gonadotropins have also been correlated with ovarian neoplasms in animal experiments (12).

Epidemiologic studies also suggest that ovarian cancer might be an endocrine-related tumor. Among women with ovarian cancer, an increased incidence of nulliparity and a lower mean number of pregnancies were detected (13-17). Several authors noted a decreased incidence of ovarian cancer after the use of oral contraceptives (18-21). Casagrande *et al* (15) found an increasing risk in ovarian cancer; the ovarian cancer incidence rising steadily with the total time in a woman's life during which her ovulations were not suppressed by pregnancy, lactation or the use of oral contraceptives. It has been suggested that each ovulation causes a minor trauma of the ovarian surface, which cumulatively could contribute to ovarian cancer (22).

During pregnancy and during the use of oral contraceptive agents, ovulation is suppressed and the levels of gonadotropins are low. The high levels of gonadotropins in women in the early post-menopause may play a role in the development of ovarian neoplasms (23, 24). This is supported by the dramatically increased incidence of ovarian cancer in women above the age of 45 years when gonadotropins reach high levels (23). In this connection, high levels of androstenedione, the precursor of estrogen, in the postmenopausal years may also have a role in the development of ovarian cancer.

In some studies estrogen replacement did not seem to effect the incidence of ovarian cancer (25, 26). However, others reported that more women with endometrioid cancer of the ovary were found among those who used estrogens during menopause (27). A two - to three - fold excess of ovarian carcinoma was found in woman who used conjugated estrogens and diethylstilbestrol (DES) (28). In animal studies, exposure to DES *in utero* could induce cystadenocarcinoma of the ovary (29). To date, no relationship has been demonstrated between intra-uterine DES exposure and ovarian cancer in humans. In a long term follow-up study of women who used medroxy-progesterone acetate for contraception, no increased risk of ovarian carcinoma was observed (30).

Other factors. The incidence of ovarian cancer is higher in most industrialized countries, except Japan. Dietary or other environmental factors may be responsible for this phenomenon, since in Japanese women who emigrated to the U.S. an increasing incidence of ovarian neoplasms is seen (26, 31). In the U.S. white women have a significantly greater risk of developing ovarian malignancy than black women (26). However, the results of studies of dietary factors in relationship to ovarian cancer are contradictory. An increasing risk for ovarian cancer with increasing consumption of animal fat has been reported (32). Carcinogenic substances in fat are presumed to promote tumor growth directly, or via the ability to increase estrogen production by bacteria in the gut (32). Other investigators could not confirm this observation (33). Coffee drinking, smoking, and alcohol consumption do not seem to increase the risk of ovarian cancer (24, 32, 33).

There is controversy about a link that has been suggested between ovarian cancer and talc (34, 35). Cancer of the ovary has also been linked to asymptomatic mumps infection (36). It has been suggested that the infection might lead to premature depletion of oocytes, resulting in higher levels of gonadotropins (36).

Table I. *Stages of primary carcinoma of the ovary, according to the International Federation of Gynecology and Obstetrics (1974).*

Stage I		Growth limited to the ovaries.
Stage Ia		Growth limited to one ovary.
	(i)	No tumor on the external surface; capsule intact.
	(ii)	Tumor present on the external surface; and/or capsule ruptured.
Stage Ib		Growth limited to both ovaries; no ascites.
	(i)	No tumor on the external surface; capsule intact.
	(ii)	Tumor present on the external surface; and/or capsule ruptured.
Stage Ic		Tumor at either Stage Ia or Ib, but with obvious ascites present or positive peritoneal washings.
Stage II		Growth involving one or both ovaries with pelvic extension.
Stage IIa		Extension and/or metastases to the uterus and/or fallopian tubes.
Stage IIb		Extension to other pelvic tissues
Stage IIc		Tumor at either Stage IIa or IIb, but with obvious ascites present or positive peritoneal washings.
Stage III		Growth involving one or both ovaries with intrapentoneal metastases outside the pelvis and/or positive retroperitoneal nodes. Tumor limited to the true pelvis, with histologically confirmed extension to small bowel or omentum.
Stage IV		Growth involving one or both ovaries, with distant metastases or pleural effusion is present, with positive cytology or metastasis to liver parenchyma.
Special category		Unexplored cases which are thought to be ovarian carcinoma.

Table II. *Summary of the classification of malignant ovarian tumors, according to W.H.O. (3).*

I	Common "epithelial" tumors
	A. Serous tumors.
	B. Mucinous tumors
	C. Endometrioid tumors.
	D. Clear cell (mesonephroid) tumors.
	E. Brenner tumors.
	F. Mixed epithelial tumors.
	G. Undifferentiated carcinoma.
	H. Unclassified tumors.
II	Sex cord stromal tumors.
III	Lipoid cell tumors.
IV	Germ cell tumors.
V	Gonadoblastoma.
VI	Soft tissue tumors not specific to the ovary.
VII	Unclassified tumors.
VII	Secondary (metastatic) tumors
VIII	Tumor-like conditions.

In an attempt to derive a common link between different explanations of the etiology of ovarian cancer, Cramer and Welch (24) described a model in which inclusion cysts are formed in the ovary by incessant ovulation or by foreign body stimulation. Stimulation by gonadotropins or estrogens may lead to differentiation, proliferation and malignant transformation of the inclusion cysts. The factor responsible for the final step, *i.e.* malignant transformation, remains unknown (24).

Epithelial cancer of the ovary can also be familial (37, 38). The hereditary pattern was found to be consistent with an autosomal dominant gene with variable penetration (37). Some genetic syndromes are also associated with ovarian cancer. The occurrence of tumors in dysgenetic ovaries is well known (39). In patients with Peutz-Jeghers syndrome more sex cord stromal tumors are seen, and in patients with multiple basal cell carcinomas, ovarian fibromas are seen more frequently (31). Ovarian cancer has also been related to the ABO-system (40). In a study of 1930 patients *versus* 24120 controls, more women with ovarian cancer were seen with blood group A than expected (40). The reason for this is unknown.

Diagnosis

Symptoms. Early ovarian cancer has an asymptomatic character. For this reason, ovarian cancer is frequently called a silent killer. Complaints arise when there is expansion of the tumor, adherence to the surrounding tissue and formation of ascites The presenting symptoms are usually of short duration and most frequently consist of pain and abdominal distension (41-43). Less often, abnormal vaginal bleeding is noted or an abdominal mass is found (42, 43). Extensive abdominal distension can cause dyspnea. Asymptomatic prolapse of the uterus or vagina can become symptomatic. Tumor deposits on the small bowel can change its motility (44). Ascites or large omental metastases can lead to dyspepsia and decreased food intake. The weight loss caused by decreased food intake may not be apparent due to abdominal distension by ascites and tumor volume.

Tumor markers. Tumor markers might be useful for the diagnosis of ovarian cancer and for monitoring the disease status during and after treatment. Elevated plasma levels of carcinoembryonic antigen (CEA) are noted in 46% of patients with ovarian cancer (45). Elevated levels of CEA have also been noted in patients with carcinoma of the cervix (53%) and endometrium (37%), patients with benign gynecologic diseases (18%), and in healthy volunteers (11%) (45). In patients with ovarian cancer, the plasma levels of CEA are related to stage of disease and tumor DNA content (45). A relationship with the histological type has also been described (46). In patients with CEA-positive tumors, determined by immunohistochemical staining, plasma CEA levels could be used in the follow-up (46), especially in cases of minimal tumor lesions

after surgery (47). Furthermore, the use of radiolabeled antibodies against CEA can be helpful in the detection of primary and metastatic ovarian tumors (48, 49).

Tumor-associated antigens are another group of tumor markers. They are glycoproteins that are present on the cell surface of cancer cells, but not on the surface of normal cells. Radioimmunoassays, using polyclonal reagents, have been developed for the detection of ovarian carcinoma-associated antigen (OCAA) (50) and ovarian carcinoma antigen (OCA) (51). OCAA and OCA could be demonstrated in the serum of approximately 70% of patients with ovarian cancer, but the value of OCAA and OCA in the surveillance of patients with ovarian cancer was disappointing (50, 52). However, serum levels of NB/70K, isolated from OCA, correlated well with stage of disease and the residual tumor burden in ovarian cancer patients (53).

Bast and co-workers developed a monoclonal antibody (OC125) against the antigen CA125, and reported elevated levels of CA125 in 82% of patients with ovarian cancer (54). Although determination of CA125 is not useful as a screening method for ovarian cancer, CA125 has been found to be an useful parameter in monitoring the disease (54-58). In patients with recurrent or progressive ovarian cancer, increased CA125 levels were seen in more than 90% of the cases, whereas in only 34% of these cases increased levels of CEA were noted (56). Rising levels of CA125 may indicate tumor progression, but decrease of the serum levels to normal ranges does not always indicate complete tumor eradication (54, 57, 58).

A number of other monoclonal antibodies that react with epithelial ovarian cancer have been investigated (52). The monoclonal antibody OV-TL3, which in contrast to OC125 recognizes a common antigen on most ovarian carcinomas, including the mucinous, may be of value for rapid diagnosis of ovarian cancer (59). This is yet to be demonstrated.

At present, tumor markers are not specific enough for the diagnosis of epithelial ovarian cancer. However, presently available markers can be useful in the surveillance of patients during and after treatment. The use of radiolabeled antibodies against tumor-associated antigens may become valuable in the detection of metastases (60-62). In addition, tumor markers may be used as targets for antibody-directed treatment (63).

Preoperative evaluation. Among the various well known procedures, ultrasonography recently, has been used for the diagnosis of ovarian cancer (64). In general, finding a cystic lesion without septae suggests a benign tumor, while tumors with multiple internal echoes or solid parts must be considered as malignant (65, 66). Furthermore, ascites can be detected by ultrasound. In addition, computerized tomography can detect upper abdominal lesions and retroperitoneal lymph nodes from the pelvis to the diaphragm (67). Nuclear magnetic resonance imaging and computerized tomography appear to give similar results in patients with gynecological tumors (68).

Lymphangiography has advantages over other methods of assessing retroperitoneal nodes. The nodes do not have to be enlarged and metastases as small as 3 mm can be recognized (67). The overall accuracy of lymphangiography using strict criteria is generally reported to be in the range of 80 to 90% (67). Evaluation of lymphangiography has shown that nodes which were positive at lymphangiography were also positive when surgically biopsied and was false-negative in 19% (9/48) of the cases (69). In doubtful cases, lymphangiographic findings may be confirmed by percutaneous transperitoneal fine needle biopsy (70).

Prognostic factors

The prognosis of patients with cancer of the ovary depends on several more or less independent factors, namely stage, histology and amount of residual tumor after surgical resection. Furthermore, the age and the condition of the patient are of importance.

Stage. Until the standardization of classification of ovarian cancers by the FIGO (Table I), various classifications, based on operability of the tumor and on anatomic extent of disease, have been used (71). It is clear that because of this variety of classifications, comparison of results from different studies was often impossible.

The clinical stage of tumor growth at the time of diagnosis is generally considered the best prognostic indicator (72). The increasing accuracy of staging of ovarian cancer led to increased survival rates of both patients with low-stage cancer and patients with advanced cancer (73). The latter can be explained by the fact that with more accurate staging, higher stages not only include patients with obvious advanced disease, but also patients with minimal abdominal lesions, resulting in a better prognosis for the whole group of patients. This information must be taken into account in comparing current survival rates for different stages with previously reported rates.

Stage III ovarian cancer (Table I) includes patients with abdominal metastases varying from microscopic lesions to large tumor deposits. In a recent revision of the FIGO staging system (Table III), stage III disease is divided into stage IIIA with microscopic seeding to the peritoneal surface, stage IIIB with peritoneal implants not exceeding 2 cm in diameter, and stage IIIC with larger abdominal implants and/or positive inguinal or retroperitoneal nodes (74).

Peroperative rupture of the tumor in stage I disease does not seem to be of great influence on the prognosis (75, 76). However, decreased 5-year survival rate of patients with stage I cancer and peroperative ruptured cysts was reported in another study (77).

Histology. The histological type of the tumor seems to be less important in prognosis. In general, mucinous, endometrioid and clear-cell tumors have a better prognosis than other epithelial tumors (78). When tumors are analysed for histological type, after correction for stage and/or grade, the prognostic value loses its importance (78, 79). Thus mucinous adenocarcinomas tend to be well differentiated and low stage, while serous adenocarcinomas are more often poorly differentiated and high stage (79).

Table III. *Stages of primary carcinoma of the ovary, according to the International Federation of Gynecology and Obstetrics (1987).*

Stage I	Growth limited to the ovaries.
Stage Ia	Growth limited to one ovary; no ascites. No tumor on the external surface; capsule intact.
Stage Ib	Growth limited to both ovaries; no ascites. No tumor on the external surface; capsule intact.
Stage Ic	Tumor either Stage Ia or Ib, but with tumor on the surface of one or both ovaries; or with capsule ruptured; or with ascites present containing malignant cells or with positive peritoneal washings.
Stage II	Growth involving one or both ovaries with pelvic extension.
Stage IIa	Extension and/or metastases to the uterus and/or fallopian tubes.
Stage IIb	Extension to other pelvic tissues.
Stage IIc	Tumor at either Stage IIa or IIb, but with tumor on the surface of one or both ovaries; or with capsule(s) ruptured; or with ascites present containing malignant cells or with positive peritoneal washings.
Stage III	Tumor involving one or both ovaries with peritoneal implants outside the pelvis and/or positive retroperitoneal or inguinal nodes.
Stage IIIa	Histologically confirmed microscopic seeding of abdominal peritoneal surfaces. Nodes negative.
Stage IIIb	Histologically confirmed implants of abdominal peritoneal surfaces, none exceeding 2 cm in diameter. Nodes negative.
Stage IIIc	Abdominal implants > 2 cm in diameter and/or positive retroperitoneal or inguinal nodes.
Stage IV	Growth involving one or both ovaries, with distant metastases. Parenchymal liver metastasis equals Stage IV.

The histological grade of the tumor is of prognostic value in patients with ovarian cancer (71, 78, 80-82). Although well differentiated tumors are more frequently associated with low stage cancer and poorly differentiated tumors with higher stages (82), the tumor grade is of importance as an independent factor in the prognosis especially of low stage ovarian cancer (78, 79, 82, 83).

Special attention must be paid to a group of tumors with atypia, pleomorphism, stratification of the epithelial lining of the papilla, and mitotic activity > 1/high power field, but without stromal invasion: the so-called "borderline tumors" or "adenomas with low potential malignancy" (3, 84, 85). Approximately 15% of the ovarian tumors are of the borderline type (86). In 11 to 36% of the cases, the tumor is diagnosed in stage III or IV (87-89). Borderline tumors have a favourable prognosis, regardless of spread of tumor (86, 90). A 5-year survival rate of 91% for serous borderline tumors and of 81% for mucinous borderline tumors has been reported (87). The 10-year survival rates were 83% and 73%, respectively (87). Because of this favourable prognosis, tumors of low potential malignancy should be regarded as a specific entity.

In the determination of histological type and grade of ovarian tumors, a significant variability has been reported (91-93). Discrepancy was seen particularly in the discrimination between borderline and well differentiated tumors (91, 92) and between undifferentiated and serous tumors (92). It has been shown that differences between different pathologists in grading ovarian tumors also reflect differences in prognosis (93). Apart from an interobserver variation, an intraobserver variation was noted (93). For these reasons the assessment of more objective and reproducible techniques is required. Quantitative morphometric methods have shown a good correlation with prognosis of ovarian cancer (94). Morphometry is especially important in the differentiation of borderline and malignant tumors and in identifying those borderline tumors which are likely to pursue an unusual malignant course (94). Flow cytometric and cytophotometric analysis of DNA levels in the tumor have been reported to be of prognostic value (95-97).

Tumor load after surgery. In patients with advanced ovarian cancer, the size of residual tumor lesions after treatment is the most important prognostic factor (79, 83, 98-101). An increased complete response rate to chemotherapy, confirmed at second-look laparotomy, was observed in patients with residual lesions ≤ 3 cm compared to those with residual tumors > 3 cm (98). In another study the survival rates of patients with metastases smaller than 1.6 cm after surgery were found to be significantly higher than those of patients with larger metastases (99).

The survival rate for patients with large abdominal metastases, surgically reduced below a certain limit, was found to be identical to the survival rate of patients whose metastases were below that limit without any treatment (99, 101). However, no beneficial effect could be demonstrated in patients with initial metastases larger than 10 cm (101). This is consistent with the hypothesis that spontaneous mutation in large tumors will have already resulted in cell clones resistant to systemic treatment (102).

As a result of all these observations, patients with advanced ovarian cancer are now divided into those with minimal residual disease (largest residual lesion < 2 cm) and those with bulky residual disease (largest residual lesion > 2 cm) (103).

Surgery

Surgery is the primary treatment in the management of patients with ovarian cancer. It is performed initially for the determination of the extent of cancer spread and for the reduction of tumor load. Second-look procedures are performed to determine the status of the disease and the response to treatment, and to remove detectable remaining tumor

masses. In case of complications such as bowel obstruction, intervention laparotomy may be necessary.

Primary surgery. A (para)median abdominal incision is made, extending from the symphysis pubis to above the umbilicus. After opening the peritoneum, ascites is aspirated for cytologic examination. In the absence of ascites, peritoneal washing is performed. Then, careful inspection and palpation of the intraabdominal organs and peritoneal sufaces is carried out. Suspicious lesions are biopsied, and the pelvic and paraaortic nodes are palpated. The next step is removal of the primary tumor, along with a total hysterectomy and bilateral-salpingo-oophorectomy. In case of extension of the tumor to the bowel, bladder or cul-de-sac, the retroperitoneal approach may be of benefit, since deep infiltration of ovarian carcinoma in the peritoneum and other structures is unusual (104). In some cases, resection of bowel with reanastomosis or colostomy may be indicated. The greater omentum is removed below its attachment to the transverse colon (infracolic omentectomy). In cases of gross involvement of the omentum, a total omentectomy should be performed (105). When feasible, an appendectomy should be carried out. If no metastases are seen or palpated, biopsies are taken from the peritoneum of the cul-de-sac, the vesico-uterine pouch, the parietal peritoneum below the arcuate line, the pelvic walls, the paracolic gutters, the sigmoid colon, the hepatic and splenic flexures, and the right diaphragm (100). Tumor adhesions are also biopsied. Clinically suspicious pelvic or paraaortal lymph nodes are removed (100). Clinically negative paraaortic nodes are sampled in the area near the insertion of the ovarian veins (100). The surgical findings and treatment should be accurately documented. Inoperable tumor masses may be marked with clips. Such a procedure may be helpful in subsequent tumor identification for radiation treatment, computerized tomography and during second-look procedures.

The right hemi-diaphragm is susceptible to implantation of tumor (105-108). Metastases to diaphragm in patients with presumed stage I or II disease who underwent laparoscopy within one month after exploratory laparotomy, were observed in up to 44% of the cases (105-108).

Spread of tumor via the lymphatics is important in ovarian cancer (8). In presumed stage I ovarian cancer, paraaortal lymph node involvement is reported in 10 to 18%, and pelvic node involvement is reported in about 9% of the cases (107, 109). The frequent involvement of paraaortic and pelvic lymph nodes in apparently low stage disease supports the view that extirpation of paraaortic and pelvic nodes should be a part of any staging laparotomy for ovarian cancer.

Since the size of residual tumor masses after treatment is found to be the most important prognostic factor in patients with advanced ovarian cancer (82, 83, 98-101), the importance of cytoreductive surgical treatment cannot be overstated.

Debulking of the tumor may achieve as much as 99.9 per cent tumor volume reduction (99), and restore gastrointestinal function and improve the patient's nutritional status (44). The residual small lesions appear to be more vulnerable to chemotherapy and radiotherapy since they are better vascularized and oxygenated and contain a higher fraction of actively proliferating cells (110). Resection of tumor bulk may be easy to perform in patients with minimally invasive growing tumors. In other cases, debulking with "maximum effort" is necessary. No difference in survival was found between patients in whom debulking, leaving masses less than two cm, was readily accomplished, compared to those who had "maximum effort" debulking (100). In a study of 70 patients, optimal cytoreductive surgery could be achieved in 87% of the cases (111). In 20% of the patients bowel resection was required (111). The morbidity and mortality of patients was found to be acceptable (111, 112). In some patients, however, cytoreductive surgery is not feasible, because of the patients' poor general condition, or due to the site of metastases. Sometimes debulking surgery may become easier after a few successful chemotherapeutic cycles.

In young women with a desire and a potential for childbearing, with a well differentiated, unilateral, encapsulated, nonadherent, common epithelial ovarian carcinoma, the uterus and contralateral ovary may be preserved if metastases are excluded (73, 105, 113). Suspicious areas on the preserved ovary should always be biopsied. Blind biopsy of the preserved ovary has also been recommended, especially in cases of serous tumors, since these tumors are frequently bilateral (73). If there is any doubt, a conservative approach should be chosen. If necessary, extensive surgical treatment can be performed after detailed histological examination of the resected tissues (73). Close patient follow-up is mandatory. After completion of childbearing, resection of the residual internal genitalia should be reconsidered (73). This concept is supported by the study of Munnell *et al* consisting of 190 cases of stage Ia ovarian cancer (114). They concluded that there was no statistically significant difference in 5 year survival rates between patients in whom unilateral salpingo-oophorectomy was performed, compared to those in whom the reproductive organs were removed (114).

Second-look laparotomy. Second-look surgery is defined as an exploratory laparotomy to assess the cancer status in patients who are clinically free of disease following a planned course of chemotherapy (115). The term "second-look operation" is also used to refer to restaging operations and secondary cytoreductive surgery.

Second-look laparotomy follows the same principles as the staging laparotomy. Ascites or peritoneal washing fluid is examined. Remnants of ovaries, uterus, or omentum are removed. Residual tumor masses are removed as far as possible. Sites, known to have residual cancer at the initial laparotomy are biopsied. Multiple biopsies are obtained from the peritoneum of the bladder, the cul-de-sac, the pelvic walls, the paracolic spaces, and from the diaphragm. Enlarged pelvic and paraaortal lymph nodes are removed. If there are no enlarged nodes, sampling can be performed. However, if

Table IV. *Frequencies of persistent disease at second - look laparoscopy in patients with advanced ovarian cancer with clinical complete response.*

Author	Year	Number	Percentage
Dauplat (116)	1986	7/24	29
Curry (117)	1981	9/20	45
Kavoussi (118)	1986	9/19	47
Webb (119)	1982	32/59	54
Copeland (120)	1985	161/246	65
Zoccerato (121)	1984	13/20	65
Barnhill (122)	1984	37/55	67
Berek (123)	1984	38/56	68
Steiner (124)	1985	7/10	70
Roberts (125)	1982	19/24	79
Raju (126)	1982	53/65	82
Phibbs (127)	1983	26/30	87

biopsies or peritoneal washings already were positive, lymphadenectomy is not required.

The diagnostic value of second-look laparotomy in patients with a clinically complete response has been justified in several studies. Persistent disease, diagnosed by second-look laparotomy, has been observed in 29 to 87% of patients with advanced ovarian cancer who were clinically free of disease (Table IV) (116-127). This clearly demonstrates that second-look surgery is useful in monitoring the response to treatment. Negative findings at second-look laparotomy are not a definitive sign of tumor eradication. In patients with moderately and poorly differentiated tumors with either negative or microscopically positive findings at second-look surgery, a recurrence rate of approximately 50% was reported after a follow-up period of at least 4.5 years (128). In another study, consisting of patients with advanced disease and a negative second-look laparotomy, 24% recurred within 5 to 32 months after laparotomy (129).

Second-look laparoscopy. Attempts have been made to use laparoscopy in the monitoring of the disease status (105, 106, 130-133). A major advantage of laparoscopy is the possibility of performing repetitive procedures with minimal morbidity and discomfort to the patient. In some studies, laparotomy was performed in case of negative findings at second-look laparoscopy. A tumor was found in 20 to 55% of the cases (106, 130, 132, 134). These data indicate that laparoscopy is of limited value in the monitoring of ovarian cancer. However, performing laparoscopy prior to second-look laparotomy may be valuable, since the finding of an unresectable tumor mass or diffuse intraperitoneal spread may obviate the need for laparotomy (130, 132, 133).

Secondary debulking surgery. The benefit of cytoreductive surgery at second-look laparotomy is a matter of dispute. The primary unresolved question is whether the association between bulky disease and poor prognosis is merely due to the presence of a great tumor load or to the fact that bulky disease is associated with more aggressive tumors with decreased sensitivity to chemotherapy. Only in the former situation might secondary cytoreductive surgery be beneficial.

In some studies, secondary debulking operations were found to be of value (135-137). In the study of Schwartz and Smith (136), survival after second-look surgery varied directly with the amount of tumor found at second-look laparotomy and the size of residual masses after operation. In patients with residual tumor masses of more than 2 cm in diameter, the 2-year survival rate was 9%, compared to a significantly higher response percentage of 47.5 when all residual tumor was removed at second-look operation (136). In another study, an average survival of 44 months in patients with residual tumor masses of 2 cm or less was reported, while in patients with larger residual lesions the average survival was 4 to 7 months (137). However, in most of these studies, no cisplatin-containing chemotherapeutic regimens were used. In studies of patients who had received cisplatin-containing chemotherapy, no beneficial effect of secondary cytoreductive surgery could be demonstrated (126, 130, 139). On the basis of the above cited studies, it can be concluded that surgical resection of bulky tumor should be performed early in the course of ovarian cancer to be effective.

Radiotherapy

Radiotherapy has been used as a primary treatment for non-resectable ovarian cancer, as an adjuvant to surgery, for palliation in advanced cases, and for the treatment of recurrent disease. However, the role of radiotherapy in the treatment of ovarian cancer has been the subject of considerable controversy, and is yet to be defined (72, 73, 140).

In the Gynecologic Oncology Group study of adjuvant treatment in stage I ovarian cancer, no beneficial effect of pelvic irradiation could be demonstrated (141). In a study of 54 patients with stage Ia2 disease, Dembo *et al* concluded that postoperative pelvic irradiation was an inappropriate treatment, because relapses occurred throughout the peritoneal cavity (142). Since ovarian cancer is considered to be a disease of the whole peritoneal cavity, intraperitoneal administration of chromic phosphate (^{32}P) and colloidal gold (^{198}Au) has been used in patients with stage I disease (143). The colloids are taken up by the lymphatics of the diaphragm, thus following a well recognized pattern of metastases. However, no significant differences, in relapse or survival were found between patients treated with intraperitoneal ^{32}P and patients treated with melphalan (144). At two-years, the disease-free survival was about 80% in both groups (144).

In a prospective study of patients with stage Ib, II, and III ovarian cancer with small or no macroscopic tumor residuum after surgery, the effect of postoperative radiotherapy and/or chemotherapy was evaluated. It was concluded that abdominopelvic irradiation, using the moving strip method without diaphragmatic shielding, significantly improved patient survival rate and long-term control of occult upper abdominal

disease in 25 to 30% more patients than did pelvic irradiation alone or together with chlorambucil treatment (142, 145). In patients with stage II or III with large residual disease, cures were rarely obtained with radiotherapy. However, no careful initial staging was performed in this study, the extent of cytoreductive surgery and the amount of residual tumor masses were not reported, and chemotherapy was not optimally administered.

In another prospective and randomized study, whole abdominal irradiation, using a moving strip method with liver and kidney shielding, was compared to melphalan treatment in patients with stage I-III tumors with no residual tumor or residual masses less than 2 cm (146). In both groups, the same 5-year survival rates were found, being 45 to 65 per cent (146). However, the morbidity in the group of irradiated patients was much higher.

Hacker *et al* (147) found whole abdominal radiation to be useful in patients who responded to primary chemotherapy and had minimal residual disease at second-look. The use of radiotherapy after chemotherapy, however, is often limited by the fact that a high percentage of patients is unable to tolerate this treatment (147, 148). Other investigators could not demonstrate a positive effect of salvage treatment with whole-abdominal irradiation (149, 150).

Reviewing the literature, it can be concluded that whole-abdominal irradiation is more effective than irradiation limited to the pelvis, and that the beneficial effect depends on the amount of residual tumor. Although whole-abdominal radiotherapy may be curative in selected patients, the role of radiotherapy in the treatment of ovarian cancer is limited and is still to be defined.

Chemotherapy

Single agent treatment. Alkylating agents were the first group of drugs widely used in ovarian cancer. Most series have reported response rates of 40% to 60% (72). For patients with advanced disease, median survival varied from 3.5 to 21 months (151). Melphalan, chlorambucil, cyclophosphamide and thiotepa have shown comparable response rates (72, 152, 153). Special care should be taken with these agents, because of the increased incidence of acute nonlymphocytic leukemia after long-term use (154).

The response rate of doxorubicin in untreated patients was found to be similar to that of alkylating agents (155).

Hexamethylmelamine is also effective in ovarian cancer (156, 157). A response rate of 31% has been reported in previously untreated patients with advanced disease. Although this is less than the rates which can be achieved with alkylating agents, hexamethylmelamine can be useful in combination with alkylating agents, since they have different mechanisms of action and no cross-resistance (157).

Cis-diamminedichloroplatinum (II) (cisplatin) is the most active non-alkylating drug in ovarian cancer. Response rates as high as 50% (158) and 67% (159) have been recorded after initial treatment with cisplatin in advanced stages of ovarian carcinoma. In previously treated patients, response rates of 33% and 52% were achieved with doses of 30 mg/m^2 and 100 mg/m^2 respectively (160).

Analogues of cisplatin with reduced toxicity have been developed. Carboplatin (JM8) and iproplatin (JM9) have shown similar response rates to those of cisplatin, with a reduced neuro-, nephro-, oto- and gastrointestinal toxicity, but an increased myelotoxicity (161-163). Newer drugs with activity against ovarian cancer further include ifosfamide, dihydroxybusulfan, prednimustine, galacticol, and mitomycin (163). Antimetabolites such as 5-fluorouracil and methotrexate are shown to be of little value as single agent treatment in ovarian cancer (151).

Combination chemotherapy. The use of combinations of drugs with different toxicities and mechanisms of action could theoretically lead to better results of treatment. Only drugs that are effective as a single agent should be used, and ideally, at their optimum dose and time interval (152).

Using non-cisplatin-based combination chemotherapy, a response rate of 47% and a median survival of 14 months was recorded (152). This is comparable to those obtained with monotherapy with alkylating agents. Only a few prospective randomized studies showed superiority of combination chemotherapy without cisplatin over melphalan (164, 165). In a study in which a therapeutic regimen consisting of hexamethylmelamine, cyclophosphamide, methotrexate and 5-fluorouracil (Hexa-CAF) was compared with melphalan treatment, a significantly higher overall response rate (75 *vs.* 54%) with more complete responses (33 *vs.* 16%), longer median survival (29 *vs.* 17 months), but also an increased toxicity was seen in the group of patients treated with Hexa-CAF (165). Other workers failed to confirm the therapeutic superiority of Hexa-CAF (166, 167).

Several cisplatin - containing regimens have been studied in prospective randomized trials. The combination of cisplatin and adriamycin was associated with a clinical response rate of 80% (168). This was significantly higher than in patients treated with cisplatin alone, or with a combination of thiotepa and methotrexate (168).

In a comparison of a combination of cisplatin and cyclophosphamide (CP) with cyclophosphamide alone in patients with advanced disease, the 2-year survival of patients treated with the combination regimen was 62%, compared to 19% for the patients treated with cycluphosphamide alone (169).

The use of a combination regimen containing cisplatin, chlorambucil and adriamycin led to similar results, as did the combination without adriamycin (170). Another three-drug regimen, consisting of cyclophosphamide, adriamycin and cisplatin (CAP), has been used by several workers, and an overall response rate of approximately 80% was recorded (171-173). In a prospective randomized trial, the CAP-regimen was compared with the CP-regimen (173). Objective response rates were similar for the two regimens (81% for CAP

vs 76% for CP), but a higher percentage of survival complete response was recorded in the CAP-group (62 *vs* 39%) (173). This advantage was especially observed in patients with small residual disease (50% *vs* 15%). However, survival was not statistically better for the CAP-group, since in some the tumor eventually recurred (173).

Using a combination of cyclophosphamide, hexamethylmelamine, adriamycin and cisplatin (CHAP) in patients with advanced ovarian cancer, an overall response of 92% was recorded, with a complete response rate of 42%, and a median survival of 24 months (174). However, in patients with tumor lesions larger than 2 cm, response was comparable to that of single agent treatment (174). In another study, an overall response rate of 79% was reported using CHAP, with a median survival of 31 months, compared with 50% response and 20 months' median survival in patients treated with Hexa-CAF (175). High response rates after the use of CHAP were also reported by others (98, 176, 177). In the group of patients treated with CHAP, earlier and more severe neurotoxicity was noted. A clinical trial to compare the effectiveness of a combination of cyclophosphamide, hexamethylmelamine, adriamycin and carboplatin (CHAC) is in progress (179).

Second-line chemotherapy. Second-line or salvage chemotherapy is used after failure of initial chemotherapy. The best results are obtained when limited prior chemotherapy is given. To date, the highest rates of clinical response are achieved using cisplatin alone or in combination treatment as a second-line treatment. Preliminary results of high-dose cisplatin administration, with hypertonic saline hydration to reduce nephrotoxicity, showed a response rate of 35% in patients who had relapsed on standard dose cisplatin (180). In another study, the use of high-dose cisplatin in 31 patients who did not have prior cisplatin treatment resulted in a response rate of 55% (181). Of nine patients who had already been exposed to lower doses of cisplatin, only 2 (22%) responded (181).

With some combination chemotherapeutic regimens, relatively high response rates (40 - 60%) were recorded in patients who received previous chemotherapy (182 - 190), but most responses were only partial and of short duration (4-6 months). Using a combination of hexamethylmelamine and cisplatin, a clinical response rate of 55% was reported (182). With adriamycin and cisplatin a response rate of 42% was recorded (183). The CAP-regimen showed rates of 30% (184) and 48% (185). The combination of hexamethylmelamine, adriamycin and cisplatin led to response rates of 40% (185) and 58% (186). In the trial of the Southwest Oncology Group, a response rate of 48% was achieved with a combination of adriamycin, 5-fluorouracil, hexamethylmelamine and cisplatin (187). Using the CHAP regimen, response rates of approximately 50% were recorded (188, 189). After schedule modification and intensification of the CHAP-regimen, a response rate of 72% was achieved (190).

Resistance to chemotherapeutic agents is a major problem in the treatment of patients with ovarian cancer. Recently, experimental model systems were developed in which mechanisms of resistance could be investigated (191). In some resistant human ovarian cancer cell lines a decreased accumulation of adriamycin was observed, which could be reversed by a calcium channel blocker (192). This has led to a clinical trial with adriamycin plus verapamil in refractory patients (193). Resistance to melphalan and cisplatin is found to be associated with increased levels of glutathione (191). The use of a specific inhibitor of glutathione may be useful (191).

Intraperitoneal chemotherapy Intraperitoneal (i.p.) delivery of chemotherapeutic agents offers the possibility of exposing the abdominal cavity to much higher concentrations than those which can be achieved by intravenous administration (194). This difference is caused by the fact that peritoneal clearance is generally slow in comparison to the total-body drug clearance. Drugs should be administered in a large volume (usually 2 liters) to make distribution through the whole intraperitoneal cavity possible (194 - 196). The i.p. distribution can be visualized using computerized tomography or radio-isotopes. The distribution may be limited by adhesions following surgery and the presence of multiple tumor masses. The effectiveness of i.p. treatment further depends on the size of the tumor lesions. Furthermore, the effectiveness depends on the ratio of plasma clearance to peritoneal permeability of the drug A high ratio is required for successssful treatment (194). The ideal i.p. drug should have a large peritoneal to plasma concentration range to be used, no local peritoneal toxicity and no cross-resistance with agents used for systemic treatment (195). In pharmacokinctic studies, a marked pharmacological advantage has been demonstrated for 5-fluorouracil, adriamycin, methotrexate, melphalan, cisplatin, carboplatin and some other drugs (196).

For i.p. delivery of chemotherapeutic agents a Tenckhoff catheter has commonly been used. Complications, including bowel perforation, infection, hemorrhage, abdominal pain, and catheter leakage, blockage, and displacement are reported in 67% of the cases (197). With a single-use Tenckhoff catheter, the occurrence of complications may be reduced (197). Another system used for i.p. administration of drugs is the totally implantable Port-A-Cath system (198). The major problem in i.p. treatment – the formation of a fibrous sheath around the catheter – occurs in both systems (199).

Cisplatin is currently the drug of choice in i.p. treatment (199). In general, no severe intra-abdominal complications of this drug are seen. In two studies of patients with minimal residual disease, i.p. cisplatin treatment resulted in surgically complete response in approximately one-third of the patients evaluated (200, 201). In i.p. cisplatin treatment, thiosulfate may be used to decrease nephrotoxicity (194).

Promising results using i.p. treatment have been reported in patients with small residual disease and in patients with malignant ascites. Probably i.p. treatment may also be of benefit in patients with a negative second-look laparotomy and in patients with early stage disease who are at high risk for relapse.

Controlled clinical trials should define the advantages of i.p. treatment in ovarian cancer.

Immunotherapy. The addition of an immunopotentiating agent should increase the capacity of tumor rejection via non-specific cellular activation and increase the recognition of and response to specific tumor antigens (203). In this aspect, *Corynebacterium parvum*, BCG, and ovarian cancer antiserum have been used in addition to chemotherapy in the treatment of patients with advanced ovarian cancer with promising results (203 - 206). Recently, the potential of other biologicals, such as interferons (207, 208) and interleukin-2 (209) has been investigated. Surgically proven responses were recorded in 5 of 11 patients (45%) with relapsing ovarian cancer using i.p. interferon-α2b (207). The responding patients all had residual tumors less than 5 mm, whereas none of the patients with larger residual masses responded.

Prediction of response to chemotherapy. Attempts have been made to predict the sensitivity of tumor cells to drugs in *in vitro* experiments. In this aspect, the effectiveness of therapeutic agents to those cells which are responsible for growth of the tumor by cell proliferation, *i.e.* the clonogenic cell population, is of special importance. The tumor stem-cell assay, first described by Hamburger and Salmon (210), may be used for predicting response of ovarian cancer to chemotherapy. In this technique, suspensions of monodispersed tumor cells are incubated with a number of drugs. After washing and incubation in a soft-agar medium for 2-3 weeks, the number of colonies is counted and compared with colony formation in the absence of drugs. Inadequate growth of tumor cells has been a major problem using this technique (211, 212).

In 90 to 99% of the cases where drug resistance was predicted, no clinical responses were seen (213, 214). In approximately 40% of the cases in which drug sensitivity of the tumor cells was predicted, it was found to be false-positive (213). At this moment, clonogenic cell assay directed treatment is still an experimental approach. Its value has to be established in prospective clinical trials. The tumor stem-cell assay may further be used for the screening and identification of new anticancer agents (215). Additionally, it may be employed in the detection of the presence of viable tumor cells, for example in the peritoneal fluid.

Non-clonogenic methods for studying drug sensitivity include the subcutaneous implantation of ovarian tumor tissue in nude mice, a congenitally athymic mouse mutant (216), and the subrenal capsule assay (217, 218). In the subrenal capsule assay, tumor tissue is transplanted under the renal capsule, a site which appears to enhance growth of xenografts by providing a rich vascular bed, where it proliferates during a short period. After six days tumor growth is determined.

Other approaches. Other interesting approaches in the management of ovarian cancer are the use of antifibrinolytic agents (219) and the application of platinum as a potentiator for radiotherapy (220). New experimental approaches may also result from the experimental studies with Mullerian inhibiting substance (MIS), that induces regresssion of the Mullerian duct in the male mammalian embryo. It has been demonstrated that MIS inhibits the growth of human ovarian cancer cell lines in the soft-agar assay (221) and in nude mice (222). Further studies on these approaches are indicated.

Table V. *Treatment with progestins in advanced ovarian cancer.*

Investigator	Agent[1]	Route[2]	Response rate	
Jolles (223)	A	i.m.	1/10	(10 %)
Varga (224)	A	i.m.	1/ 6	(17 %)
Ward (225)	B	i.m.	3/15	(20 %)
Timothy (226)	B	i.m.	1/ 7	(14 %)
Malkasian (227)	C	p.o.	2/ 9	(22 %)
Kauffman (228)	D	p.o.	1/11	(9 %)
Malkasian (229)	D	p.o.	1/19	(5 %)
Bergqvist (230)[3]	D	i.m.	3/ 4	(75 %)
Slayton (231)	D	i.m.	0/19	(0 %)
Mangioni (232)	D	i.m.	5/33	(15 %)
Mangioni (232)	D	p.o.	0/30	(0 %)
Aabo (233)	D	p.o.	1/27	(4 %)
Rendina (234)	D	i.m.	18/33	(55 %)
Tropé (235)	D	i.m.	1/25	(4 %)
Hamerlynck (236)	D	i.m.	1/41	(2 %)
Landoni (237)	D	i.m.	9/67	(7 %)
Weeth (238)	E	p.o.	0/ 2	(0 %)
Geisler (239)	E	p.o.	10/22	(45 %)
Sikic (240)	E	p.o.	4/47	(9 %)
Total			53/360	(15 %)

[1] A = 17-α-hydroxyprogesterone - 17-n-caproate.
B = 17-α-hydroxy - 19-norprogesterone - 17-n-caproate.
C = 6, 17-α-dimethyl - 6-dehydroprogesterone.
D = medroxyprogesterone acetate.
E = megestrol acetate.
[2] i.m.: intramuscular.
p.o.: per os.
[3] All stages.

Endocrine therapy

The fact that the ovary is not only the main source of estrogens and progesterone but also a target organ for these and other hormones suggests that epithelial ovarian cancer may be an endocrine-related tumor. Investigations to determine the role of hormones in ovarian cancer include empirical treatment with hormonal agents in patients with advanced disease, studies of steroid receptors in ovarian cancer and animal experiments.

Steroidal and anti-steroidal agents. Estrogens were thought to be possible promoters of ovarian carcinoma and estrogen counter-acting progestins have therefore been tested in the treatment of ovarian cancer (223 - 240) (Table V). In most of

Table VI. *Treatments with progestin in combination with other agents in advanced ovarian cancer.*

Investigator	Agents[1]	Response rate	
Ward (225)	Prog. + Estrogen	0/ 8	(0%)
Julius (241)	Prog. + Estrogen	2/11	(18%)
Freedman (242)	Prog. + Estrogen	9/65	14%)
Myers (243)	Prog. + Tamoxifen	1/ 1	(100%)
Jakobsen (244)	Prog. + Tamoxifen	0/17	(0%)
Guthrie (245)	Prog. + Endoxan	41/54	(76%)
Bergqvist (230)[2]	Prog. + Melphalan	28/33	(85%)
Prospective controlled studies:			
Bonomo (246)	MPA + CAF		(59%)
(32 patients)	CAF		(55%)
Jungi (247)	MPA + Cyclophosphamide		(43%)
(71 patients)	Cyclophosphamide		(58%)
	CF		(42%)
Mennince (248)	MPA + CAP		(0%)
(12 patients)	CAP		(50%)

[1] MPA Medroxyprogesterone acetate.
CAF Cyclophosphamide, Adriamycin, and 5-Fluorouracil.
CF Cyclophosphamide, 5-Fluorouracil.
CAP Cyclophosphamide, Adriamycin, and cis-Platin.
[2] All stages.

Table VII. *Treatment with anti-estrogens in advanced ovarian cancer.*

Investigator	Agent	Response rate	
Myers (243)	Tamoxifen	2/ 2	(100%)
Schwartz (249)	Tamoxifen	1/ 13	(8%)
Landoni (237)	Tamoxifen	0/ 55	(0%)
Shirey (250)	Tamoxifen	0/ 22	(0%)
Weiner (251)	Tamoxifen	3/ 31	(10%)
Slevin (252)	Tamoxifen	0/ 22	(0%)
Total		6/145	(4%)

the early reports, both subjective and objective improvement have been reported as response. In Table V, however, only the objective responses by current standards are listed.

In most studies, true objective response was recorded in only about 10 to 15% of the patients, with an additional 10% of patients with stabilization of disease. It has been shown that intramuscular administration of medroxyprogesterone acetate (MPA) is more effective than oral administration (232). In the study of the EORTC Gynecological Cancer Cooperative Group of MPA (500 mg daily i.m. for four weeks, with a maintenance dosage of 1000 mgMPA weekly), only one partial response was recorded among 41 patients (236). The duration of this response was only 20 weeks. With high-dose megestrol acetate treatment, 1 complete and 3 partial responses were recorded among 47 patients with advanced disease. The survival for patients who responded to treatment was significantly longer than for nonresponding patients (240).

In a study of 33 patients with advanced endometrioid ovarian cancer treated with MPA, 18 responses (55%) were recorded (234). It must be noted, however, that 79% of these tumors were well differentiated. In the same study, 10 patients with well differentiated stage I-II endometrioid ovarian carcinomas were treated with MPA. Five of them had a complete response (234). Geisler (239) recorded 6 complete and 4 partial responses among 22 patients treated with megestrol acetate. Survival of patients who achieved a complete response was 5 to 36 months. Patients with partial remissions survived 4 to 10 months (239).

Progestins have also been used in combination with other agents (225, 230, 241-248) (Table VI). Freedman *et al* (242) studied the effect of a sequential combination of MPA and ethinylestradiol in 65 patients with refractory ovarian carcinomas. Nine (14%) patients responded and 13 (20%) had stable disease. Vascular complications were seen in three patients. In one of the patients hemiplegia occurred. In a phase-II study of cyclic treatment with MPA and tamoxifen, no objective responses were recorded (244). In a review of patients postoperatively treated with MPA plus melphalan, a response rate of 85% was reported (230). However, about half of these patients had early stage disease. Guthrie (245), studying 54 patients treated with Gestronol and continuous oral cyclophosphamide, recorded 25 complete (46%) and 16 partial responses (30%). None of these patients had received previous radio- or chemotherapy. However, these high response rates with a combination of progestins and alkylating agents could not be achieved in three prospective, controlled studies, in which treatment with chemotherapy plus MPA was compared with chemotherapy alone (246 - 248).

The synthetic anti-estrogen tamoxifen has also been used in the treatment of ovarian cancer (237, 242, 249-252) (Table VII). Landoni *et al* (237) recorded no responses among 55 patients with advanced disease treated with tamoxifen. However, 19 patients (35%) had stable disease (237). Another study also recorded no objective responses, but objective stabilization of disease was seen in 80% of 22 refractory patients treated with 20 to 40 mg tamoxifen daily (250). In a study of 31 patients with recurrent disease, 1 complete and 2 partial responses were seen (251). Six patients (19%) had stable disease. Median survival was 16 months for responders and 7 months for nonresponders (251).

Recently, the androgen fluoxymesterone was used in the treatment of 16 ovarian cancer patients (253). No responses were recorded (253). The LHRH agonist D-Trp-6-LHRH (Decapeptyl) has also been used in the treatment of ovarian cancer (254, 255). In a study of 10 women in whom agonist treatment was started after failure of chemotherapy, tumor stabilization or shrinkage was seen in 50% of the cases (255). The rationale for this treatment is the down regulation of gonadotropins and thus the minimization of the steroid output, if any, of the ovarian carcinoma. It is not yet known whether total down regulation of gonadotropins can be achieved with this form of treatment in postmenopausal women.

Summarizing the literature, a response rate of 15% was recorded among 360 unselected patients treated with progestins. Most responses were seen in patients with serous and endometrioid tumors. Duration of response was mostly short. Stabilization of disease was seen in approximately 10% of the patients. Using progestins in combination with chemotherapeutic agents, high response rates were recorded (76% and 85%), but these could not be achieved in prospective controlled studies. The combination of MPA with ethinylestradiol showed response rates of 14 to 18%. It has been postulated that simultaneous and sequential administration of a potent estrogen and progestin may provide therapeutic advantage and increase responsiveness of the tumor to treatment (242). A response rate of only 4% was noted in the six studies of tamoxifen treatment. Stabilization of disease was seen in up to 80% of the patients (250).

It must be stated, however, that the meagre response rates using endocrine treatment may be partly due to the fact that endocrine treatment was often initiated after failure of other therapeutic approaches, as a last resort. Since endocrine treatment has lower toxicity than current chemotherapeutic agents and seems to improve the quality of life in patients with advanced ovarian cancer, further investigations are necessary to determine its role in ovarian cancer. Additionally, endocrine treatment deserves to be evaluated as a primary treatment due to the increase in the information available, discussed in the following section.

Steroid receptors. Steroids interact with specific receptor proteins inside the target cell, after entering the cell probably by diffusion. The complex of hormone and receptor is translocated to the nucleus and interacts with specific genomic sites on the chromatin, resulting in the stimulation of specific RNA and proteins. In this classical "two-step" model, receptors are thought to be localized in the cytoplasm in an inactive form. After activation by binding with the ligand, the complex is transported into the nucleus (256, 257). Recently, receptors have also been localized specifically in the nucleus (258, 259). Irrespective of this controversy as to the precise location of the steroid receptors in the cell, it is clear that their presence in specific cells indicates that those cells may be sensitive to specific hormones.

Information of the predisposition of tumors to hormonal control is an important parameter for selecting a specific endocrine treatment. To date, a number of studies have been performed in the management of breast cancer, based on the presence of specific steroid receptors. Additionally, estrogen (ER) and progesterone receptors (PR) have been convincingly demonstrated in cancer of the breast and their importance as a predictor of response to hormone treatment and as a prognostic indicator has been extensively recorded (260, 261). Breast tumors containing ER are likely to respond to endocrine treatment, whereas tumors without ER respond less frequently. In the presence of both ER and PR the likelihood of response is higher (262).

In the last decade, several reports on the presence of steroid receptors in ovarian cancer have been published (230, 234, 263-304) (Table VIII). The criteria for positivity vary among the series. Furthermore, differences in reported frequencies may be attributed to different assay methods, different tumor types, and the limited number of tumors studied. If the criteria of the authors are accepted and the overall frequencies are analysed, estrogen receptors are seen in 63%, progesterone receptors in 48%, and androgen receptors in 69% of the tumors. The presence of both ER and PR is seen in 36% of the epithelial ovarian cancers. Estrogen receptors without PR are seen in 28% of the cases, whereas in 25% neither ER nor PR are present. The presence of PR in the absence of ER is seen in only 11% of the tumors (Table IX). This may be partly due to the fact that PR synthesis is an estrogen related process (305).

Compared to ER and PR, the presence of AR has been investigated in only a few studies (265, 269, 276, 277, 282, 294, 304). In the study of Kuhnel *et al* (304), 85 of 94 tumors (90%) contained AR. In 19% of the cases PR were seen in combination with AR and in the absence of ER (304), suggesting that androgens may also induce PR synthesis. The fact that ovarian cancer is predominantly seen in postmenopausal women might be attributed to the predominance of AR in ovarian tumors, since postmenopausal ovaries produce little or no estrogen but continue to produce androgens. Since AR are regulated by androgens, it is likely that accumulation of androgens takes place in ovarian tumors without conversion to estrogens. This androgen to estrogen conversion is partly controlled by the enzyme aromatase. It has been demonstrated that tumors without aromatase activity are AR positive (306). In these tumors, androgens are not converted to estrogens and can influence the tumor cells through the AR. In these patients treatment with anti-androgens might be of value.

An inverse correlation between the presence of ER and tumor growth kinetics has been demonstrated in breast cancer (307). In endometrioid cancer of the ovary, the presence of ER and PR is reported to be a reliable criterion in the selection of patients for progestin treatment (234). However, further studies are required to determine the correlation between receptor content and response to treatment in ovarian cancer. In these studies, not only the presence or absence of receptors should be compared to response to treatment, but also their concentrations.

Differences in results of receptor studies may be partly attributed to the fact that within one tumor, the cell population may be heterogeneous for hormone binding (308). This might also influence endocrine treatment. Probably, only the proliferation of receptor containing cells can be influenced and not the proliferation of receptor negative cells.

Since endocrine treatment is most frequently used after failure of chemotherapy, it is of importance to know the effect of chemotherapeutic agents on receptor levels. It has been demonstrated that ER continues to be expressed in patients with advanced disease after chemotherapy (291, 295). If failure of hormonal treatment is due to low concentrations of PR,

Table VIII. *Estrogen (ER), progesterone (PR), and androgen receptor (AR) presence in epithelial ovarian cancer.*

Investigator	Number of patients	ER + no.	(%)	PR + no	(%)	AR + no.	(%)
Taylor (263)	8	0	(0)				
Kang (264)	5	2	(40)				
Friberg (265)	4	1	(25)			2	(50)
Ribeo (266)	29	29	(100)				
Dupont (267)	57	29	(50)	18	(32)		
Friedman (268)	34	34	(100)	34	(100)		
Grilli (269)	8	5	(63)	5	(63)	6	(75)
Hähnel (270)	4	1	(25)				
Holt (272)	16	8	(50)	3	(19)		
Kauppila (272)	25	10	(40)	10	(40)		
Jänne (273)	21	15	(71)	8	(38)		
Stedman (274)	11	5	(45)				
Stedman (274)	4			1	(25)		
Bergqvist (230)	11	8	(73)				
Bergqvist (230)	8			3	(38)		
Greasman (275)	18	11	(61)				
Greasman (275)	15			4	(27)		
Galli (276)	9	6	(67)	5	(56)	8	(89)
Hamilton (277)	12	5	(42)			11	(92)
Holt (278)	29	24	(83)				
Farley (279)	2	1	(50)	2	(100)		
Hähnel (280)	22	10	(45)	3	(14)		
Pfleiderer (281)	44	27	(61)	18	(41)		
Quinn (282)	37	17	(46)				
Quinn (282)	36			11	(31)		
Quinn (282)	31					10	(32)
Rendina (234)	43	35	(81)	31	(72)		
Saarikoski (283)	7	4	(57)	2	(29)		
Schwartz (284)	30	16	(53)				
Ford (285)	39	15	(38)	6	(15)		
Jones (286)	42	35	(83)	18	(43)		
Kauppila (287)	68	60	(88)	55	(81)		
Leibach (288)	21	13	(62)	13	(62)		
Pollow (289)	43	31	(72)	20	(47)		
Spona (290)	68	40	(59)	27	(40)		
Teufel (291)	153	97	(63)				
Teufel (291)	150			69	(46)		
Vierriko (292)	45	40	(89)	41	(91)		
Willcocks (293)	49	28	(57)	14	(29)		
Würz (294)	32	28	(88)	9	(28)	10	(31)
Geyer (295)	171	112	(64)	84	(49)		
Gronroos (296)	21	13	(62)	12	(57)		
Lantta (297)	50	24	(48)	22	(44)		
Richman (298)	52	38	(73)				
Schwartz (299)	113	58	(51)				
Schwartz (299)	99			50	(51)		
Iversen (300)	31	16	(52)	15	(48)		
Sutton (301)	32	22	(69)				
Sutton (301)	20			7	(35)		
Toppila (302)	60	27	(45)	32	(53)		
Agarwal (303)	16	6	(38)	11	(69)		
Kühnel (304)	94	52	(55)	49	(52)	85	(90)
Total	1686	1058	(63)				
	1470			712	(48)		
	190					132	(69)

Table IX. *Presence of estrogen (ER) and progesterone receptors (PR) in combinations in epithelial ovarian cancer.*

Investigator	Number of patients	ER+PR+ no.	(%)	ER+PR− no.	(%)	ER−PR+ no	(%)	ER−PR− no	(%)
Holt (271)	16	3	(19)	5	(31)	0	(0)	8	(50)
Kauppila (272)	25	10	(40)	9	(36)	1	(4)	5	(20)
Jänne (273)	21	8	(38)	7	(33)	0	(0)	6	(29)
Bergqvist (230)	8	3	(38)	2	(25)	0	(0)	3	(38)
Greasman (275)	15	4	(27)	5	(33)	0	(0)	6	(40)
Galli (276)	9	5	(56)	1	(11)	0	(0)	3	(33)
Hähnel (280)	12	3	(25)	7	(58)	0	(0)	2	(17)
Pfleiderer (281)	44	11	(25)	16	(36)	7	(16)	10	(23)
Quinn (282)	32	8	(25)	8	(25)	3	(10)	13	(41)
Jones (286)	42	15	(36)	20	(48)	3	(7)	4	(10)
Kauppila (287)	68	52	(76)	8	(13)	3	(5)	5	(7)
Leibach (288)	21	9	(43)	4	(19)	4	(19)	4	(19)
Pollow (289)	43	17	(40)	14	(33)	3	(7)	9	(21)
Spona (290)	68	22	(32)	18	(26)	5	(7)	23	(34)
Teufel (291)	150	54	(36)	44	(29)	16	(11)	36	(24)
Willcocks (293)	49	10	(20)	18	(37)	4	(8)	17	(14)
Würz (294)	32	9	(28)	19	(59)	0	(0)	4	(13)
Geyer (295)	171	67	(39)	47	(28)	19	(11)	38	(22)
Lantta (297)	50	14	(28)	10	(20)	8	(16)	18	(36)
Iversen (300)	31	10	(32)	6	(19)	5	(16)	10	(32)
Toppila (302)	60	20	(33)	7	(11)	12	(20)	21	(34)
Agarwal (303)	16	5	(31)	1	(6)	6	(38)	4	(25)
Kühnel (304)	94	31	(33)	21	(22)	18	(19)	24	(26)
Total	1077	390	(36)	297	(28)	117	(11)	273	(25)

induction of receptors might increase activity (301). Tamoxifen may increase the number of PR and decrease the number of ER. From this point of view, alternating treatment with tamoxifen and progestins might be useful (301). However, as discussed previously, in a trial of 29 patients with advanced disease, no beneficial effect of this combination could be demonstrated (244).

In metastases or recurrences of ovarian carcinomas steroid receptors are rarely seen when the primary tumor is receptor negative (271, 278, 284, 289, 291, 292, 295, 299, 309).

Several authors correlated the presence of receptors with prognostic factors as histology, stage and age. No correlation between receptor content and stage of disease could be demonstrated (266, 284, 286, 295, 300, 301, 310). Some workers found no correlation between the presence of ER and histologic type or grade (266, 284, 286, 289, 295, 298, 310). Others reported endometrioid tumors to contain more PR, often in association with ER (234, 268, 285, 291, 301, 304), while serous tumors are often ER positive (282, 291). In mucinous (282, 285, 287) and in clear cell tumors (291, 295) lower amounts of steroid receptors were detected. Friedman *et al* (268) reported that most ER positive tumors were seen among postmenopausal women with poorly differentiated tumors, whereas PR positive tumors were associated with well differentiated tumors in premenopausal women. The association

between ER and poorly differentiated tumors has also been described by other investigators (291). Others found that well differentiated tumors more frequently contained ER (285, 300) or both ER and PR (275, 282). The occurrence of higher levels of ER (294, 301) and lower levels of PR (294, 295) in tumors of postmenopausal women are also reported. Steroid receptor presence has also been correlated with response to chemotherapy and prognosis. Although some investigators could not demonstrate such a relationship (284), others found PR negative tumors to have a better (295), or worse (275, 290, 294, 300) prognosis. Agarwal *et al* (303) reported that patients with low ER and PR had a better response to chemotherapy, whereas the other tumors showed a definite response to progestins *in vitro*. Recently, we also investigated the relationship between receptor content and survival and found that patients with tumors containing high levels of PR had better survival than patients with tumors that were low positive or negative for PR (to be published).

Since clinical and experimental data show a role for gonadotropins in the development of ovarian cancer, gonadotropic hormone (311, 312) and gonadotropic releasing hormone (313) binding has also been demonstrated in ovarian tumors. However, the clinical significance of these observations has to be further determined.

At present, hormone receptors are not used routinely for the characterization of gynecologic tumors or selection of treatment. However, information about receptor content may become useful for the selection of the appropriate treatment. Patients with aromatase positive tumors might benefit from progestins, anti-estrogens and aromatase inhibitors. Since all aromatase negative tumors are found to be AR positive, anti-androgen treatment might be of value in these cases. The combination of gonadotropin down regulation using chronic LHRH administration along with anti-androgen treatment may be even more effective in the treatment of ovarian cancer, compared to either of them alone.

The response of *in vitro* cultured cells to different hormones and anti-hormones may also be helpful in the selection of the adequate treatment and it may provide a basis for rational use of endocrine treatment. Furthermore, steroid receptors estimation may be used in the future in cases of metastases with unknown primary tumor site, while radiolabeled ER ligands may be used for imaging and treatment of ER-rich cancers (314, 315).

Conclusions

Ovarian cancer ranks first among the causes of death from gynecologic malignancies. Although there is evidence that endocrine, environmental, and genetic factors may be of importance in the development of ovarian cancer, the precise pathogenesis has still to be clarified. As a result of this, it is difficult to identify a population at risk and to develop guidelines for prevention. In most cases, the disease is diagnosed at a time when dissemination has already taken place. Currently, ultrasound is the only useful technique which may lead to earlier diagnosis of the disease. Tumor markers are not specific enough to be used in the screening for ovarian cancer. However, they can be of great help in monitoring the disease.

Stage, histologic grade, and the size of residual tumor lesions after surgery are important prognostic factors.

Treatment of ovarian cancer generally consists of surgery, followed by radio- or chemotherapy. At surgery, the stage of disease should be accurately determined and tumor masses should be removed as far as possible. The use of second-look surgery in the management of patients with ovarian cancer makes it possible to evaluate response to treatment with more accuracy. With cisplatin containing chemotherapeutic regimens, response rates of 80% are achieved. However, these response rates have not yet resulted in improvement of long-term survival. New approaches such as intraperitoneal chemotherapy and immunotherapy may be of value in this aspect.

Endocrine treatment has frequently been used empirically in ovarian cancer. Despite the fact that in most cases endocrine treatment was administered after failure of previous treatments, response rates of approximately 10% were recorded. In general, although endocrine treatment has the least amount of side effects, it has not been fully exploited. Among estrogen, progestin, and androgen receptors recent investigations have emphasized the presence of androgen receptors in ovarian cancers, suggesting that the tumor may be dependent on androgens, particularly in postmenopausal women. The available information strongly supports a need for initiation of clinical trials to evaluate the efficacy of antiandrogens alone, and in combination with LHRH analogues.

New promising leads such as predicting drug sensitivity and methods for reversal of drug resistance may further improve the outlook for patients with ovarian cancer. However, managing ovarian cancer remains difficult, and at present many important questions about ovarian cancer cannot be answered.

Acknowledgements

This work was supported by The Netherlands Cancer Foundation Academisch Ziekenhuis Vrije Universiteit, Maurits en Anna de Kock Fonds, The Netherlands.

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Received January 14, 1988
Accepted March 31, 1988

ANTICANCER RESEARCH 8: 435-442 (1988)

Radioimmunolocalisation of Bladder Tumors Xenotransplanted in Nude Mice

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Abstract. *We have previously reported on the derivation of mouse monoclonal antibodies (Mabs), identifying several cell surface antigens selectively associated with cancer of the urinary bladder (TCC) (1-4). Three of these Mabs (4E8, SK4H and 8F4) have now been assessed for their ability to localise TCC-tumor xenografts in nude mice. The biodistribution of ^{125}I-labeled intact Mabs as well as the corresponding Fab and F(ab')$_2$ fragments from two of them were investigated in animals carrying TCC tumors or antigen negative control tumors. Using direct measurements of excised tissues, all three antibodies were seen to accumulate specifically in the TCC tumors, giving tumor to normal tissue ratios of between 3 and 20 depending on the Mab used and the time after injection. Antibody fragments were generally more efficient in their localisation, mainly due to a dramatic reduction in the blood background as compared to intact Ig. One of the antibodies, 4E8, was also employed for external imaging with gamma camera scintigraphy using ^{111}In or ^{131}I as tracers. Excellent visualisation of the tumor sites could be obtained both with Fab fragments and intact antibody within 12-24 hours after injection. As expected, background radioactivity was significantly lower with fragments than with whole molecules. ^{111}In labeled antibodies appeared in all instances to be superior to the corresponding ^{131}I conjugates. In conclusion, the present study indicates that the three anti TCC antibodies may become useful for the in vivo diagnosis of bladder cancer in man.*

Immunolocalisation has gradually reached a stage where it has begun to fulfill some of the expectations associated with it. Of major importance in this progress has been the derivation of monoclonal antibodies, not only for their proven potential of defining new and suitable target antigens but also for their convenient accessibility in the amounts and homogeneities required. Although the technique of *in vivo* localisation is becoming a clinically useful option for the diagnosis of certain tumors, this is still far from the case for most tumors. One type of malignancy that has not been extensively investigated in this respect is cancer of the urinary bladder. However, recent developments in defining a number of antigens associated with this disease (for review see 5) have permitted such studies, and a few reports on the localisation of this tumor type in the nude mouse model have been recently published (6-8).

Using the same model, we have now investigated the *in vivo* behavior and tumor targeting capacity of three of our anti-TCC antibodies (4E8, SK4H ad 8F4). The target antigens for these Mabs have all been defined as cell surface antigens with a very selective expression on bladder tumor tissue (1-4). Localisation was performed with both intact Mab as well as antibody fragments and was measured either directly on excised tissue or by immunoscintigraphy. The biodistribution of ^{111}In- and ^{131}I-conjugated antibodies was compared.

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Key Words: Radioimmunolocalisation, human bladder cancer, monoclonal antibodies, nude mice.

Materials and Methods

Cell lines. Three established TCC cell lines T24, TCCSuP and HU549 (1) were used. Antigen negative cell lines used as controls included the colon carcinoma line LS174T (9) and the melanoma line, 1585 (10).

Monoclonal antibodies. Three different monoclonal antibodies, 4E8, SK4H and 8F4 (all IgG2a), directed against different TCC associated antigens were used. The tissue specificity of these have been described previously by us (1-4). In addition, a mouse Mab (IgG1) against human growth hormon (11) was used as a negative control. All atibodies were purified from culture supernatants by affinity chromatography on Protein A-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden).

Preparation of F(ab')2 and Fab fragments. F(ab')2 fragments of Protein A purified Mab were obtained by incubation for 12 hrs at 37°C with 3% (wt/wt) pepsin (Worthington Biochemicals, Freehold, N.J.) in 0.12 M acetate buffered saline (pH 4.0) (12). The digests were centrifuged (3000 rpm, 10 min), dialyzed against phosphate buffered saline (PBS) (pH 7.0) and centrifuged (3000 rpm, 10 min). Before use, the fragments were separated on a FPLC gel column (Superose 12, Pharmacia).

Monovalent Fab fragments were obtained by incubation for 3 hrs at

Pergamon

0278-6915(94)00105-7

Fd Chem. Toxic. Vol. 32, No. 12, pp. 1173–1184, 1994

0278-6915/94 $26.00 + 0.00

Review Section

BIOLOGICAL EFFECTS OF COSMETIC TALC

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(Accepted 16 June 1994)

Summary—A review of the literature reveals two primary issues: (1) a weak, but not causal, association of hygienic use of cosmetic talc and ovarian cancer; (2) lung changes in animals exposed to talc aerosol concentrations that resulted in lung overload. The evidentiary weight of the most significant of the epidemiological and laboratory studies and their biological significance for human risk assessment are briefly discussed. Publications describing granulomatous lesions attributed to talc on surgical gloves, and consequences of accidental inhalation of baby powder by infants are also reviewed. The literature reviewed does not provide any convincing evidence that pure cosmetic talc, when used as intended, presents a health risk to the human consumer.

Introduction

Hildick-Smith (1976 and 1977) and Lord (1978) have reviewed the older literature relating to talc. Hildick-Smith concluded that the normal use of cosmetic-grade talc does not present a health hazard. In his review of biological effects of talc in laboratory animals, Lord found the mineral to be fibrogenic but he observed that the fibrotic response was a function of the administered dose and 'that there are levels of exposure that are tolerable'. In none of the studies reviewed was there any indication of neoplasia.

A computer search of the literature of the last 15 years provided well over 700 titles/abstracts on various aspects of talc and talc issues. Of these, 137 were selected for review as they describe epidemiological, clinical and relevant animal and *in vitro* studies. Papers on exposures to *industrial* talc, often containing more toxic impurities, such as asbestos fibres, were excluded as irrelevant to the safety of *cosmetic* talc when used as intended. Studies of lesions following intravenous injection of talc by drug addicts were also excluded because of unintended use. The term 'cosmetic talc' in this review refers to talc of high purity as produced by today's responsible manufacturers.

A review of the selected titles reveals two primary issues, namely (1) hygienic use of cosmetic talc and ovarian cancer risk, and (2) health effects of inhaled cosmetic talc. In addition, a number of papers describe granulomatous lesions attributed to talc on surgical gloves, and consequences of accidental inhalation of baby powder by infants. This review focuses on publications dealing with these and related issues.

Abbreviations: BAL = bronchoalveolar lavage fluid; CI = confidence interval; NTP = National Toxicology Program; OR = odds ratio; RR = relative risk; SCE = sister chromatid exchange; UDS = unscheduled DNA synthesis.

Hygienic use of talc and ovarian cancer risk

The Harlow paper

Publication of a paper by Harlow *et al.* (1992) revived concern that chronic talc use in genital hygiene might be associated with an increased risk of epithelial ovarian cancer. That concern was originally raised 21 years earlier in a paper by Henderson *et al.* (1971) on the same subject. Harlow *et al.* (1992) provided the most detailed results to date on the relationship between perineal talc exposure and ovarian cancer. The study is the only one to date designed specifically to address this issue. It is among the largest studies (greatest statistical power) and one of the few to use closely age-matched neighbourhood controls.

The authors interviewed 235 white women diagnosed with epithelial ovarian cancer between 1984 and 1987 in Boston and 239 population-based matched controls. Approximately 50% of cases and 40% of controls used talc on the perineum, undergarments, sanitary napkins or diaphragms. This resulted in a 1.5 odds ratio (OR) for ovarian cancer [95% confidence interval (CI) 1.0–2.1]. Perineal exposure to talc resulted in significantly elevated risks in the subgroups who applied it directly as a body powder (OR 1.7, 95% CI 1.1–2.7), on a daily basis (OR 1.8, 95% CI 1.1–3.0), and for more than 10 years (OR 1.6, 95% CI 1.0–2.7). The greatest risk was

observed in women with more than 10,000 talc applications during years when they were ovulating and had an intact genital tract (OR 2.8, 95% CI 1.4–5.4); however, this applied to only 14% of the women with ovarian cancer.

The authors acknowledge the inherent limitations of epidemiological studies. In their statistical analyses they adjusted for several confounding variables and conceded the existence of additional but intangible variables. In-person interviews included enquiries into dietary history but it is not clear whether, for example, lactose consumption and coffee consumption were determined and adjusted for; both substances have been associated with a significant increase in the risk of ovarian cancer (Cramer *et al.*, 1989; Whittemore *et al.*, 1988). Except for these two cases, no diligent efforts appear to have been made to identify other confounding variables that could account for increased incidence of ovarian cancer, such as vulvovaginal diseases (e.g. yeast infections and trichomoniasis) and obesity. McGowan *et al.* (1979) reported a higher incidence of rubella infection between the ages of 12 and 18 years in ovarian cancer patients than in controls, but there was a lower frequency of mumps (West, 1966) and of pneumonia and influenza (Joly *et al.*, 1974).

Harlow *et al.* (1992) mention in one sentence that they used meta-analysis in the statistical evaluation of their epidemiological data. Meta-analysis requires rigid criteria that must be strictly observed to yield meaningful results (Chalmers *et al.*, 1989; Yusuf *et al.*, 1985). Without details on the meta-analytical procedures used by the authors it is not possible to determine whether the use of meta-analysis was appropriate, and if so, whether it was used appropriately.

The sample population in the Harlow study is limited in numbers and restricted to white women in the Boston area. With an overall adjusted OR of 1.5 (CI 1.0–2.1) the results are statistically barely significant. Extrapolating from results in that small, restrictive group to the general population will require caution.

The authors conclude that their "data support the concept that a life-time pattern of perineal talc use may increase the risk for epithelial ovarian cancers", but they do not claim to have established a cause–effect relationship.

Related literature

Harlow *et al.* (1992) cite a number of literature references on talc translocation and putative association between hygienic use of talc and ovarian cancer. These references contribute to the ambiguity of the database as some of them suggest translocation or support an association whereas others do not; again, others suggest alternative explanations for greater-than-average incidences of ovarian cancer. For example, Cramer *et al.* (1989) suggested that lactose consumption might be a dietary risk factor and transferase a genetic risk factor. Whittemore *et al.* (1988) observed in a well-designed study (188 cases, 539 controls) that the ovarian cancer risk in women who had consumed coffee for more than 40 years was 3.4 times that of women who had never regularly consumed coffee. This relationship is more robust ($P = 0.01$) than the marginal significance of the relationship with perineal talc use [relative risk (RR) = 1.40, $P = 0.06$].

A causal relationship between talc and ovarian cancer requires that talc particles, administered to the perineum or to the vagina, can translocate to the ovaries. Whether or not inanimate particles without locomotion of their own can do this without assistance has been the subject of a number of investigations in humans and animals. Results have been inconsistent and ambiguous. For a discussion of relevant findings published before 1986, reference is made to Wehner *et al.* (1986).

Henderson *et al.* (1986) observed talc particles in the ovaries of all eight ex-breeder rats after *intrauterine* deposition of a talc suspension. Following *intravaginal* deposition of talc suspension, talc particles were found in the ovaries of only two of six ex-breeder rats. Retrograde flow of menstrual products into the peritoneal cavity through the oviducts is well known; therefore, translocation to the ovaries of talc particles deposited in the uterus in 250 μl saline, perhaps aided by the hydrostatic pressure of the saline solution during and after deposition, is not surprising. Yet in two of six rats the inanimate talc particles managed to cross the cervical barrier and reach the ovaries, perhaps also aided by hydrostatic pressure during deposition. Henderson *et al.* (1986) express a guarded opinion on the association of talc with cancer, stating that "carcinogenic activity of talc has not been established although its ubiquitous presence in the environment and its elemental similarity to asbestos has brought it under suspicion". Elemental similarity, of course, does not necessarily mean similarity in biological effects. It is well known that differences in physical properties, such as shape and surface characteristics, between elementally similar substances are responsible for significant differences in their biological effects. The pharmacological action of certain elementally and structurally identical agents (isomers) depends on whether they are dextrorotatory or levorotatory.

A remarkably large number of talc particles were found by Henderson *et al.* (1979) in human ovarian tissue, namely from 6900 to 55,100 particles/g wet weight tissue in three normal ovaries, from 17,400 to 24,300 in three cystic ovaries, and from 6400 to 24,300 in three ovarian adenocarcinomas. In another reference (Henderson *et al.*, 1978), 2×10^5 particles/mm^3 are reported from oxygen incineration studies of human ovarian tissue.

Talc particles in human and animal tissues can be identified by X-ray fluorescence and X-ray diffraction

(Wehner *et al.*, 1977c) and by neutron activation of talc before animal exposure and subsequent γ-ray analysis of animal tissues for at least two suitable radionuclides (Wehner *et al.*, 1986 and 1977b). The neutron activation technique eliminates contamination problems during sample collection and processing.

Talc particles found, for example, *on* ovarian tissue might be contaminants deposited during sample collection and processing. For talc particles *in* ovarian tissue, contamination during sample collection and processing can be ruled out.

Wehner *et al.* (1985 and 1986) investigated talc translocation in Cynomolgus monkeys (*Macaca fascicularis*). The female of this species resembles the human female closer than any other animal model in the physiological and anatomical parameters of interest. The authors deposited a suspension of neutron-activated talc in the posterior fornix of the vagina on 30 consecutive work days (45 calendar days), that is, through at least one menstrual cycle (Wehner *et al.*, 1986). 2 days after the 30th talc application the animals were killed. The following samples underwent γ-ray analysis: vagina with the cervix of the uterus; uterus; the entire oviduct in three sections; ovaries; and peritoneal lavage fluid. Only the vagina-with-cervix samples—the site of deposition—contained varying quantities of talc. No talc was detected in any of the other samples. The detection limit of the neutron activation/γ-ray analysis technique under the described experimental conditions was approximately 0.5 μg talc (Wehner *et al.*, 1986) or about 1/230,000–1/245,000 of the estimated talc deposition in the posterior vaginal fornix (Wehner *et al.*, 1985).

Harlow and Weiss (1989) interviewed 116 patients with borderline ovarian tumours on their use of hygienic powders and found 'no appreciably altered risk' in the use of baby powder or cornstarch. However, the unspecified smaller number among those women who use deodorizing powders alone or in combination with other talc-containing powders had a 2.8-fold higher risk than 158 women without perineal exposure to powder. In the light of this newly discovered confounding variable, Harlow and Weiss recommend that the specific type(s) of powder used should be identified in future studies on hygienic powder use and ovarian tumours.

Cramer *et al.* (1982) observed a statistically significant ($P < 0.003$) relationship between epithelial ovarian cancer and talc used for dusting the perineum or sanitary napkins in 215 women. However, Cramer *et al.* (1982) found no relationship between ovarian cancer and talc exposure from dusting condoms or diaphragms, even though talc, in the latter applications, is deposited close to the cervical os. Hartge *et al.* (1983) made a similar observation from their epidemiological study. Their data indicate that the use of talc on a diaphragm did not appear to increase risk and that there was no overall association between talc use and risk of ovarian cancer although a small group of women who specifically reported 'genital use' of talc showed an unspecified excess relative risk.

Mostafa *et al.* (1985) observed in 175 grossly normal, surgically removed human ovaries a 9% incidence of magnesium silicate granulomas and an additional 9% incidence of histological changes very similar to these granulomas.

Booth *et al.* (1989) reported an association between infertility as well as late onset of menopause and increased risk of ovarian cancer. In their opinion, the evidence linking talc with ovarian cancer was controversial, and they stated that more studies are needed to clarify this issue.

The association between 'fibre' exposure and ovarian cancer has been described by Rosenblatt *et al.* (1992). Cases were ascertained between 1981 and 1985. The authors define fibre exposure "as exposure to asbestos, talc (which may contain asbestos), and fiberglass". The authors observed elevated, but statistically not significant, risks with the use of condoms, powdered diaphragms and genital bath-talc. The risk for use of talc on sanitary napkins was significantly greater than unity (RR = 4.8; 95% CI = 1.3–18.0). The authors conclude that "The results of our study and others suggest that genital fiber exposure may be associated with an adverse effect ... but further study is needed to determine if this relationship is causal in nature."

Tzonou *et al.* (1993) examined the use of analgesics, tranquillizers and perineal talc application as risk factors for ovarian cancer in 189 cases and 200 controls. Talc use was determined on a no/yes basis without attempts to quantify application. The authors adjusted for a number of confounding variables, among them age, years of schooling, body weight, age at menarche, menopausal status, parity (nulliparous/parous, parous age at first birth, smoking (non-smoker/ever smoker), consumption of alcohol (glasses/day) and coffee (cups/day). There was a marginally significant inverse association with an apparent dose–response trend between frequency of analgesics use and ovarian cancer, and a highly significant dose-dependent positive relation between hair dyeing and ovarian cancer. As to talc, the authors state, "although the number of talc users is in general small and the respective confidence interval fairly large, there is clearly no evidence of an increased risk associated with perineal application of talc."

Chen *et al.* (1992) investigated risk factors for epithelial ovarian cancer in Beijing in 112 cases and 224 age-matched community controls. Among a number of other risk factors, the authors reported in their abstract an elevated risk in women with a history of long-term (>3 months) application of talc-containing dusting powder to the lower abdomen and perineum (RR 3.9, 95% CI 0.9–10.63).

Examination of the full paper reveals that these figures are based on seven (!) cases and five (!) controls.

Kupryjanczyk (1989) described multiple talc granulomas, inclusion cysts and adhesions in close proximity to an adenomatoid tumour of the left ovary of a 41-year-old patient. She suggests that talc crystals, as well as repair and inflammatory processes, should be taken into account as initiating factors in the development of ovarian adenomatoid tumours in susceptible patients.

In their paper on the aetiology of ovarian cancer, Baylis *et al.* (1986) conclude, "It certainly cannot be said at present that talc causes ovarian cancer." However, because the cause of ovarian cancer is unknown, and because of the "unexpected and unwarranted" presence of talc in ovarian cancer tissue and talc's "chemical similarity with asbestos", the authors are pursuing further investigations.

In their review paper on the epidemiology of ovarian cancer, Greene *et al.* (1984) summarize the role of talc and asbestos as follows: "while the above observations [reviewer's comment: referring to some of the publications reviewed here] are provocative, a conclusive role for talc or asbestos or both in the genesis of human ovarian cancer has yet to be demonstrated in either cohort or case–control studies."

There are other papers in which an association between hygienic talc application and ovarian cancer is mentioned. Most are reviews, referring to the literature reviewed here. None provide new information on the talc/ovarian cancer issue.

Hamilton *et al.* (1984) injected 100 μl saline containing 10 mg talc into ovaries of rats and observed no evidence of neoplasia.

In their review, Longo and Young (1979) point out that "epidemiological data could be interpreted as showing that the risk of developing cancer from an occupational talc exposure was due to contaminating asbestos." This could equally apply to chronic exposure from hygienic use of talc. The authors support this view by subsequently stating "... consumer talc products marketed before 1973 were variably contaminated by asbestos", and, later, "... data collected on populations exposed before 1976 may reflect the hazard of contaminating asbestos rather than talc. Unfortunately, adherence to the revised Cosmetic, Toiletry and Fragrance Association guidelines is voluntary ... so that, even now [1979; year added], commercial talcs are not certain to be asbestos-free." Cralley *et al.* (1968) found fibre contents ranging from 8 to 30% in cosmetic talc products available at the time of their investigation.

In considering mechanisms that might be involved in ovarian carcinogenesis, Venter (1981) points to the extremely high concentrations of gonadotrophins and potent steroids in the follicular fluid that is released monthly into the pelvic cavity by the rupture of the ovarian follicles. The concentration of these chemicals correlates with the mitotic and biosynthetic activities of granulosa cells, of which approximately 50 million accumulate in a follicle during the follicular phase with about 6.5 ml of antral fluid before the follicle is transformed into a corpus luteum. Given the fact that oestrogens cause proliferation of certain cells, Venter proposes the hypothesis that the antral fluid could act as an ovarian cancer promoter. Dietl *et al.* (1986) emphasize that the ovarian surface epithelium is a dynamic tissue with distinct morphological differentiations: it may proliferate inwards and form crypts and inclusion cysts or it may develop superficial papillary excrescences. In addition, constant metaplastic changes may take place in various parts of the müllerian epithelium. These growth processes appear to be influenced by endogenous and exogenous factors. It is conceivable that these factors, in combination with the physiological, biochemical and morphological characteristics of the ovarian tissue, can induce neoplastic lesions in the ovarian surface epithelium. The repeated breaks in the epithelium that occur during ovulation apparently increase the risk of developing neoplasia.

Biological effects of inhaled talc

The literature search revealed that publications on biological effects of inhaled cosmetic talc are sparse and basically fall into two categories: (1) findings in animal studies; (2) accidental inhalation of large quantities of talc by infants and small children.

Talc pneumoconioses in adults

Feigin (1989) distinguished between three forms of pulmonary disease caused by talc inhalation, namely (1) talcosilicosis, (2) talcoasbestosis and (3) pure talcosis. Feigin writes: "Talcosilicosis is produced by exposure to talc usually from Italy but also from California associated with silica and other non-asbestiform minerals.... The clinical and radiographic manifestations resemble those of silicosis.... The only documented difference between silicosis and talcosilicosis is the presence of talc, as demonstrated histologically. No other differences have been described. The radiographic changes are indistinguishable from those of silicosis." Talcoasbestosis is caused by inhalation of talc containing asbestiform fibres, such as tremolite and anthophyllite, as mined, for example, in upper New York state. On pure talcosis, Feigin writes: "Symptoms and pulmonary function test results consistent with restrictive pulmonary disease are well documented in pure talcosis; airway obstruction may also occur. The clinical form of the disease has most often been documented in miners and other workers involved in the obtaining and processing of pure talc. It has also been documented in people exposed to cosmetics, but only when the exposure was very heavy and prolonged".

This reviewer could find only two literature references on talcosis in consumers. Wells *et al.* (1979)

describe a case of talcosis due to talc abuse, initially suspected to be tuberculosis, in a 41-year-old housewife. On direct questioning the patient admitted to very heavy (at least once a day) use of talc powder over her whole body in an unventilated room for many years. The other reference is to a paper by Tukiainen *et al.* (1984) who describe two cases. One of them involved an elderly female smoker of 20 years with a history of several operations. When she presented years later with non-productive cough, dyspnoea, tachycardia and low-grade fever, talcosis was considered a possibility on account of her 10-year use of talcum powder two or three times a day in an unventilated room. The authors eventually diagnosed chronic sarcoidosis with talc deposition in the lungs. The other case was an elderly female smoker of 30 years who had been occupationally exposed to *industrial* talc from 1958 to 1968.

Scatarige and Stitik (1988) reviewed the literature on the induction of thoracic malignancies in inorganic dust pneumoconiosis. They concluded that "talc pneumoconiosis does not appear to be associated with an increased risk of lung carcinoma or mesothelioma, and animal studies have failed to convincingly demonstrate the carcinogenicity of talc."

Accidental inhalation of large quantities of talc by infants

A number of reports describe consequences of accidental inhalation of large quantities of baby talc powder by infants. Although the cases do not constitute talc use as intended, they are nevertheless included in this review for the sake of completeness as they present a form of—albeit accidental—talc inhalation.

Brouillette and Weber (1978) describe the case of a prematurely born 1-month-old girl, presented at a hospital in cardiac arrest. She was covered with talc powder which had been poured into her mouth and nose by her 3-year-old brother. Following appropriate treatment of her severe pneumonia, she was discharged after 12 days of hospitalization without apparent consequences. The authors refer in their paper to at least 24 previous cases of massive talc powder inhalation by infants. Most of the children were older than 6 months, and those old enough to play with the powder container were considered at risk. The mortality in these cases was 20%.

Mofenson *et al.* (1981) point out the potential hazard of careless use of baby powder. They reviewed the experience of the Poison Control Center at the Nassau County Medical Center, New York, with baby powder inhalation in children less than 5 years of age, in whom most of the incidents occur. Of approximately 4300 calls in a 6-month period, 40 concerned baby powder inhalation. Symptoms included cough in 14 children, dyspnoea in five, sneezing in five, vomiting in six, and cyanosis in one.

McCormick *et al.* (1982) describe the hazards associated with diaper (nappy) changing, based on statistics from the Massachusetts Poison Control System. Of 138 cases of exposure to various 'poisons' during diaper change in a 3-month period, powders accounted for 47%. Symptoms such as coughing, wheezing and shortness of breath were described as mild, occurring most often with powders.

Motomatsu *et al.* (1979) report the clinical case of two baby girls who died following accidental inhalation of baby powder and unsuccessful treatment. "In order to investigate the harm of baby powder," the authors placed eight mice in a box, the bottom of which was covered with baby powder which was then "blown up" with compressed air. Neither aerosol characterization nor other measurements or experimental data are provided by the authors. Four mice, removed from exposure after 30 and 60 min, "recovered completely". Two other mice, removed after 90 min exposure, died within 6 hours and the last two mice died after 2 hours of exposure. Histopathological findings include haemorrhage, oedema and desquamation of bronchial epithelium.

Pfenninger and D'Apuzzo (1977) describe two cases of powder inhalation. One was a 7.5-month-old girl who had inhaled Fissan baby powder containing talc, zinc oxide and other unspecified substances. The patient responded only slowly to treatment and required 19 days of hospitalization but eventually recovered fully. The second case involved a 13-month-old boy who had inhaled Merfen powder containing talc and borate of phenylmercury. The patient responded well to treatment and recovered completely within 4 days.

De La Rocha and Brown (1989) report a case of baby powder inhalation followed by adult respiratory distress syndrome in a 16-month-old girl who recovered fully after 20 days of hospitalization.

Gutermuth *et al.* (1980) describe accidental talc inhalation and treatment in an 11-month-old female infant and in a 21-month-old boy. The first patient recovered after a 16-day hospital stay, the second one after a 13-day hospital stay.

Mussi *et al.* (1979) report a fatal case of powder inhalation in a 14-month-old girl. After a characteristic fairly asymptomatic initial period—in this case 12 hours—the girl did not respond to treatment and died 41 hours after the accident.

Swanson-Bicarman *et al.* (1991) report the case of a 10-month-old boy who was hospitalized for 16 days following massive talc inhalation. The patient had recovered completely by the time of his discharge.

Butenandt *et al.* (1981) treated a 9-month-old male infant who had accidentally inhaled several grams of Penanten powder which contained 96% talc. Prompt bronchial lavage plus appropriate treatment resulted in an asymptomatic status after only 4 days of hospitalization.

Cotton and Davidson (1985) describe the case of a prematurely born 4-month-old male infant with a tracheotomy tube in place to maintain airway patency. During diaper change at home, baby powder was spilled accidentally and apparently blocked the infant's airway. At arrival in the hospital the boy was in cardiac arrest. He was resuscitated but died the following day.

Pairaudeau *et al.* (1991) report respiratory arrest in a 12-week-old boy, following a talc spill on his face during diaper change. He recovered during several days of hospitalization and treatment.

Cruthirds *et al.* (1977) diagnosed progressive diffuse pulmonary fibrosis (talc pneumoconiosis) in a 10-year-old girl who had inhaled a considerable quantity of baby powder at 2 years of age at which time hospitalization was not thought indicated.

Articles by Rumack (1982), Hayden and Sproul (1984), Wagner and Hindi-Alexander (1984) and Hollinger (1990) are mainly brief reviews which do not contribute new scientific information above and beyond the papers reviewed in this report.

Animal studies

Wehner *et al.* (1977c) exposed hamsters to a respirable talc aerosol concentration of approximately 8 mg/m^3 for 3, 30, or 150 minutes/day, 5 days/week for 30 days, or for 30 or 150 minutes/day either until they died naturally or for a maximum of 300 days. The hamsters received cumulative exposures ranging from about 15 to more than 6000 mg/hr/m^3. Estimates based on a pulmonary deposition and clearance study with neutron-activated talc (Wehner *et al.*, 1977b) indicate that 0.05–6 μg talc, depending on the length of exposure, was deposited in the hamster lungs at each exposure. Estimates based on infant-dusting experiments (J. N. Sivertson, personal communication, 1976) show that the weekly hamster exposures, expressed in mg/hr/m^3, exceeded the average weekly infant exposures by some 30 to 1700 times, depending on the hamster exposure group. At death, the lungs, trachea, larynx, liver, one kidney, stomach, uterus, one ovary, or one testis, and all tissues showing gross lesions were collected for histopathological examination. The talc exposures did not affect body weight, survival or the type, incidence or degree of histopathological changes in the exposed groups compared with sham-exposed controls.

Wehner *et al.* (1977b) determined pulmonary deposition, translocation and clearance of inhaled talc in hamsters by a single 2-hour nose-only exposure to neutron-activated talc and subsequent serial killing. Lungs, liver, kidneys, ovaries, skinned carcass and 2-day and 4-day excreta were subjected to γ-ray analysis. The isotope ^{60}Co was used to estimate talc quantities in the samples; the isotope ^{46}Sc was used to check the validity of ^{60}Co as a tracer for talc. From 20 to 80 μg talc (approx. 6–8% of the quantity inhaled) was deposited in the deep lung with a biological half-life of 7–10 days. Alveolar clearance was essentially complete 4 months after exposure. No translocation of talc to liver, kidneys, ovaries or other parts of the body was found.

To validate the interpretation of the pulmonary deposition, translocation and clearance data (Wehner *et al.*, 1977b), Wilkerson *et al.* (1977) investigated whether radionuclides leached from the neutron-activated talc in serum and in dilute hydrochloric acid. Leaching was negligible in both liquids, but somewhat higher in dilute hydrochloric acid than in serum.

A gavage study with neutron-activated talc showed that talc absorption in the gastro-intestinal tract of hamsters is also negligible (Wehner *et al.*, 1977a).

In 1992, the National Toxicology Program (NTP, 1992) prepared for public review and comment a draft technical report on a comprehensive chronic inhalation study conducted at the Lovelace Biomedical and Environmental Research Institute. The study, designed to investigate toxicology and carcinogenesis of talc in rats and mice, followed the standard NTP experimental protocol for chronic inhalation studies. F344/N rats and B6C3F$_1$ mice were exposed for 6 hours/day, 5 days/week to an intended talc aerosol concentration of 0, 6 or 18 mg/m^3. In rats, the exposures resulted in impaired respiratory function; increased lung weights; inflammatory, reparative and proliferative processes in the lungs; hyperplasia of alveolar epithelium; interstitial fibrosis; accumulation of macrophages in lymphoid tissue and regional lymph nodes; and occasionally squamous metaplasia. Incidence and severity of these changes generally were a function of dose. The incidences of alveolar/bronchiolar adenomas and carcinomas were significantly higher in female rats (but not in males) of the 18 mg/m^3 exposure group than in the controls. A significantly increased incidence of phaeochromocytomas of the adrenal medulla in talc-exposed rats of both sexes cannot be explained, as there is no known mechanism by which talc particles deposited in the lungs can affect the adrenal medulla, with the possible exception of a stress-related effect owing to a high pulmonary particle load. In mice, the talc exposures produced chronic inflammation and macrophage accumulation in the lungs, but no hyperplasia, metaplasia or interstitial fibrosis, and no pulmonary neoplasms.

As a relatively recent, comprehensive study that yielded interesting—even puzzling—results, the Lovelace study deserves special comment. Although it was generally well conducted, the study has flaws that can interfere with the interpretation of its results. Inclusion of negative and positive dust control groups would have allowed unambiguous determination of relative toxicity/carcinogenicity of inhaled talc. As it is, the question remains whether the observed pulmonary lesions and other changes in the talc-exposed rodents of the Lovelace study are

talc-specific or a non-specific foreign-body (dust) reaction that is to be expected as a consequence of inhalation exposures at concentrations that result in lung overload.

The investigators were unable to maintain target aerosol concentrations for the 18 mg/m^3 rat exposure group during 19 of the 113–122 weeks of exposure. For 7 of these weeks the rats were exposed to approximately twice the intended aerosol concentration. Even the two intended exposure concentrations led to an impairment of lung clearance mechanisms; both of them, therefore, meet the criteria for a maximum tolerated dose or maximum functionally tolerated dose. On the basis of present knowledge and standards for conducting chronic inhalation studies to investigate carcinogenicity, the chosen talc aerosol concentrations in the Lovelace study were too high. The carcinogenic response observed in female rats of the high-dose exposure group might therefore be attributable to a secondary effect of carcinogenesis, based on a high particle load in the lung (lung overload condition). The Lovelace study can be considered irrelevant for assessing the pulmonary oncogenicity of inhaled talc in humans and the authors of the NTP draft report do not imply any such relevance. Talc aerosol doses received by users of cosmetic talc are several orders of magnitude lower (Aylott *et al.*, 1979; Russell *et al.*, 1979) with no danger of reaching a lung overload condition. The increased incidence of phaeochromocytomas in male and female rats at the high talc exposure concentration remains a perplexing phenomenon that needs to be independently confirmed. Its relevance for humans is questionable, last but not least, again because of the excessive amount of talc accumulating in the rat lungs and the possibility of subsequent stress-related effect. Background incidences of phaeochromocytomas in control rats were already rather high, and no significant increase was observed in the low exposure groups, although these groups also clearly showed symptoms of lung particle overload with impairment of alveolar macrophage clearance function.

The benign pulmonary lesions in the talc-exposed animals generally were those typically observed in inhalation exposures of laboratory rodents to high concentrations of a variety of dusts. There was a significant incidence of bronchiolar/alveolar adenomas and carcinomas in the female rat group exposed to 18 mg/m^3, but not in the males, and not in mice of either sex. The biological significance of this observation remains uncertain.

Pickrell *et al.* (1989) investigated the relationship between the inhalation exposure concentration of talc and the resulting lung burdens and histological lesions. Rats were exposed to 0, 2.3, 4.3 and 17 mg talc/m^3 for 6 hours/day, 5 days/week, for 4 weeks. Lung burdens were 0, 0.07, 0.17 and 0.72 mg talc/g lung, respectively. Mice were exposed to 0, 2.2, 5.7 or 20.4 mg talc/m^3, which resulted in lung burdens of 0, 0.10, 0.29 and 1.0 mg talc/g lung. Histological changes consisted of a modest increase in talc-containing free macrophages within alveolar spaces in both rat and mouse groups exposed to the highest concentration of talc.

Wagner *et al.* (1977) exposed groups of rats to mean respirable concentrations of 11 mg SFA chrysotile or Italian talc/m^3, 7.5 hours/day, 5 days/week, for 3, 6 or 12 months, respectively. Some rats were killed 10 days after termination of exposures, others after one year. Minimal to slight fibrosis as a function of exposure duration occurred in the dust-exposed rats.

In vitro studies

Beck *et al.* (1987) investigated the toxicity of quartz- and asbestos-free talc and of granite (12% quartz), collected from worksites, in hamsters that received the dusts by intratracheal instillation. Dose–response (0.15, 0.75 and 3.75 mg/100 g body weight) and time-course (1–14 days) studies were conducted in bronchoalveolar lavage fluid (BAL). One day after exposure, both talc and granite caused elevated enzyme levels, pulmonary oedema, and increased cell numbers in BAL. Macrophage phagocytosis was inhibited. Response levels were either between 'nontoxic' iron oxide and toxic alpha-quartz or comparable with alpha-quartz. The response to granite dust decreased fairly rapidly as a function of time, but talc exposure resulted in longer elevated enzyme levels and decreased macrophage phagocytosis. The authors conclude that, given similar mass deposition in the lungs, talc causes more lung injury than granite.

Endo-Capron *et al.* (1993) studied genotoxicity of three talc samples in rat pleural mesothelial cells, using genotoxicity assays for unscheduled DNA synthesis (UDS) and sister chromatid exchanges (SCEs). Attapulgite and anatase served as negative controls, chrysotile and crocidolite as positive controls. The positive asbestos controls enhanced UDS or SCEs in treated cultures compared with untreated control cultures, but the talc samples and the negative controls did not.

Davies *et al.* (1983) tested the cytotoxicity of seven specimens of high-purity talc dust in mouse peritoneal macrophages. All samples consistently showed moderate macrophage cytotoxicity, suggesting that they would be fibrogenic *in vivo*.

Talc granulomas from powdered surgical gloves

Talc powder is fibrogenic when administered by various routes to many species of animals (Lord, 1978). When introduced into open wounds it may induce talc granulomas. This phenomenon is used therapeutically in pleurodesis, the deliberate production of adhesions between the parietal and visceral pleura by (usually surgical) deposition of talc or kaolin to treat recurrent pneumothorax. Chappell

et al. (1979) published a survey of the long-term effects of talc and kaolin pleurodesis. There was no increased incidence of lung cancer and no case of mesothelioma in 199 traceable patients who underwent pleurodesis 14 to 40 years previously. Weissberg and Kaufman (1986) successfully used talc for pleurodesis in the treatment of resistant empyema in five patients who completely recovered from empyema with no undesirable side-effects. However, in the vast majority of cases, talc granulomas are undesirable effects of wound contamination with talc, usually from surgical gloves.

Sparrow and Hallam (1991) implicate talc powder in the aetiology of an appreciable number of umbilical granulomas excised from infants and young children. In view of these cases and with reference to the sometimes disastrous consequences of accidental inhalation of baby powder by infants, the authors strongly discourage the routine use of talc powder in the care of infants.

Healey and McDonald (1977) observed a talc granuloma in the right hemiscrotum of a 3-year-old boy who had undergone a right hydrocelectomy 3 months previously. The authors state that the interval for granuloma formation following contamination is extremely variable, ranging from 2 weeks to 45 years, with an average of 2 months after surgery. The extent of granuloma formation depends mainly on the dose and antigenicity of the contaminant and on host response.

Pratt *et al.* (1985) consistently found birefringent particles, which they identified as talc, in the subserosal stroma of hernia sacs. Cellular response was remarkably mild, perhaps owing to the small particle size (about 10 μm) compared with about 50 μm of particles in talc granulomas. The authors further hypothesize that the particles were ingested with medication or food and reached the peritoneal cavity by migration through the intact intestinal wall.

Al-Sheikhli (1978) observed talc powder in granulomas of the vocal cords of a 16-year-old girl and suspects as the cause accidental talc contamination of an endotracheal tube with which she was intubated 4 months previously.

Simsek *et al.* (1992) report a case of severe obstruction of the urinary tract due to a talc granuloma in a 70-year-old male patient 7 years after a suprapubic transvesical prostatectomy.

Pelling and Evans (1986) experimentally tested in rats long-term peritoneal tissue response to mould-release agents and lubricant powder used on surgical gloves. Intraperitoneal implantation of talc produced significantly more adhesions and more severe, persistent, granulomatous reaction than starch powder and calcium carbonate.

Sheikh *et al.* (1984) describe tissue reactions to talc and Keoflo (low cross-linked cornstarch) which they tested as contaminants on the surface of surgical sutures or as pellets implanted in the abdominal muscle of rats. Keoflo caused a strong acute inflammatory reaction which, together with the starch, had essentially disappeared by the fourth week, leaving minimal lesions and scar formation. By contrast, the implanted talc initially induced only mild to moderate acute inflammation followed by chronic inflammatory response, and granuloma formation by the third day. The results suggest that low cross-linked cornstarch is a bioabsorbable substance, whereas talc is not. The authors therefore conclude that low cross-linked cornstarch "is a safe material for use as surgical glove powder", a conclusion not supported by findings of cornstarch-induced lesions reported independently by several other investigators (see below). Kaiser *et al.* (1982) reported similar results with intraperitoneal tests in rats.

Talc may contaminate surgical gloves as a mould-release agent during the manufacturing process or it may be deliberately added as a lubricant for easier donning. In a letter to the editor, Henderson and Griffiths (1979) state: "The large number of talc particles observed on certain American-produced surgeons' gloves, on occasions in excess of 6×10^7 particles/cm^2, would suggest that it is this material that is being employed as the releasing dusting powder in the molding process." Tolbert and Brown (1980) and Khan *et al.* (1983) point out that removal of talc particles from gloves is difficult using recommended washing and wiping procedures, and that a shedding hazard might exist by mechanical dislodging of the particles during surgery.

Rather than reviewing all publications of the last 15 years on talc granulomas, it is the limited objective of this section to show that talc granulomas following surgery can and do occur at various locations and that they can be induced experimentally in animal models. For relatively recent comprehensive reviews of various aspects of glove-related issues, reference is made to the papers by Ellis (1990) and Beck (1992), Fay and Sullivan (1992), White (1992) and Witmeyer (1992). Parenthetically, replacement of talc with cornstarch powder as a glove lubricant has resulted in starch granulomas (Wilson and Garach, 1981), starch peritonitis (Ellis, 1990; Loup *et al.*, 1979; Urdiales Cabal *et al.*, 1989) and intraperitoneal adhesions in rats (Kamffer *et al.*, 1992). With the trend since the 1980s towards powder-free gloves gaining momentum, complications from wound contamination with surgical glove powders may become rarer but not eliminated. To avoid allergic reactions (including anaphylactic shock) in sensitive individuals exposed to water-extractable latex proteins on medical gloves (Beezhold, 1992), vinyl, nitrile, neoprene and copolymer gloves are now available; generally, these gloves are powdered (Witmeyer, 1992).

Ingested talc

Under conditions of normal use, talc can be ingested in one of two ways. First, when used as intended as dusting powder, very small amounts of

talc dust may be inhaled. That portion of the talc particles which is deposited on the ciliated part of the respiratory tract will be transported cranially by the mucociliary escalator mechanism and then swallowed. There are no reports in the literature describing biological effects of these minute quantities of ingested talc. Secondly, talc accounts for the bulk of filler materials in tablets. Appreciable amounts of talc can be ingested with chronic heavy consumption of pills.

Anani *et al.* (1987) describe the case of a 46-year-old male who presented with abdominal pain. Further examination and a right hemicolectomy showed marked fibrosis of the intestinal wall in which birefringent particles with energy-dispersive spectra typical of those for talc were found. The anamnesis revealed that, at the age of 27 years, the patient was treated over a period of 28 months for pulmonary tuberculosis with tablets containing talc (183 g talc per 2670 g total tablet ingestion). The authors speculate that the tablets ingested during this antituberculosis therapy were the source of the talc found in the intestinal fibrosis. If this assumption is correct, the potential consequences of daily multiple pill use, as can be the case with vitamin and mineral supplements, should be more closely examined. Moderate pill consumption does not appear to present a risk. In their review of pharmaceutical excipients, Golightly *et al.* (1988) state, "Ingestion of talc is very unlikely to be toxic."

The Joint Expert Committee of Food Additives of the Food and Agriculture Organisation of the United Nations and the World Health Organization stated in its report on food additives that talc was not mutagenic *in vitro* or *in vivo* and allocated an acceptable daily intake "not specified" classification to food-grade (i.e. free of asbestiform particles) talc (FAO/WHO, 1987).

Discussion

The literature reviewed reflects several areas of concern regarding biological effects of cosmetic talc use.

Hygienic talc use and ovarian cancer

The presence of large numbers of talc particles in normal and diseased ovarian tissue seems indisputable although it is difficult to imagine how inanimate particles without locomotion of their own can breach the formidable cervical barrier and migrate 'upstream' against the ciliary beat of the fallopian epithelium to the ovaries. Several investigators have reported an association between hygienic use of talc and ovarian cancer; none claims to have established a causal relationship. The epidemiological evidence linking hygienic talc use with an increased risk of ovarian cancer generally is weak and sometimes inconsistent: confounding variables were often ignored; the reported increased risk ratios, in most cases less than 2, are barely statistically significant, and epidemiological studies are not sensitive enough to estimate risk ratios less than 2. Talc use might be a causally unrelated marker for confounders associated with increased ovarian cancer risk such as, for example, vulvovaginal disease or obesity. There appears to be a consensus of opinion that more and better designed studies are needed before valid scientific judgement can be passed on whether or not there exists a causal relationship between hygienic talc use and increased ovarian cancer risk.

An interesting question remains. Talc is a recognized fibrogen. If there are sufficient numbers of talc particles in ovarian tissues long enough to cause cancer, where is the ovarian fibrosis which one would expect to develop long before cancer occurs? The only references to ovarian granulomas are those by Mostafa *et al.* (1985) and Kupryjanczyk (1989).

Talc inhalation

In contrast to individuals occupationally exposed to industrial talc, talc pneumoconiosis from personal use of cosmetic talc appears to be extremely rare, occurring only following chronic abuse. When talc, a fibrogenic substance, is chronically deposited at doses sufficiently high to overwhelm the bronchopulmonary clearance mechanism, a fibrotic/ granulomatous tissue response can and will occur, as observed in humans and animals. Animal studies suggest that natural defence mechanisms, such as macrophages and mucociliary clearance, can cope with exposure to talc concentrations considerably exceeding estimated infant exposures, without lesion development (Wehner *et al.*, 1977c). If doses in animal experiments are increased substantially so as to result in pulmonary overload, the results can no longer be considered relevant for human risk assessment. The Lovelace study (NTP, 1992) is a case in point.

Paracelsus recognized some 450 years ago, "*dosis solum venenum facit*" (only the dose makes a poison). Lee *et al.* (1985) confirmed this dramatically by inducing a significant incidence of squamous cell carcinoma in lungs of female CD rats, exposed for up to 104 weeks to 250 mg titanium dioxide/m^3, a substance long considered by many to be biologically inert and frequently used as negative dust control. Should titanium dioxide therefore be considered a carcinogen in rats—and in humans? There is no evidence in the literature to suggest that cosmetic talc, inhaled under conditions of normal use, can cause cancer in the human respiratory system.

Accidental inhalation of baby powder by infants presents a largely avoidable problem if physicians, nurses, parents and other individuals handling the infants are made aware of the potential danger and observe appropriate precautions, such as keeping the powder can out of reach of children. Considering the very large number of dustings administered daily to babies, these incidences fortunately are very rare.

Other studies

In vitro studies have shown that talc is fibrogenic and not genotoxic. The number of papers reporting talc granulomas caused by powdered surgical gloves indicates a certain concern. Calls for talc-free surgical gloves have been voiced. Cornstarch powder does not appear to be a suitable substitute as it, too, causes lesions. The use of talc on surgical gloves presents a special problem that does not affect the typical consumer of cosmetic talc. Ingestion of food-grade talc is unlikely to be toxic.

Conclusion

There is no conclusive evidence in the literature reviewed to indicate that cosmetic talc, when used as intended, presents a health hazard.

Acknowledgements—Johnson & Johnson Consumer Products, Inc. granted permission to publish this manuscript which is based on a critical literature review previously prepared for Johnson & Johnson.

The Cosmetic, Toiletry and Fragrance Association (CTFA) authorized the inclusion of excerpts in this review from BEC's 1992 technical report to CTFA, written by E. L. Alpen, A. J. Gross, G. Oberdörster, F. J. C. Roe, R. K. Ross and A. P. Wehner.

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American Journal of Epidemiology
Vol. 136, No. 10

Printed in U.S.A.

Characteristics Relating to Ovarian Cancer Risk: Collaborative Analysis of 12 US Case-Control Studies

IV. The Pathogenesis of Epithelial Ovarian Cancer

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Two hypotheses have been proposed to explain the reduced risk of epithelial ovarian cancer associated with pregnancy and oral contraceptive use. The first states that some sequelae of ovulation increase the likelihood of malignancy and that pregnancies and oral contraceptives protect by suppressing ovulation. The second hypothesis states that circulating levels of pituitary gonadotropins increase the risk of malignancy and that pregnancies and oral contraceptives protect by suppressing secretion of these hormones. The authors evaluate the two hypotheses in light of combined data from 12 United States case-control studies of epithelial ovarian cancer in white women conducted from 1956 to 1986. While a number of observations support both hypotheses, there are exceptions. Differential risk reduction associated with pregnancy and oral contraceptive use (pregnancy being the more effective in young women and the less effective in older women) conflicts with the first "ovulation" hypothesis, while reduced risk associated with breast feeding and absence of altered risk associated with estrogen replacement therapy conflicts with the second "gonadotropin" hypothesis. Several findings would not have been predicted by either hypothesis, e.g., only weak trends relate cancer risk to age at menarche, and, among older women, no clear trends relate risk to age at menopause. Odds ratio attenuation due to errors in reporting personal characteristics may be responsible for some of these inconsistencies. Multidisciplinary research is needed to clarify the etiologic roles of ovulation and gonadotropin stimulation, both of which may enhance carcinogenesis in the ovarian epithelium. *Am J Epidemiol* 1992;136:1212–20.

gonadotropins; ovarian neoplasms; ovulation; reproduction

Pregnancy and oral contraceptive use are associated with reduced risk of epithelial ovarian cancer. These associations appear to reflect more than a correlation between low parity and some form of infertility that predisposes to the disease. Two hypotheses propose direct protective effects of these factors. Fathalla (1, 2) hypothesized that ovarian carcinogenesis involves some mechanical sequelae of ovulation, such as trauma or mitotic stimuli to the ovarian epithelium. This "ovulation" hypothesis suggests that pregnancy and oral contraceptive use protect against ovarian cancer by inhibiting ovulation. Alternatively, Gardner (3) and Stadel (4) hypothesized that exposure of the ovarian epithelium to persistently high circulating levels of pituitary gonadotropins increases the likelihood of malignancy. This "gonadotropin" hypothesis suggests that pregnancy and oral contraceptive use protect against ovarian cancer by inhibiting pituitary secretion of gonadotropins.

Animal experiments provide evidence to support both hypotheses. In domestic fowl, stimulating egg production induces ovarian adenomas (5), and in rodents, anatomic alterations that result in increased gonadotropin secretion enhance ovarian tumorigenesis (6). However, because these experimentally induced tumors are either uncommon or nonexistent in humans, their relevance to the epithelial cancers that comprise the majority of human malignancies is unclear. Here we evaluate the hypotheses in light of combined data from 12 United States case-control studies of epithelial ovarian cancer in white women conducted from 1956 to 1986 and described in the previous two papers.

PREGNANCIES

Pregnancy induces both anovulation and suppression of pituitary gonadotropins (7, 8). Thus, both hypotheses predict that pregnancies reduce ovarian cancer risk. In support of this prediction, each additional term pregnancy (even among the highly parous) is associated with reduced risk of both invasive cancer (9, table 4) and cancers of low malignant potential (10, table 1). The data suggest that each additional pregnancy after the first confers the same percent reduction in risk of invasive cancer, estimated to be about 14 percent. For the population-based studies, this reduction is smaller ($p < 0.001$) than the 40 percent reduction found for the first term pregnancy. In terms of the two hypotheses then, the population-based data suggest that some factor in addition to a period of anovulation or gonadotropin suppression distinguishes uniparous women from nulliparous women.

Both the ovulation and the gonadotropin hypotheses predict that failed pregnancies protect against ovarian cancer, perhaps to a lesser degree than do term pregnancies. The data for both invasive and borderline cancers support this prediction. Failed pregnancies are associated with reduced risk among the parous (9, table 5 and 10, table 1), although no clear effect of gravidity is evident among the nulliparous. The risk reduction per pregnancy is smaller in magnitude for failed pregnancies than for term pregnancies. However, data on gestational length for each pregnancy, available from a subset of the studies, suggest that the decreased protection associated with a failed pregnancy is due to its shorter length: Among the gravid, odds ratios per month of pregnancy do not depend on pregnancy outcome. In conclusion then, findings from the combined data concerning the relation of pregnancies to ovarian cancer risk support both hypotheses.

Both hypotheses, and especially the gonadotropin hypothesis, also receive support from the observed increased risk of both invasive and borderline ovarian cancer associated with use of fertility drugs (9, table 3 and 10, table 1). Such drugs stimulate ovulation by increasing follicular-phase levels of follicle-stimulating hormone (FSH). Clues to pathogenesis are provided by the complications of fertility drugs, which include multiple gestations (supportive of the

Received for publication August 21, 1991, and in final form July 23, 1992.

Abbreviation: FSH, follicle stimulating hormone.

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Supported by National Cancer Institute grants CA 43689, CA 47427, and CA 47448.

The authors are grateful to Drs. Sally Glaser, Esther M. John, Pamela Horn-Ross, and Carolyn Westal for helpful comments on earlier versions of the manuscript.

ovulation hypothesis) and ovarian enlargement from FSH hyperstimulation (supportive of the gonadotropin hypothesis) (11, 12).

BREAST FEEDING

Breast feeding induces partial inhibition of ovulation in lactating women (13, 14). Thus,the ovulation hypothesis predicts that breast feeding reduces ovarian cancer risk. Breast feeding also induces increased secretion of FSH and reduced secretion of luteinizing hormone (15). In lactating women, FSH rises after childbirth to normal follicular phase values and remains elevated until the return of ovarian estrogenic function (16). Thus, the gonadotropin hypothesis predicts that breast feeding, with its concomitant elevated FSH levels, increases risk. Yet the data for both invasive cancers (9, table 7) and cancers of low malignant potential (10, table 1) show reduced risk associated with breast feeding. So the data on breast feeding, while consistent with the ovulation hypothesis, conflict with the gonadotropin hypothesis. It should be noted however, that lactation is also associated with ovarian refractoriness to FSH stimulation (15), and this characteristic may be responsible for its apparent protective effect.

The effectiveness of lactation in suppressing ovulation is strongest during the first few months after delivery and wanes thereafter (15). The ovulation hypothesis thus predicts that a month of lactation shortly after delivery reduces a woman's ovarian cancer risk more than does a month of subsequent lactation. Data available from six of the studies support this prediction.

AGE AT MENARCHE AND AGE AT MENOPAUSE

Studies in the United States (17) and Finland (18) suggest that the later menarche occurs the longer it takes to establish regular ovulatory cycles. Thus, the ovulation hypothesis predicts that women who began menstruating before their teens have higher ovarian cancer risk than do women with later menarche because the former started ovulating earlier. By contrast, cessation of menstruation at menopause is a poor surrogate for cessation of ovulation, since ovulatory cycles occur sporadically during the perimenopausal years. The ovulation hypothesis therefore predicts that late age at menopause is weakly associated with increased ovarian cancer risk. In agreement with the prediction for menarche, the data show trends of increased risk associated with early menarche, although the trends are weak (9, table 8). However, in disagreement with the prediction for menopause, the data show no clear trends with later cessation of menstruation (9, table 8 and 10, table 3). Among older women, those with late menopause have no altered risk of invasive cancer in the hospital-based studies and slightly decreased risk in the population-based studies. The slight decrease in risk in the population data supports, instead, the gonadotropin hypothesis because late menopause postpones exposure to elevated gonadotropin levels that accompany the menopause (19). This absence of increasing risk with increasing age at menopause among older women conflicts with the less rapid rise with age of ovarian cancer incidence rates after age 55 years. Such deceleration is seen even in countries where hysterectomy is uncommon and therefore unlikely to bias postmenopausal rates downward by removing women from the population at risk (20, 21).

Imprecision in estimates of age at first and last menses could explain at least some of the discrepancies. Consistent with this interpretation are the stronger trends for menarche and menopause in young women compared with older women, who are less able to recall accurately menstrual events in the distant past (22–24). Indeed, the total age span within which women undergo menopause is relatively narrow, so reporting of menopausal age with large error (23) may misclassify women from one extreme of age at menopause to the other.

EXOGENOUS ESTROGENS

Estrogen-containing oral contraceptives suppress ovulation and reduce pituitary secretion of gonadotropins (25). Thus, both ovulation and gonadotropin hypotheses pre-

dict that oral contraceptive use reduces ovarian cancer risk. This prediction is supported by trends of decreasing risk with increasing duration of oral contraceptive use, even among the highly parous, as seen in reference 9, table 9 and reference 10, table 2.

Conjugated estrogens also suppress pituitary hormone levels in postmenopausal women, although not to premenopausal levels (25–29). However, the data show no association between estrogen replacement therapy and risk for ovarian cancer, regardless of tumor behavior (9, table 10 and 10, table 2). The failure of estrogen replacement therapy to reduce risk argues against the gonadotropin hypothesis, unless gonadotropin levels in estrogen users are still above a threshold needed to facilitate carcinogenesis.

PELVIC SURGERIES

Tubal ligation and hysterectomy with ovarian conservation may impair ovarian function, possibly by compromising blood supply to the ovaries (30–35). Thus, they should protect against ovarian cancer, according to the ovulation hypothesis. Moreover, hysterectomy during the reproductive years should protect more than hysterectomy after the menopause. These predictions are confirmed by the observed reduced risks associated with both types of surgery (9, table 11 and 10, table 3), although several sources of bias (9) must be considered in interpreting the findings. In contrast, the risk reductions conflict with an increased risk predicted by the gonadotropin hypothesis, unless any estrogen deficiency after surgery (34, 36) fails to elevate gonadotropin levels above a threshold needed for carcinogenesis.

TOTAL YEARS OF ANOVULATION

We next evaluate predictions of the two hypotheses for associations between risk of epithelial ovarian cancer and periods of anovulation and for the variation in strength of these associations with factors such as reference age (i.e., age at diagnosis or interview), source of anovulation, age at anovulation, and adiposity. Here we use the term "anovulation" to mean suppression of menses due to pregnancy, oral contraceptive use, delayed menarche, or early menopause. In this section, we restrict attention to invasive cancers (9), whose numbers were large enough to permit evaluation of such interactions.

Reference age

Pike (37) hypothesized that ovarian cancer incidence rates I are proportional to ovarian epithelial "tissue age" t, measured in units of cell mitoses:

$$I(t) = ct.$$

where c is a constant. Ovulation induces a transient increase in mitotic activity in the ovarian epithelium (1); therefore, a year of anovulation reduces ovarian tissue age by the number t_0 of mitoses avoided. Pike's hypothesis then predicts that each year of anovulation reduces the ovarian cancer incidence rate by a fixed (absolute) amount:

$$I(t) - I(t - t_0) = ct - c(t - t_0) = ct_0.$$

Since a woman's tissue age increases as she ages, the prediction implies that the proportional risk reduction associated with a year of anovulation wanes with age:

$$[I(t) - I(t - t_0)]/I(t) = ct_0/ct \rightarrow 0$$

as t increases. This implication is supported by the attenuated odds ratios among older women (reference age, greater than 55 years) compared with younger women, shown in table 1. This table presents odds ratios among younger and older women according to total years of ovulation, estimated by subtracting age at menarche from age at last menstrual period and then further deducting total time pregnant in term pregnancies (assuming 9 months per pregnancy), breast feeding, or taking oral contraceptives. Women hysterectomized before the menopause were assumed to continue ovulating until age 55 years. Table 1 shows strong and statistically significant trends of increasing risk with increasing years of ovulation for younger, but not older women. The trend in older women showed interstudy heteroge-

TABLE 1. Odds ratios (OR) for invasive epithelial ovarian cancer according to estimated years of ovulation, by reference age

Years of ovulation	Reference age (years) <55†							Reference age (years) ≥55‡						
	Cases		Controls		OR§	95% CI‖	p value	Cases		Controls		OR§	95% CI	p value
	No.	%	No.	%				No.	%	No.	%			
<25	208	30	2,099	47	1.0			24	5	90	6	1.0		
25–29	131	19	804	18	1.8*	1.4–2.5		24	10	160	11	1.3	0.72–2.3	
30–34	198	29	962	22	2.6*	1.9–3.6		121	23	363	25	1.2	0.72–2.1	
≥35	145	21	595	13	2.9*	2.0–4.1		318	62	826	58	1.5	0.87–2.4	
Overall trend per year					1.08¶		<0.001					1.02#		0.10

*$p < 0.001$.
† Based on studies 2, 6, 8, 9, 11, and 12 (see part I, table 1 (50)).
‡ Based on studies 2, 6, 8, 9, and 12.
§ Adjusted for age and study.
‖ CI, confidence interval.
¶ Test of OR homogeneity across studies: $\chi^2_5 = 5.8$, $p = 0.38$.
# Test of OR homogeneity across studies: $\chi^2_4 = 11.9$, $p = 0.04$.

neity ($p = 0.04$); data from one study showed a stronger trend than did data from the others. Similar trends were obtained when women were classified by the complementary years of anovulation (relative to a maximum 45 years of ovulation for a nulligravid woman with menarche and menopause at ages 10 and 55 years, respectively, who never used oral contraceptives).

In additional age-specific analyses (not shown), measures of risk associated with pregnancy showed strong and consistent trends of attenuation with increasing reference age. Specifically, the percent risk reduction associated with parity relative to nulliparity declined steadily, ranging from 28 percent for women aged less than 40 years to only 1 percent for women aged 70 or more. Indeed, there was little difference in risk by parity among women aged 60 years or more. Similarly, the risk reduction among the parous associated with each term pregnancy declined with age. These trends of decreasing percent risk reduction with increasing age were present ($p < 0.01$) for both hospital- and population-based studies. Such attenuation supports Pike's hypothesis. It also is consistent with a transient protective effect of gonadotropin suppression during pregnancy. However, it contradicts the conjecture that pregnancy induces changes that continue to reduce ovarian cancer risk throughout life. If the conjecture were true, then the percent risk reduction per pregnancy would remain constant or increase, rather than decrease, with age.

The percent risk reduction associated with a year of delayed menarche also tended to wane with increasing reference age, but the trends are more readily explained by chance. Risk reductions associated with breast feeding did not vary by reference age, while those associated with oral contraceptive use showed a nonsignificant increase with age.

Source of anovulation

According to the ovulation hypothesis, ovarian cancer risk depends on a woman's reproductive and menstrual history only as a function of her total years of anovulation, rather than as a function of the individual components from the various sources. Table 2 compares estimates of the percent risk reduction per year of anovulation due to delayed menarche, term pregnancy among the parous, breast feeding among those who had breast-fed, oral contraceptive use among users, and early menopause among those who were naturally postmenopausal or premenopausal. Among younger women, the magnitude of risk reduction per year of term pregnancy exceeds that associated with the other sources. Among older women, however, pregnancy is less protective than is oral contraceptive use. Reporting accuracy is probably greater for time spent pregnant than for time from all other anovulatory sources given in table 2. Since random recall error attenuates regression coefficient estimates toward zero, such error could explain some of the observed differences.

TABLE 2. Percent reduction of invasive epithelial ovarian cancer risk per year of anovulation according to source, by reference age

Source of anovulation	Reference age (years) <55		Reference age (years) ≥55	
	% reduction	SE†	% reduction	SE
Delayed‡ menarche	2.1	2.4	0.8	3.0
Term pregnancy§	27.9**	4.3	12.2	5.2
Breast feeding‖	8.5	9.2	10.3	8.7
Oral contraceptive use¶	8.0**	1.7	20.4*	7.9
Early menopause#	−1.0	1.7	0.4	1.0

*$p < 0.01$; **$p < 0.001$.
† SE, standard error.
‡ After age 10 years.
§ Among the parous, adjusted for age, study, and oral contraceptive use, and assuming 9 months per term pregnancy.
‖ Among women who had breast-fed, adjusted for age, study, parity, and oral contraceptive use.
¶ Among users, adjusted for age, study, and parity.
# Before reference age for those aged <55 years and before age 55 years for those aged ≥55. Women with artificial menopause were excluded.

In developed countries, breast feeding suppresses ovulation less effectively than does pregnancy; some 40–75 percent of lactating women menstruate while nursing (13). The findings in table 2 are thus consistent with the ovulation hypothesis, which predicts that a month of breast feeding is associated with a smaller reduction in ovarian cancer risk than is a month of pregnancy.

Although the differences in risk reduction between pregnancy and breast feeding do not achieve statistical significance, those between pregnancy and oral contraceptive use are significant ($p < 0.01$) among younger, but not older women. This difference in risk reduction in younger women, if not due to reporting errors in duration of oral contraceptive use (38) or other bias, conflicts with the ovulation hypothesis, which predicts equal protection per year of pregnancy and oral contraceptive use, since these conditions are equally effective in suppressing ovulation. The difference is more consistent with the gonadotropin hypothesis because the low potency oral contraceptives may be less effective than pregnancy in inhibiting pituitary secretion of gonadotropins (7, 39–41). The relatively large risk reduction associated with oral contraceptive use among older women has a large standard error because few older women had used oral contraceptives. Nevertheless, it suggests that the early high-potency formulations used by these women (42, 43) may be especially protective. Such extra protection also would argue against the ovulation hypothesis since all formulations suppress ovulation at similar rates (44), but would support the gonadotropin hypothesis since the high-dose formulations may have been particularly effective in reducing pituitary gonadotropins (39–41). Alternatively, the large risk reduction accompanying previous oral contraceptive use in older women suggests that the biologic effects of oral contraceptives may increase with time. Like the previous explanation, such a latency effect would conflict with the ovulation hypothesis, but not necessarily with the gonadotropin hypothesis.

The data in table 2 suggest that for all women, neither a year of delayed menarche nor a year of early menopause is associated with the same risk reduction as is a year of pregnancy, breast feeding, or oral contraceptive use. Among younger women, breast feeding and oral contraceptive use appear equally effective in reducing ovarian cancer risk, albeit less effective than pregnancy.

Age at anovulation

The probability that a menstrual cycle is ovulatory varies with age, being highest in the age range 25–40 years and lowest at either end of the reproductive years (45). Thus, the ovulation hypothesis predicts less protection per year of menses suppression due to delayed menarche and early menopause than per year of such suppression at the height of reproductive life. The differences in table 2 support this prediction. Further, since women with late first birth tend to complete their childbearing later than do women with early first birth, the ovulation hypothesis predicts less protection per pregnancy to women with late first birth than to women with early first birth. This second prediction received weak support from the hospital-based data, but not from the population-based data. Future studies should obtain the timing of all pregnancies and episodes of lactation and oral contraceptive use, as well as information on menstrual cycle length, in order to compare ovarian cancer risk reduction per unit time at the height of the reproductive years with that at either extreme.

Adiposity

Extreme obesity before the menopause is associated with increased incidence of anovulatory cycles (46) and lower circulating levels of pituitary gonadotropins (47). Thus, both hypotheses predict that pregnancy, lactation, and oral contraceptive use are more protective to the lean than to the obese, for whom they prevent fewer ovulations and less exposure to FSH. Only the data for breast feeding support this prediction; the risk reductions associated with pregnancy and oral contraceptive use did not vary with adiposity. However, the reduction associated with breast feeding was confined to women whose "usual" value of Quetelet index was less than 35 ($p < 0.05$ for both hospital-based and population-based studies).

SUMMARY

The data relating ovarian cancer risk to pregnancies, oral contraceptive use, and use of fertility drugs support both ovulation and gonadotropin hypotheses. Yet there are inconsistencies: The observed protective effect of delayed menarche is weaker than predicted by either hypothesis, and the lack of clear trends with age at menopause, if not due to recall error or other bias, argues against both hypotheses. The greater risk reduction associated with pregnancy than with oral contraceptive use argues against the ovulation, but not the gonadotropin, hypothesis. Some of these inconsistencies may be due to differential measurement error among the sources, since pregnancies probably are recalled more accurately. Evaluation of this issue in cohort studies is of interest in light of evidence (21, 22) that contemporaneous report of menopausal status and estrogen use may be more accurate than recall of these events later in life.

The findings for breast feeding, estrogen replacement therapy, and pelvic surgeries are consistent with the ovulation hypothesis, but conflict with the gonadotropin hypothesis. However, the endocrine profiles associated with these characteristics and their contributions to ovarian carcinogenesis are poorly understood. Pending further research to clarify these issues, the findings cannot be interpreted as strong evidence against the gonadotropin hypothesis.

Both hypotheses may be valid, with each explaining some fraction of all epithelial ovarian cancers. Histology-specific analysis, although not feasible for these data, may help to distinguish the relative contributions of each. Research involving both epidemiology and the basic sciences will be needed. For example, ovarian cancer has a strong familial component (48), and work is under way to determine genetic loci that predispose to the disease. Markers of genetic susceptibility will permit refined analyses that may elucidate mechanisms. Clinical research will also be useful. For example, epithelial ovarian cancers have been observed in dysgenetic gonads that lack ova and are, therefore, incapable of ovulating (49). This observation demonstrates that ovulation is not necessary for epithelial carcinogenesis. However, dysgenetic gonads are exposed to elevated cir-

culating levels of pituitary gonadotropins and contain binding sites to gonadotropins.

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American Journal of Epidemiology
Vol. 136, No. 10

Printed in U.S.A.

Increased Risk of Breast Cancer with Alcohol Consumption in Postmenopausal Women

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The association between breast cancer incidence and alcohol consumption among postmenopausal women was examined in the Iowa Women's Health Study. In January 1986, a cohort of 41,837 postmenopausal women, aged 55–69 years, completed a questionnaire that included alcohol intake and other information. Through December 1989, 493 incident breast cancer cases were identified. Age-adjusted relative risks of consumption of less than 1.5, 1.5–4.9, 5.0–14.9, and 15.0 g or more of alcohol per day compared with abstention were 1.08, 1.10, 1.08, and 1.28, respectively (p for trend = 0.11). After controlling for age, body mass index, age at first livebirth, age at menarche, and family history of breast cancer, the relative risks were 1.18, 1.20, 1.25, and 1.46 (p for trend = 0.04). Multivariate modeling, using Cox proportional hazards regression, revealed a significant multiplicative interaction between alcohol intake and noncontraceptive estrogen use. The relative risks of breast cancer associated with average daily alcohol consumption of 5.0–14.9 and 15.0 g or more were 1.88 (95% confidence interval 1.30–2.72) and 1.83 (95% confidence interval 1.18–2.85), respectively, among ever-users of estrogen; no association between alcohol and breast cancer was observed among never-users of estrogen. *Am J Epidemiol* 1992;136:1221–31.

alcohol drinking; breast neoplasms; cohort studies; estrogen replacement therapy

Breast cancer is the most common cancer among women in the United States and is a major public health concern. It is estimated that one in nine women will develop breast cancer in their lifetimes (1). Thus, identifying those risk factors amenable to modification would be useful for implementing primary prevention strategies. In 1977, Williams and Horm (2) reported results from the Third National Cancer Survey, a population-based cross-sectional study of incident cancers, suggesting for the first time that alcohol consumption may be related to breast cancer. This report lead to an extensive examination of the alcohol-breast cancer relation.

In a recent review, Hiatt (3) summarized most of the studies that examined the association between breast cancer and alcohol consumption. He noted that 11 of 18 case-control studies (2, 4–20), five of the six cohort studies (21–26), and one meta-analysis (27) have shown a positive association. Three of four case-control studies (28–31) and one meta-analysis (32) published between 1989 and 1991 also reported a positive association. In general, the relative risks and odds ratios for developing breast cancer ranged from 1.4 to 2.0 for women who

Received for publication February 21, 1992, and in final form May 22, 1992.
Abbreviation: CI, confidence interval.
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Supported by grant R01-CA 39742 to Dr. Aaron R. Folsom from the US National Cancer Institute. Susan M. Gapstur was supported by National Institutes of Health training grant T32 CA099607 to Dr. John D. Potter.
The authors thank Dr. Susan Kaye, Dr. Robert Wallace, Dr. Larry Kushi, Kathleen McKeen, and Ching Ping Hong for their important contributions. They also thank Dr. Walter Willett and Laura Sampson for permission to use the Harvard food frequency questionnaire.

AN JOURNAL OF EPIDEMIOLOGY
Vol. 128, No. 6

Printed in U.S.A.

RSONAL AND ENVIRONMENTAL CHARACTERISTICS RELATED TO EPITHELIAL OVARIAN CANCER

EPRODUCTIVE AND MENSTRUAL EVENTS AND ORAL CONTRACEPTIVE USE

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In two case-control studies conducted in the six-county San Francisco Bay Area, 111 women diagnosed with epithelial ovarian carcinoma in 1974–1977 and 188 women diagnosed in 1983–1985 were interviewed concerning their menstrual, sexual, and reproductive histories. For comparison, interviews were conducted with 752 control women admitted to the same hospitals within six months of the cases; for cases diagnosed in the later period, interviews were also conducted with an additional 259 population-based controls selected by random digit dialing. Controls were matched to cases by age and race. Qualitative and quantitative findings were similar for the two studies. In the combined data, cases were more likely than their matched controls to have been nulliparous, to have undergone menarche at an early age, and to have refrained from using oral contraceptives. Menopause occurred slightly later for cases than for controls, but the differences were not statistically significant. Neither age at first term pregnancy (20 or more weeks gestation) nor number of term pregnancies was predictive of ovarian cancer risk. The protection afforded by oral contraceptive use was independent of parity, and it increased with increasing duration of use. There were no trends in risk with time since last oral contraceptive use or with time since first use, after adjustment for duration of use. These observations suggest that oral contraceptive use decreases risk for ovarian cancer, rather than merely indicates fertility, which may itself decrease risk of developing the disease. The authors combined reproductive characteristics and oral contraceptive use to estimate a woman's total duration of ovulation, which was positively associated with ovarian cancer risk ($p < 0.001$ for trend). These observations support the concept that the greater the duration of ovulation or accompanying endocrinologic phenomena, the greater a woman's risk for ovarian cancer.

contraceptives, oral; menarche; menopause; ovarian neoplasms; ovulation; parity

a from several studies suggest that earing and use of oral contraceptives protect against epithelial tumors of the ovary (1–16). Two hypotheses have been

ved for publication May 29, 1987, and in final ril 11, 1988.
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proposed as possible explanations for these protective effects. The first states that childbearing and oral contraceptive use protect by suppressing ovulation (2, 17, 18). According to this hypothesis, each ovulation increases risk of neoplastic change, possibly by traumatizing the ovarian epithelium or by exposing it to endogenous promoting agents, such as estrogen-rich follicular fluid or high serum levels of pituitary gonadotropins.

The second hypothesis states that parity and oral contraceptive use are not causally related to ovarian cancer but that the three conditions are common correlates of subfertility. According to this hypothesis, women who have difficulty conceiving are at increased ovarian cancer risk and also are less likely to be parous or to use oral contraceptives. The first part of this hypothesis is supported by some studies showing that women with ovarian cancer are more likely than control women to report difficulty in conceiving (11) or to have eschewed contraceptives during marriage (12).

Here we present data from two case-control studies concerning the effects on epithelial ovarian cancer risk of childbearing, oral contraceptive use, and other reproductive and menstrual factors. The first study, conducted from 1974 to 1977, will be called the 1970s study; the second study, conducted from 1983 to 1985, will be called the 1980s study.

MATERIALS AND METHODS

1970s study

Cases for the 1970s study were white English- or Spanish-speaking residents of northern California who were 20 to 85 years of age at diagnosis. These included 111 women with histologically verified epithelial ovarian cancer, newly diagnosed from 1974 through 1977 at one of 34 hospitals in the five counties included in the San Francisco-Oakland Bay Area Surveillance and End Results (SEER) reporting system (19). Cases with a history of surgical menopause were excluded.

Controls in the 1970s study were white women hospitalized in one of the 34 admitting hospitals for the cases. Control patients were matched to cases by age (within five-year groups), hospital of admission, and year of admission. Women were excluded if they had undergone surgical menopause or if they had been admitted or treated for psychiatric, obstetric, gynecologic, or malignant conditions. A total of 472 control patients participated. Further details of the study design can be found elsewhere (20, 21).

1980s study

Cases in the 1980s study were residents of northern California aged 18 to 74 years who were diagnosed with epithelial ovarian cancer or with a borderline epithelial ovarian tumor between January 1983 and December 1985 at one of the seven hospitals in Santa Clara County or at the University of California, San Francisco, Medical Center. Slides of cases' surgical specimens were reviewed and classified by one of us (M. H.). Of the 317 eligible cases identified during the study period, eight (3 per cent) were not interviewed because their physicians denied permission, 30 (9 per cent) declined to participate, and 44 (14 per cent) were too ill to participate or died before they could be contacted. The remaining 235 women (74 per cent) constituted the cases for this study. The tumors of 47 (20 per cent) of these women were classified as borderline; these tumors were excluded from this analysis, leaving 188 cases for the 1980s study.

Two sets of control women were selected for the 1980s study. The first set consisted of women hospitalized at one of the eight admitting hospitals for the cases. These women were matched to the cases by age (five-year groups), race (white, black, other), hospital, and date of admission

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This work was supported by NIH Grant CA 35067.

The authors thank Dr. Gerald M. Shefren for his help in the study.

thin ±3 months). We attempted to lo-
e two controls (one medical admission,
surgical admission) for each case; how-
r, only one control was available for
ne nonwhite women. Women were ex-
ded if both their ovaries had been re-
ved or if they had been admitted for
chiatric, obstetric, gynecologic, or ma-
ant conditions. Of 524 eligible women,
(9 per cent) were not interviewed be-
se their physicians denied permission,
) (19 per cent) declined to participate,
123 (4 per cent) were too ill or died prior
being contacted. The remaining 354
men (68 per cent) constituted the set of
spital controls for the 1980s study.
venty-four of these women were matched
cases with borderline tumors and are
luded from this analysis, leaving 280
spital controls.

The second set of controls consisted of
men who were selected from the general
pulation by random digit dialing tele-
one contacts, with an attempt to reach
controls per case. These women were
tched to cases by age (within five-year
ups), race (white, black, other), and tele-
one area code and prefix. Women known
have a previous bilateral salpingo-
ohorectomy were excluded. We con-
ted a total of 2,917 households. Of these,
25 were business numbers, were not in
vice, gave no answer after 10 attempts,
were households containing no eligible
men. An additional 228 households re-
ed to provide information about poten-
l eligibility of their members. Of 464
gible women contacted by telephone, 408
per cent) consented to receive a letter
plaining the study, and 329 (71 per cent)
eed to participate. Seventy of these were
tched to cases with borderline tumors
l are excluded from these analyses, yield-
a balance of 259 population controls.

Interviews

ll cases and controls participated in
ictured personal interviews conducted
their homes by trained interviewers.
Most case interviews were conducted within three months of diagnosis. The questionnaire used for the 1980s study was similar to the 1970s questionnaire but included more questions. Both instruments were designed to assess menstrual, reproductive, medical, and family history prior to a reference date (one year before diagnosis for cases; one year before interview for controls). In addition, the 1980s questionnaire included questions about exposures to environmental agents such as talc, asbestos, coffee, tobacco, alcohol, and radiation. To facilitate recall of oral contraceptive use, interviewers used photographs of all oral contraceptives marketed in the United States prior to 1983. To facilitate recall of reproductive events, interviewers provided subjects with a chart organized by calendar year.

Data analysis

Conditional logistic regression for matched case-control studies (22) was used to estimate odds ratios (hereafter called relative risks) and to test for trend. Analyses were conducted by means of the programs EGRET (23) and RISK (24). Approximate 95 per cent confidence limits were obtained by the Wald method (22). All p values are two-tailed. Differences in relative risks for the two components were tested by likelihood ratio methods. Under the null hypothesis of no difference, the likelihood ratio statistic (defined as minus twice the difference in log likelihood between regressions with and without a common relative risk) has approximately a chi-squared distribution (22).

RESULTS

Demographic and reproductive characteristics of study participants are shown in table 1. Cases in both studies were more likely to be nulliparous than were their matched controls. However, cases and controls did not differ in average age at first term pregnancy (20 or more weeks gestation) or in average number of abortions.

TABLE 1

Characteristics of study participants, San Francisco Bay Area, 1974–1977 and 1983–1985

Characteristic	1970s participants		1980s participants		
				Controls	
	Cases (n = 111)	Controls (n = 472)	Cases (n = 188)	Hospital (n = 280)	Population (n = 259)
Age (mean years)	55.4	56.8	52.8	53.4	52.0
Race (% white)	100	100	94.7	97.1	94.2
Oral conceptive use (% ever use)	22.5	21.6	45.7	50.0	57.9
Parity (% nulliparous)	27.9	19.7	20.7	17.1	10.0
Term pregnancies* (mean no.)	1.8	2.1	2.2	2.5	2.5
Age at menarche (mean years)	12.8	13.0	12.5	12.7	12.8
Age at first term pregnancy* (mean years)	24.8	24.2	23.2	23.1	23.5
Abortions (mean no.)	0.4	0.5	0.6	0.6	0.6

* 20 or more weeks gestation.

A prior history of oral contraceptive use was less prevalent among participants in the 1970s study than among those in the 1980s study. This difference reflects temporal trends of increasing oral contraceptive use in the United States. Since women in the 1970s study were interviewed at an earlier date and were older at interview than were those in the later study, a greater fraction of their reproductive years occurred before the introduction of oral contraceptives in the early 1960s.

Characteristics considered below were similar for the two sets of 1980s controls. Therefore, we report only results obtained by comparing 1980s cases with the combined 1980s set of hospital and population controls.

Table 2 gives the distribution of cases and controls and relative risks by number of term pregnancies. Parous women were at lower risk than were nulliparous women in both the 1970s and 1980s studies, although the difference achieved statistical significance only for the 1980s data. The relative risk associated with ever having a term pregnancy was 0.64 for the combined 1970s and 1980s data ($p < 0.001$). Both data sets also indicated decreased risk with increasing number of term pregnancies among the parous women, although in neither study was the decrease statistically significant. According to the logistic function fit to the combined data, each term pregnancy conferred a 7 per cent reduction in risk ($p = 0.18$).

Table 2 also gives relative risks by age at first term pregnancy. The table shows no trend in risk with age at first term pregnancy, regardless of the decade of data collection. This lack of trend was also evident in both data sets after adjustment for number of term pregnancies. Similarly, neither data set showed any trend in risk with age at last term pregnancy, either before or after adjustment for number of term pregnancies.

The effects of age at menarche and of menopausal status are examined in table 3. Ovarian cancer risk tends to decrease with increasing age at menarche. This trend was statistically significant in the 1980s data ($p = 0.04$) and in the combined data ($p = 0.03$). Overall, each year of delayed menarche beyond age 12 years conferred a 9 per cent reduction in risk.

By contrast, menopausal status and age at natural menopause had no effect on risk, regardless of the decade of interview. Women who had undergone surgical or natural menopause were not at altered risk in comparison to premenopausal women of the same age. Among postmenopausal women, older age at natural menopause was associated with slightly increased risk, but the trend was not statistically significant.

TABLE 2

Ovarian cancer risk, by number of term pregnancies and by age at first term pregnancy, San Francisco Bay Area, 1974–1977 and 1983–1985

	1970s participants						1980s participants						All participants					
	Cases		Controls		RR†	95% CI†	Cases		Controls		RR	95% CI	Cases		Controls		RR	95% CI
	n	(%)	*n*	(%)			*n*	(%)	*n*	(%)			*n*	(%)	*n*	(%)		
No. of term pregnancies‡																		
None	31	(28)	93	(20)	1.00		39	(21)	74	(14)	1.00		70	(23)	167	(17)	1.00	
One or more	80	(72)	379	(80)	0.67	0.40–1.11	149	(79)	465	(86)	0.61	0.39–0.96	229	(77)	844	(83)	0.64*	0.45–0.89
1	18	(16)	71	(15)	0.80	0.40–1.58	22	(12)	65	(12)	0.64	0.34–1.20	40	(13)	136	(13)	0.71	0.45–1.12
2	31	(28)	136	(29)	0.67	0.37–1.21	51	(27)	163	(30)	0.62	0.37–1.03	82	(27)	299	(30)	0.64*	0.44–0.95
3+	31	(28)	172	(36)	0.58	0.31–1.08	76	(40)	236	(44)	0.59	0.36–0.98	107	(36)	408	(40)	0.60*	0.40–0.88
Unspecified	0	(0)	0	(0)			0	(0)	1	(0)			0	(0)	1	(0)		
Overall trend per pregnancy (if parous)					0.88	0.72–1.08					0.96	0.84–1.08					0.93	0.84–1.04
Age (years) at first term pregnancy‡																		
Nulliparous	31	(28)	93	(20)	1.00		39	(21)	74	(14)	1.00		70	(23)	167	(17)	1.00	
<20	9	(8)	66	(14)	0.39	0.16–0.92	30	(16)	99	(18)	0.61	0.34–1.09	39	(13)	165	(16)	0.55	0.34–0.88
20–24	33	(30)	146	(31)	0.70	0.38–1.28	68	(36)	189	(35)	0.72	0.44–1.19	101	(34)	335	(33)	0.73	0.49–1.07
25–29	20	(18)	103	(22)	0.65	0.33–1.25	31	(16)	115	(21)	0.52	0.30–0.92	51	(17)	218	(22)	0.57	0.37–0.88
30+	18	(16)	60	(13)	0.99	0.49–2.00	18	(10)	60	(11)	0.56	0.28–1.12	36	(12)	120	(12)	0.73	0.44–1.19
Unspecified	0	(0)	4	(1)			2	(1)	2	(0)			2	(1)	6	(1)		
Overall trend per 5 years (among the parous)					1.20	0.92–1.55					0.95	0.78–1.15					1.03	0.88–1.20

* $p < 0.001$.

† RR, relative risk; CI, confidence interval.

‡ 20 or more weeks gestation

TABLE 3

Ovarian cancer risk, by age at menarche and by menopausal status, San Francisco Bay Area, 1974–1977 and 1983–1985

	1970s participants						1980s participants						All participants					
	Cases		Controls		RR*	95% CI*	Cases		Controls		RR	95% CI	Cases		Controls		RR	95% CI
	n	(%)	*n*	(%)			*n*	(%)	*n*	(%)			*n*	(%)	*n*	(%)		
Age (years) at menarche																		
<12	18	(16)	85	(18)	1.00		48	(26)	111	(21)	1.00		66	(22)	196	(19)	1.00	
12–14	79	(71)	303	(64)	1.19	0.67–2.12	120	(64)	347	(64)	0.79	0.53–1.17	199	(67)	650	(64)	0.90	0.65–1.25
15+	12	(11)	75	(16)	0.81	0.36–1.84	20	(11)	79	(15)	0.56	0.31–1.03	32	(11)	154	(15)	0.63	0.39–1.02
Unspecified	2	(2)	9	(2)			0	(0)	2	(0)			2	(0)	11	(1)		
Overall trend per year					0.94	0.82–1.07					0.89	0.81–0.99					0.91	0.84–0.99
Menopausal status																		
Premenopausal	37	(33)	139	(29)	1.00		63	(34)	196	(36)	1.00		100	(33)	335	(33)	1.00	
Surgical menopause†	0	(0)	0	(0)			41	(22)	154	(29)	0.93	0.54–1.60	41	(14)	154	(15)	0.89	0.54–1.47
Age (years) at natural menopause	74	(67)	333	(71)	1.16	0.50–2.69	84	(45)	189	(35)	1.47	0.80–2.69	158	(53)	522	(52)	1.36	0.83–2.22
<45	7	(6)	46	(10)	0.71	0.23–2.22	11	(6)	22	(4)	1.55	0.54–4.44	18	(6)	68	(7)	1.06	0.50–2.27
45–49	28	(25)	97	(21)	1.40	0.59–3.33	21	(11)	60	(11)	1.03	0.42–2.54	49	(16)	157	(16)	1.26	0.67–2.34
50+	33	(30)	149	(32)	1.09	0.42–2.79	51	(27)	103	(19)	1.67	0.67–4.18	84	(28)	252	(25)	1.41	0.73–2.70
Unspecified	6	(5)	41	(9)			1	(1)	4	(1)			7	(2)	45	(4)		
Overall trend per 5 years (among those naturally menopausal)					1.05	0.76–1.46					1.22	0.87–1.71					1.13	0.89–1.43

* RR, relative risk; CI, confidence interval.

† Women with surgical menopause were excluded in the 1970s study.

iere were no clear or consistent pat-
s of risk with any of several menstrual
ors examined, which included history
nenorrhea, irregular menstrual cycles,
midcycle pain.

able 4 shows the effects of oral contra-
ive use on ovarian cancer risk, after
stment for number of term pregnan-

The unadjusted relative risks and p
es were similar to the adjusted ones.
pite the different frequency distribution
al contraceptive use in the two decades,
ive risks from the two studies were
lar, with a combined risk reduction of
er cent associated with prior oral con-
eptive use. Risk decreased with increas-
luration of use. For the combined data,
ive risks ranged from 0.97 among
ien who had used oral contraceptives
ne year or less to 0.40 among users of
e than three years. The overall reduc-
in risk per year of use among ever
s was 12 per cent ($p < 0.001$).

ne protection afforded by oral contra-
ive use was greatest among women who
used them for more than one year. The
ive risk did not vary with time since
oral contraceptive use or with time
e first use, after adjustment for total
ation of use.

able 5 gives relative risks by $3 \times 3 = 9$
categories of parity and duration of
contraceptive use. The data suggest
the protective effects of oral contra-
ive use do not wane with increasing
ty. The relative risks in table 5 were
uately described by a multiplicative
ive risk function (goodness of fit $\chi^2_4 =$
$p = 0.21$). According to this function,
ty status has no effect on risk ratios in
nigher two durations of oral contracep-
use relative to the lowest duration.

ases and controls were similar with re-
t to noncontraceptive estrogen use dur-
both the 1970s and the 1980s. For the
bined data, the relative risk associated
a history of any noncontraceptive es-
en use was 0.89 ($p = 0.46$). Further-

TABLE 4

Ovarian cancer risk, by oral contraceptive use, San Francisco Bay Area, 1974–1977 and 1983–1985

Oral contraceptive use	1970s participants					1980s participants					All participants				
	Cases		Controls			Cases		Controls			Cases		Controls		
	n	(%)	*n*	(%)	RR†	95% CI†	*n*	(%)	*n*	(%)	RR	95% CI	*n*	(%)	*n*	(%)	RR	95% CI
Never used	86	(77)	370	(78)	1.00		102	(54)	249	(46)	1.00		188	(63)	619	(61)	1.00	
Ever used (months of use)																		
1–12	25	(23)	102	(22)	0.71	0.37–1.37	86	(46)	290	(54)	0.74	0.48–1.14	111	(37)	392	(39)	0.74	0.52–1.06
13–36	9	(8)	39	(8)	0.72	0.30–1.72	42	(22)	97	(18)	1.03	0.63–1.70	51	(17)	136	(13)	0.97	0.64–1.49
37+	8	(7)	19	(4)	1.26	0.46–3.42	16	(9)	50	(9)	0.66	0.32–1.35	24	(8)	69	(7)	0.81	0.45–1.44
Unspecified	8	(7)	38	(8)	0.53	0.20–1.43	20	(11)	131	(24)	0.37	0.20–0.68	28	(9)	159	(17)	0.40	0.24–0.66
	0	(0)	6	(1)			8	(4)	12	(2)			8	(3)	18	(2)		
Overall trend per 12 months of use (among users)					0.96	0.86–1.08					0.86*	0.79–0.93					0.88*	0.83–0.94

* $p < 0.001$.
† RR, relative risk, adjusted for number of term pregnancies (20 or more weeks gestation) in categories 0, 1–2, and 3+; CI, confidence interval.

TABLE 5

Ovarian cancer risk, by parity and by duration of oral contraceptive use (all participants), San Francisco Bay Area, 1974–1977 and 1983–1985

Months of oral contraceptive use	No. of term pregnancies*											
	None				1–2				3+			
	Cases	Controls	RR†	95% CI‡	Cases	Controls	RR	95% CI	Cases	Controls	RR	95% CI
0–12	57	124	1.00		99	323	0.63*	0.42–0.95	83	307	0.55*	0.35–0.84
13–36	7	11	0.82	0.28–2.47	7	34	0.38*	0.22–0.65	10	24	0.66	0.43–1.02
37+	3	26	0.13*	0.04–0.50	16	73	0.34*	0.25–0.50	9	68	0.23*	0.15–0.35
Unspecified	3	4			0	5			5	9		
Overall trend per 12 months of use (among users)			0.35	0.05–2.47			0.96	0.84–1.10			0.85	0.68–1.07

* $p < 0.01$.
† 20 or more weeks gestation.
‡ RR, relative risk; CI, confidence interval.

more, there was no clear trend with duration of use.

To investigate the hypothesis that the protective effects of late age at menarche, parity, and oral contraceptive use are due to their common suppression of ovulation, we examined the relation between risk and estimated duration of ovulation, defined as the time between menarche and last menstrual period, minus nine months for each term pregnancy and three months for other pregnancies, and minus the total duration of oral contraceptive use. Women who had undergone hysterectomy without bilateral oophorectomy and were under age 45 years were assumed to be ovulating; those aged 45–55 years ($n = 75$) were excluded from the analysis, and those older than 55 years were assigned the median menopausal age of nonhysterectomized women, specific for case and control status. We excluded breastfeeding from the calculation of ovulatory years because its effectiveness in suppressing ovulation is variable, depending on the mother's nutritional status, the extent of supplemental feeding to the infant, and the intensity of suckling (25–27). Its effectiveness for women in developed countries is weaker than that of pregnancy or oral contraceptive use; some 40–75 per cent of lactating women menstruate while nursing (25, 27).

Table 6 relates ovarian cancer risk to estimated duration of ovulation. Data from both studies show trends of increasing risk with increasing duration of ovulation, with a stronger trend in the 1980s study than in the earlier one. Compared with a relative risk of unity for those who had been ovulating for less than 25 years, women in the combined studies who had ovulated for an estimated 25–29 years had a risk of 1.58, those who had been ovulating for 30–34 years had a risk of 2.48, and those who had been ovulating longer had a risk of 3.26. According to the logistic function fit to the combined data, each additional five years of ovulation conferred a 56 per cent increase in risk ($p < 0.001$). These findings

TABLE 6

Ovarian cancer risk, by duration of ovulation, San Francisco Bay Area, 1974–1977 and 1983–1985

	1970s participants					1980s participants					All participants				
	Cases		Controls			Cases		Controls			Cases		Controls		
	n	(%)	*n*	(%)	RR†	95% CI†	*n*	(%)	*n*	(%)	RR	95% CI	*n*	(%)	*n*	(%)	RR	95% CI
Years of ovulation‡																		
<25	17	(15)	47	(10)	1.00		33	(18)	144	(27)	1.00		50	(17)	191	(19)	1.00	
25–29	15	(14)	93	(20)	0.87	0.30–2.58	28	(15)	89	(17)	1.98	0.95–4.11	43	(14)	182	(18)	1.58	0.86–2.90
30–34	35	(32)	133	(28)	1.57	0.53–4.65	46	(24)	119	(22)	2.84*	1.33–6.10	81	(27)	252	(25)	2.48*	1.33–4.64
35+	37	(33)	146	(31)	1.66	0.54–5.12	60	(32)	118	(22)	4.43*	1.96–9.99	97	(32)	264	(26)	3.26*	1.69–6.26
Unknown	7	(6)	53	(11)			21	(11)	69	(13)			28	(9)	122	(12)		
Overall trend per 5 years					1.32	1.01–1.72					1.73	1.39–2.14					1.56	1.32–1.85

* $p < 0.001$.

† RR, relative risk; CI, confidence interval

‡ See text for definition.

could be confounded by age despite the matching within five-year age groups. However, the results were unaffected when age was added to the regressions as a continuous variable.

DISCUSSION

The above results should be interpreted cautiously for several reasons including the studies' failure to interview all eligible ovarian cancer patients and a completely random sample of eligible controls, potential pitfalls in combining the two studies and the two control groups in the second study, and the possibility of confounding by unmeasured variables. Nevertheless, several conclusions seem appropriate. In particular, the present data do not support the hypothesis that an association of ovarian cancer with infrequent oral contraceptive use is a consequence of their common correlation with subfertility. This hypothesis predicts that oral contraceptive use confers no benefit on women of high parity and that long episodes of use confer little benefit above that of short episodes. These predictions are inconsistent with the present data which show oral contraceptive use to reduce risk independently of number of previous term pregnancies, with increased reduction attached to increasing duration of use.

Most studies support the present findings that nulliparous women are at elevated risk of ovarian cancer (1–4, 6–9, 11–13, 28–31). Similarly, the lack of association noted with age at first term pregnancy agrees with other data (2, 3, 13, 28, 32–34). Exceptions are provided by data from two studies (9, 35) showing that women whose first pregnancy occurred after the age of 25 years had two to three times the risk of those whose first pregnancy occurred earlier.

At least 12 studies support the current finding that oral contraceptive use protects against ovarian cancer (1, 2, 4, 5, 7, 8, 11, 13–16, 36). The strong trend noted here of increasing protection with increasing years of use also was found in a recent study

involving 492 cases and 4,228 controls (4) which found similar protective effects for each of the four major histologic subtypes and for each of several specific oral contraceptive formulations.

Other studies (4, 14) also support the constancy of relative risk with respect to time since last oral contraceptive use among women who have used oral contraceptives for a fixed duration. Such a constant relative risk model predicts that former users of oral contraceptives experience the same per cent reduction in ovarian cancer incidence rates throughout their lifetime. However, the long-term effects of oral contraceptive use cannot yet be tested in women over 60 years of age, because too few have ever used oral contraceptives. Therefore, one cannot yet exclude the possibility that the protective effect of oral contraceptive use wanes long after use ceases.

The present association between early age at menarche and increased risk is not supported by other studies, which have found cases and controls to be similar in their ages at menarche (8, 11, 13). Cases and controls also appear similar in their ages at menopause, according to data here and elsewhere (1, 11, 13). The failure of case-control studies to find a strong relation between ovarian cancer and age at menopause contrasts with the behavior of national incidence rates for the disease, which increase more slowly with age after the fifth decade (37). This drop in the rate of increase at the time of menopause suggests a protective effect for termination of ovulation. The effect may be missed in case-control studies because they measure termination of menstrual bleeding rather than ovulation. Anovulatory cycles are so common at the end of reproductive life that perimenopausal menstrual bleeding is a poor indicator of ovulation.

Late menarche, pregnancy, and oral contraceptive use decrease a woman's total number of ovulations. Therefore, the protective effects of these characteristics provide indirect support for the hypothesis of Fathalla (17) that ovulation increases risk for ovarian cancer, possibly by subjecting the ovarian epithelium to increased cellular proliferation, steroid-rich follicular fluid, or elevated levels of pituitary gonadotropins. Results from four other case-control studies that have examined this issue (2, 7, 8, 30) agree with the present finding that a woman's estimated duration of ovulation is a strong indicator of increased ovarian cancer risk. One study (30) found substantially larger magnitudes of diminished risk from each of the separate characteristics that reduce ovulatory years than would be expected solely on the basis of their inhibition of ovulation. For example, the reduction in risk per year of oral contraceptive use was roughly four times the reduction per year of early menopause. However, these findings do not provide strong evidence against the hypothesis that pregnancy and oral contraceptive use protect by suppressing ovulation. As noted above, a decrease of one year in age at menopause need not represent a decrease of one ovulatory year, since ovulation is sporadic in the perimenopausal years. Furthermore, in estimating the magnitudes of diminished risk expected according to the ovulatory suppression hypothesis, the authors assumed that the logarithm of ovarian cancer incidence rates increases in proportion to total duration of ovulation. However, it can be shown that if cancer risk depends only on duration of ovulation and the regression model relating risk to duration of ovulation is misspecified, the estimated regression coefficients corresponding to ovulatory years lost from the separate characteristics need not have equal magnitudes.

Stadel (38) and Cramer and Welch (39) have suggested that high circulating levels of pituitary gonadotropins may play a role in ovarian cancer. If so, late menarche, pregnancy, and oral contraceptive use may protect against the disease by reducing total exposure to gonadotropins (40, 41). However, in agreement with other investigators

7, 8, 39, 42, 43), we found no statistically nificant associations between ovarian ıcer risk and use of noncontraceptive rogens, a practice which also reduces ıadotropin exposure, although not to emenopausal levels (40). This lack of asiation suggests that the protective efts of oral contraceptive use may be meıted by mechanisms other than its role reducing serum levels of gonadotropins. rther work is needed to resolve this issue. se numbers in the present study were too all to investigate the finding of Weiss et (43) that noncontraceptive estrogen use reases risk for ovarian tumors of the dometrioid type.

In summary, the present observations pport the concept that oral contraceptive decreases risk for ovarian cancer, rather ın merely indicates fertility, which may elf be associated with decreased risk. rthermore, they suggest that the greater oman's duration of ovulation (or of conitant endocrinologic phenomena), the ater her risk of developing ovarian can-

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Br. J. Cancer (1989), 60, 592-598

Risk factors for ovarian cancer: a case-control study

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Summary A hospital-based case-control study of ovarian cancer was conducted in London and Oxford between October 1978 and February 1983. Menstrual characteristics, reproductive and contraceptive history and history of exposure to various environmental factors were compared between 235 women with histologically diagnosed epithelial ovarian cancer and 451 controls. High gravidity, hysterectomy, female sterilisation and oral contraceptive use were associated with a reduced risk of ovarian cancer. Infertility and late age at menopause were associated with an increase in risk. While these factors were related, they were each found to be independently associated with ovarian cancer risk after adjusting for the effect of the other factors.

While results from recent case-control studies have consistently shown that multiparity and oral contraceptive use are associated with a reduced risk of ovarian cancer, the association of the cancer with other reproductive, hormonal and related factors such as age at menopause, history of hysterectomy or use of oestrogen replacement therapy is less clear. We have conducted a hospital-based case-control study in London and Oxford which was designed to investigate the independent contributions of reproductive history and contraceptive use to ovarian cancer risk. In particular, it was planned to attempt to segregate out the effect on risk of infertility from that of voluntary limitation of family size. The association between ovarian cancer and other possible aetiological agents was also examined.

Subjects and methods

Between October 1978 and February 1983 five interviewers identified and questioned women with a diagnosis of ovarian cancer and women selected as controls at 13 hospitals in London and two in Oxford. A standard questionnaire was used to obtain information on reproductive and menstrual history and on exposure to various substances such as exogenous oestrogens, cigarettes and talc. A month by month record was made of the specific contraceptive methods used by each woman between the ages of 16 and 45 years, or, if under 45 years, up to the time of diagnosis (cases) or interview (controls). The methods were classified as sheaths, diaphragms, intrauterine devices, oral contraceptives or 'other methods' (spermicides, rhythm and coitus interruptus). Women who reported using a contraceptive diaphragm were asked if they had stored it in talc. Also recorded were months during which a woman was not using contraception due to sexual abstinence, pregnancy, menopause or because she or her partner had been sterilised. The other months when a woman reported using no method of contraception although sexually active have been classified as months of 'unprotected intercourse'. The total duration of use of each contraceptive method, of any contraceptive method, of unprotected intercourse and of pregnancy were computed for each woman.

The study was confined to women aged less than 65 years whose diagnosis of ovarian cancer had been made within two years of interview. A total of 280 cases were interviewed and pathological specimens were histologically classified by Professor C. Hudson and Dr M. Curling from St Bartholomews Hospital. A total of 235 women with epithelial ovarian cancer were included in the analyses. For these women, the tumour type was described as serous in 101 (43%) cases, mucinous in 38 (15%) cases, endometrioid in 52 (22%) cases and clear cell in 12 (5%) cases. Mixed and undifferentiated types of epithelial tumours accounted for the remaining 32 (14%) cases. Excluded from the analyses were nine women with a non-epithelial ovarian neoplasm, 11 with a primary tumour in an unknown site outside the ovary, 21 with a primary tumour in an unknown site although one consistent with an ovarian origin, one with a benign tumour and three for whom pathology material could not be obtained.

For each case it was planned to select two age-matched controls from women being treated in the same hospital. Women with bilateral oophorectomy were excluded from the control group as were women admitted with conditions that have been related to reproductive history or oral contraceptive use (all circulatory and gynaecological diseases, gallbladder and thyroid diseases, rheumatoid arthritis, malignant disease of the breast, uterus and bladder, and melanoma).

It proved logistically impossible to select two age-matched controls for each case from the same hospital and it was decided merely to ensure that the age distribution of the controls was approximately the same as that of the cases. For 63 cases recruited from a London hospital where only cancer patients are treated, controls were selected from other London hospitals. For these reasons, the data were analysed using an unmatched approach with adjustments being made to relative risk estimates for age and socio-economic status. A total of 451 controls have been included in the analyses. The admission diagnoses for these patients were gastrointestinal disease (105), bone or joint disease (70), respiratory disease (39), renal or other urinary disease (35), neurological disease (30), fractures or other injuries (28), skin or subcutaneous tissue disease (17), malignant neoplasms of the digestive organs (15) and bone or skin (2), benign neoplasms of the digestive organs (4) respiratory system (4) and other sites (8) and various other conditions and symptoms (94). This final category included patients with haemorrhoids (15) and those with symptoms relating to the respiratory system (10), gastrointestinal tract (20) and urinary system (10).

Maximum likelihood estimates of relative risk (RR) together with their 95% confidence interval (95% CI) and tests for trend where appropriate were computed by multiple logistic regression techniques (Breslow & Day, 1980) using the GLIM statistical package (Baker & Nelder, 1978). All relative risks have been adjusted for age in 5-year strata (20-24, 25-29, . . . 60-64) and for social class in six categories (I,II,III non-manual, III manual, IV and V). Age of the cases was taken as age at diagnosis of ovarian cancer and of the controls as age at interview. Social class was based on occupation (Office of Population Censuses and Surveys, 1970) using husband's occupation for ever married women and own occupation for those who had never married. Other relative risk adjustments and tests for trend have been made with the exposures as continuous variables. When the data were examined by place of interview (London or Oxford), there were no notable differences in the risk estimates

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Received 3 February 1989; and in revised form 9 May 1989

associated with the major variables of interest. The relative risks have not, therefore, been stratified by place of interview. The terms nulligravid and gravid have been used to denote, respectively, women who have never knowingly conceived and women who have had at least one pregnancy. Parity has been defined as number of live and still births.

Results

The age distributions of the cases and controls are shown in Table I. The average age of the cases was slightly higher than that of the controls. There was an excess of cases in social classes I, II, and III non-manual (58%) as compared to controls (43%) ($P = 0.05$) and, because of this, all relative risks have been adjusted for social class as well as age. Table II shows the relative risks for ovarian cancer associated with various aspects of pregnancy history. Nulligravid women had a higher risk of ovarian cancer than gravid women (RR = 1.7, 95% CI 1.1–2.6). The relative risks were elevated both in nulligravid women who had been sexually active and in those who had not, although significantly so only for the sexually active. Among those who had ever been pregnant, the relative risks decreased as the number of pregnancies increased, (χ^2 (trend) = 4.3, $P < 0.05$). Similarly, among parous women, the higher the parity the lower the relative risks (χ^2 (trend) = 3.9, $P < 0.05$). After adjusting for parity, the relative risks associated with successive numbers of incomplete pregnancies (spontaneous and induced abortions) also decreased although the trend was not statistically significant (χ^2 (trend) = 0.5). Women having their first pregnancy after the age of 35 years had a significantly higher risk of ovarian cancer than women with a first pregnancy before the age of 20 years. Their risk was also higher than that for nulligravid women. There was, however, no marked nor significant trend of increasing risk the later the age at first pregnancy (χ^2 (trend) = 1.0). Analyses by age at first livebirth gave similar findings. After adjustment for number of livebirths, women who had breastfed for more than two years in total had over three times the risk of ovarian cancer compared to women who had never breastfed ($P < 0.05$) but overall, there was no significant trend the longer the duration of lactation.

Analyses of infertility and subfertility as risk factors for ovarian cancer were restricted to the 213 (91%) cases and 240 (93%) controls who reported that they had ever been sexually active. Among these women, 30 (14%) with ovarian cancer and 34 (8%) controls reported, when so questioned, that they had had problems in becoming pregnant and, of these, 16 cases and 12 controls had never conceived. Analysis of the data on contraceptive use suggested that there were other women who might have been infertile or subfertile. Although sexually active, they had used contraception infrequently or not at all and had had few or no pregnancies. For all women who had ever been sexually active, the risk of ovarian cancer increased with increasing duration of unprotected intercourse after adjustment for gravidity (χ^2 (trend) = 10.2, $P < 0.01$). The effect was most marked among nulligravid women who reported more than 10 years of unprotected intercourse. Their risk was over six times that of nulligravid women who reported less than three months of unprotected intercourse (Table III). Among gravid women, those reporting over 10 years of unprotected intercourse had a higher risk than other gravid women. There was no significant trend in risk associated with the duration of use of any contraception (χ^2 (trend) = 1.2) although sexually active nulligravid women who had never used any method of contraception had about twice the risk of ovarian cancer compared to all other sexually active women (Table IV).

Of the specific methods of contraception studied, ever having used oral contraception and having been sterilised were associated with a statistically significantly reduced risk of ovarian cancer, while no method was associated with a significantly elevated risk (Table V). As only three cases had been sterilised it was not possible to assess whether age at sterilisation influenced the risk of ovarian cancer. Table VI shows detailed analyses of the relative risks associated with oral

Table I Age distribution and average age of cases and controls

Age (years)	*Cases (%)*	*Controls (%)*
20–34	13 (5.5)	33 (7.3)
35–44	27 (11.5)	75 (16.6)
45–54	87 (37.0)	156 (34.6)
55–64	108 (46.0)	187 (41.5)
Total	235	451
Average age (years)	52.4	51.4

Table II Relative risks for ovarian cancer associated with pregnancy history

Variable	*Cases*	*Controls*	*RR*	*95% CI.*
Gravidity[b]				
Gravid	176	376	1.0[a]	
Nulligravid	59	74	1.7	(1.1–2.6)
Nulligravid and ever sexually active	37	44	1.9	(1.1–3.1)
Nulligravid and never sexually active	22	30	1.5	(0.8–2.6)
Number of pregnancies				
0	59	74	1.0[a]	
1	43	71	0.8	(0.4–1.3)
2	63	107	0.7	(0.4–1.1)
3	37	98	0.5	(0.3–0.8)
4	13	41	0.4	(0.2–0.8)
⩾5	20	59	0.4	(0.2–0.8)
	χ^2 for trend = 4.3 $P<0.05$ (gravid women only)			
Estimated reduction in relative risk associated with each pregnancy			0.86	(0.78–0.94)
Parity[b]				
0	66	87	1.0[a]	
1	48	84	0.7	(0.4–1.2)
2	61	127	0.6	(0.4–1.0)
3	40	88	0.6	(0.3–1.0)
4	12	30	0.5	(0.2–1.0)
⩾5	8	34	0.3	(0.1–0.7)
	χ^2 for trend = 3.9 $P<0.05$ (parous women only)			
Estimated reduction in relative risk associated with each birth			0.84	(0.75–0.94)
No. of incomplete pregnancies[b,c]				
0	185	330	1.0[a]	
1	39	83	0.9	(0.6–1.4)
2	7	18	0.7	(0.3–1.8)
⩾3	4	19	0.6	(0.2–1.7)
	χ^2 for trend = 0.5			
Estimated reduction in relative risk associated with each incomplete pregnancy			0.92	(0.75–1.13)
Age at first pregnancy (years)[d]				
15–19	26	65	1.0[a]	
20–24	73	182	0.9	(0.5–1.5)
25–29	49	96	1.2	(0.7–2.2)
30–34	17	29	1.2	(0.8–2.7)
⩾35	9	4	4.1	(1.1–15.1)
Nulligravid	59	74	2.0	(1.1–3.7)
	χ^2 for trend = 1.0 (gravid women only)			
Months of lactation[c]				
None	44	107	1.0[a]	
⩽6	66	124	1.3	(0.8–2.2)
7–12	29	80	0.9	(0.5–1.6)
13–18	13	29	1.2	(0.5–2.5)
19–24	5	7	2.1	(0.7–6.7)
⩾25	12	15	3.4	(1.1–10.8)
	χ^2 for trend = 1.8			

All relative risks adjusted for age and social class. [a]Reference category. [b]Data missing for 1 control. [c]Relative risks adjusted for parity. [d]Data missing for 2 cases and 1 control. [e]Women with livebirths only. Relative risks adjusted for number of live births.

Table III Relative risks for ovarian cancer associated with duration of unprotected intercourse by gravidity

	Cases	*Controls*	*RR*	*(95% CI)*
Nulligravid women				
Duration of unprotected intercourse (months)[b]				
⩽3				
4–60	12	26	1.0[a]	(0.4–6.5)
61–120	4	6	1.5	(0.1–7.8)
>120	1	4	0.7	(2.1–20.4)
	20	8	6.5	
	χ^2 for trend = 11.2 $P<0.001$			
Gravid women				
Duration of unprotected intercourse (months)[b]				
⩽3				
4–60	78	176	1.1	(0.5–2.4)
61–120	51	113	1.1	(0.5–2.6)
>120	10	27	1.1	(0.4–3.2)
	37	60	1.6	(0.7–4.0)
	χ^2 for trend = 2.6			

Sexually active women only. Relative risks adjusted for age and social class. [a]Reference category. [b]Time when sexually active and at risk of pregnancy but using no contraception.

Table IV Relative risks for ovarian cancer associated with duration of use of contraception by gravidity

	Cases	*Controls*	*RR*	*(95% CI)*
Nulligravid women				
Duration of use of contraception				
Never used	15	10	1.0[a]	
<10 years	14	21	0.5	(0.1–1.7)
10–20 years	6	9	0.5	(0.1–2.5)
>20 years	2	4	0.4	(0.1–3.2)
	χ^2 for trend = 0.9			
Gravid women				
Duration of use of contraception				
Never used	32	47	0.4	(0.2–1.1)
<10 years	25	56	0.3	(0.1–1.0)
10–20 years	55	147	0.4	(0.1–1.2)
>20 years	64	126	0.5	(0.2–1.5)
	χ^2 for trend = 0.3			

Sexually active women only. Relative risks adjusted for age, social class and duration of unprotected intercourse. [a]Reference category.

Table V Relative risks for ovarian cancer associated with the use of different methods of contraception

Method of contraception		*Cases*	*Controls*	*RR*	*(95% CI)*
Sheath	Never used	108	205	1.0[a]	
	Ever used	105	215	1.1	(0.8–1.7)
Diaphragm	Never used	178	329	1.0[a]	
	Ever used	35	91	0.7	(0.4–1.1)
Intrauterine device	Never used	201	383	1.0[a]	
	Ever used	12	37	0.8	(0.4–1.7)
Oral contraception	Never used	178	306	1.0[a]	
	Ever used	35	114	0.5	(0.3–0.9)
Partner with vasectomy	No	203	404	1.0[a]	
	Yes	10	16	2.1	(0.9–4.9)
Female sterilisation	No	210	375	1.0[a]	
	Yes	3	45	0.2	(0.1–0.6)
Other methods[b]	Never Used	156	292	1.0[a]	
	Ever used	57	128	1.1	(0.7–1.7)

Sexually active women only. Relative risks adjusted for age, social class, gravidity and duration of unprotected intercourse. [a]Reference category. [b]Use of spermicides, rhythm or coitus interruptus.

contraceptive use. The risks decreased as duration of use increased although, among those who had ever used such contraceptives, the trend was not significant (χ^2 (trend) = 1.2). Whatever their age at first use, women who used oral contraceptives had a lower risk of ovarian cancer than those who had never used them, the risk being lowest in those who had first used oral contraceptives under the age of 25 years. The risk of developing ovarian cancer did not increase as time since discontinuing use increased. Women who had stopped using oral contraceptives more than ten years previously had a statistically significant reduced risk of 0.3 compared to women who had never used them. Women both under the age and over the age of 40 years had a reduced risk of ovarian cancer associated with oral contraceptive use, but the reduction was greater in the younger women. Gravid and nulligravid women who had used oral contraceptives had a reduced risk of ovarian cancer.

Table VII shows the relative risks associated with age at menarche and age at natural menopause. There was no trend in risk with age at menarche (χ^2 (trend) = 0.03). In contrast, risk increased the later the age at natural menopause (χ^2 (trend) = 7.1, $P<0.01$). Women having their menopause at the age of 50 years or later had nearly three times the risk of women who were menopausal before the age of 45 years. The risks and trend associated with age at menopause were similar irrespective of whether they were adjusted for age in five year or one year strata.

Table VI Relative risks for ovarian cancer associated with oral contraceptive (OC) use

	Cases	*Controls*	*RR*	*(95% CI)*
Duration of OC use (years)				
Never used	178	306	1.0[a]	
<5	24	70	0.6	(0.3–1.0)
5–10	10	29	0.6	(0.2–1.4)
>10	1	15	0.1	(0.01–1.0)
	χ^2 for trend within users = 1.2			
Age at first OC use (years)				
Never used	178	306	1.0[a]	
<25	6	39	0.1	(0.04–0.5)
25–29	6	17	0.6	(0.2–2.0)
30–34	11	27	0.7	(0.3–1.6)
⩾35	12	31	0.7	(0.4–1.5)
	χ^2 for trend within users = 5.9, $P<0.05$			
Time since discontinuing OC use (years)				
Never used	178	306	1.0[a]	
Current users	6	19	0.5	(0.2–1.5)
<5	12	24	0.8	(0.4–1.9)
5–10	9	25	0.8	(0.3–1.9)
>10	8	46	0.3	(0.1–0.7)
	χ^2 for trend within users = 2.6			
Age (years)				
<40				
OC use				
Never used	11	11	1.0[a]	
Ever used	9	35	0.2	(0.1–0.9)
⩾40				
OC use				
Never used	167	295	1.0[a]	
Ever used	26	79	0.7	(0.4–1.2)
Gravidity				
Gravid women[b]				
OC use				
Never used	149	280	1.0[a]	
Ever used	27	96	0.5	(0.3–0.9)
Nulligravid women				
OC use				
Never used	29	26	1.0[a]	
Ever used	8	18	0.3	(0.05–2.8)

Sexually active women only. Relative risks adjusted for age, social class, gravidity and duration of unprotected intercourse. [a]Reference category. [b]Relative risks adjusted for gravidity.

Table VII Relative risks for ovarian cancer associated with age at menarche and age at natural menopause

	Cases	*Controls*	*RR*	*(95% CI*
Age at menarche (years)[b]				
>14	97	197	1.0[a]	
12–13	89	185	0.9	(0.6–1.3)
<12	46	66	1.3	(0.8–2.1)
	χ^2 for trend = 0.03			
Age at natural menopause (years)[c]				
<45	10	34	1.0[a]	
45–49	47	77	2.0	(0.9–4.7)
>50	84	99	2.5	(1.1–5.8)
	χ^2 for trend = 7.1 $P < 0.01$			

Relative risks adjusted for age and social class. [a]Reference category. [b]Data on age at menarche missing for 3 cases and 3 controls. [c]Data on age at menopause missing for 2 cases and 1 control.

Women who reported hysterectomy, with or without unilateral oophorectomy, had a much reduced risk (Table VIII). Since there were only 10 women with ovarian cancer who had had a hysterectomy it was not possible to assess the effect of age at hysterectomy on ovarian cancer risk.

Total duration of ovulation was estimated as the months from menarche to diagnosis (cases) or interview (controls), or to menopause, whichever came first, minus the total months of anovulation due to pregnancy and oral contraceptive use. Women who reported a hysterectomy were excluded from these analyses as it was unknown if or when they had stopped ovulating. For all women combined, there was a strong trend of increasing risk the longer the duration of ovulation (χ^2 (trend) = 17.8, $P < 0.001$) (Table IX). In separate analyses by menopausal status, there was no significant effect of duration of ovulation after adjustment for the 'anovulatory' factors used to estimate that exposure, namely, months of pregnancy and oral contraceptive use and age at menopause for post-menopausal women and months of pregnancy and oral contraceptive use for premenopausal women. Duration of ovulation is very sensitive to age but the risks and trends were virtually unaffected when adjusted for age in one year rather than five year strata.

Five (2%) cases and 29 (6%) controls reported having taken hormone pills as a pregnancy test and five (2%) cases and 13 (3%) controls had been given hormones to prevent miscarriage. For all post-menopausal women, there was a small but non-significantly increased risk of ovarian cancer associated with ever having recieved hormone replacement therapy (Table X). The excess was confined to women who had reported a hysterectomy who had an 11-fold risk. The cases did not report more severe menopausal symptoms. Among the hormone treated women with ovarian cancer, 23% had endometrioid or clear cell tumours compared to 38% in the untreated women.

The reproductive and related factors found to be statistically significantly related to ovarian cancer risk (gravidity, duration of unprotected intercourse, use of oral contraception, having been sterilised, age at natural menopause and having had a hysterectomy) are not independent and we also computed the relative risks associated with each factor after adjusting for the others (Table XI). As sterilisation is often a consequence of high parity, the risks associated with gravidity were not adjusted for sterilisation as this was considered to be overadjustment. In this study, 40% of the sterilised women had five or more children compared with 9% of the unsterilised women. Each of the variables remained statistically significantly related to ovarian cancer risk, suggesting that each may be independently associated with the risk of developing ovarian cancer.

There was no significant difference between the percentage of cases (53%) and controls (57%) who had ever smoked cigarettes. No cases or controls reported having worked with asbestos. No cases but three controls reported a radiation-induced menopause.

Women who reported using talc more than once a week or daily had higher risks of ovarian cancer than women who reported less frequent use (Table XII). Although the relative risk of 2.0 associated with weekly use was statistically significant

Table VIII Relative risks for ovarian cancer associated with reported history of hysterectomy and or unilateral oophorectomy

	Cases	*Controls*	*RR*	*(95% CI)*
Reported womb intact	220	370	1.0[a]	
Reported unilateral oophorectomy by no hysterectomy	5	9	0.9	(0.4–2.1)
Reported hysterectomy but conserved ovaries	8	62	0.2	(0.1–0.4)
Reported hysterectomy and unilateral oophorectomy	2	10	0.4	(0.1–1.1)

Relative risks adjusted for age and social class. [a]Reference category.

Table IX Relative risks for ovarian cancer associated with duration of ovulation

	Duration of ovulation (years)	*Cases*	*Controls*	*Relative risks adjusted for age and social class*	*Relative risks adjusted for age, social class and duration of anovulation*	*Relative risks adjusted for age, social class, duration of anovulation and age at menopause*
All women[b]						
	<30	59	163	1.0[a]		
	30–34	73	106	2.0		
	35–39	68	92	2.0		
	>40	19	13	4.3		
				χ^2 for trend = 17.8 $P < 0.001$		
Post-menopausal women						
	<30	14	53	1.0[a]	1.0[a]	1.0[a]
	30–34	54	81	2.4	2.1	0.9
	35–39	58	72	2.4	1.9	0.6
	>40	14	10	5.0	4.0	0.7
				χ^2 for trend = 12.3 $P < 0.001$	7.7 $P < 0.01$	0.5
Premenopausal women						
	<30	45	110	1.0[a]	1.0[a]	
	30–34	19	25	1.5	1.1	
	35–39	10	20	1.3	0.9	
	>40	5	3	3.2	1.9	
				χ^2 for trend = 4.4 $P < 0.05$	0.6	

Women reporting a hysterectomy excluded. [a]Reference category. [b]Data missing for 6 cases and 4 controls. [c]Total months of pregnancy and oral contraceptive use.

Table X Relative risks for ovarian cancer associated with the use of hormone replacement therapy for menopausal symptoms

	Cases	*Controls*	*RR*	*(95% CI)*
All post-menopausal women				
Use of hormone replacement therapy				
No	122	249	1.0[a]	
Yes	34	44	1.5	(0.9–2.6)
Women reporting hysterectomy				
Use of hormone replacement therapy				
No	5	62	1.0[a]	
Yes	5	10	10.9	(1.7–69.0)
Post-menopausal women other than those reporting hysterectomy				
Use of hormone replacement therapy				
No	177	187	1.0[a]	
Yes	29	34	1.2	(0.7–2.3)

Post-menopausal women only. Relative risks adjusted for age and social class. [a]Reference category.

Table XI Relative risks associated with the factors found to be significantly related to ovarian cancer

Factor	*RR*	*(95% CI)*	χ^2 test for trend (1 d.f.)
Gravidity[b,c]			
0	1.0[a]		
1	0.8	(0.4 – 1.5)	
2	0.8	(0.4 – 1.4)	
3	0.6	(0.3 – 1.1)	
4	0.4	(0.2 – 1.0)	
⩾5	0.5	(0.2 – 1.0)	6.5 $P<0.05$
Unprotected intercourse (months)[c]			
<3	1.0[a]		
4–60	1.3	(0.8 – 2.0)	
61–120	1.1	(0.5 – 2.5)	
>120	1.9	(1.2 – 3.2)	7.8 $P<0.05$
Oral contraceptive use[b]			
Never used	1.0[a]		
⩽5 years	0.6	(0.3 – 1.1)	
6–10 years	0.6	(0.3 – 1.4)	
>10 years	0.1	(0.02 – 1.1)	4.6 $P<0.05$
Ever sterilized[b]	0.2	(0.05 – 0.6)*	
Age at natural menopause[d]			
<45 years	1.0[a]		
45–49 years	1.9	(0.8 – 4.5)	8.2 $P<0.01$
⩾50 years	2.6	(1.1 – 6.1)	
Ever reported hysterectomy[c]	0.2	(0.1 – 0.5)*	

The relative risks associated with each factor have been adjusted for age, social class and all the other factors in the table. [a]Reference category. [b]Sexually active women only. [c]Relative risks not adjusted for sterilization, see text for details. [d]Women reporting natural menopause only. *$P<0.001$.

Table XII Relative risks for ovarian cancer associated with reported frequency of talc use in the genital area

	Cases	*Controls*	*RR*	*(95% CI)*
Reported frequency of talc use[b]				
Never	76	178	1.0[a]	
Rarely	6	16	0.9	(0.3 – 2.4)
Monthly	7	24	0.7	(0.3 – 1.8)
Weekly	55	77	2.0	(1.3 – 3.4)
Daily	71	139	1.3	(0.8 – 1.9)
		χ^2 for trend = 3.80, $P=0.05$		

Relative risks adjusted for age and social class. [a]Reference category. [b]Data missing for 18 (8%) cases and 17 (4%) controls as questions on talc use introduced three months after study began.

($P=0.007$), there was no consistent trend of increasing risk with increasing frequency of talc use (χ^2 (trend) = 3.80, $P=0.05$). There was no significant difference between the percentages of cases (86%) and controls (81%) who had used and kept their diaphragm in talc.

Discussion

As in most previously reported studies (Booth & Beral, 1985) we found that nulligravid women had an increased risk of ovarian cancer and that risk decreased as the number of pregnancies increased. We also found that the greater the number of incomplete pregnancies the lower the risk, although the trend was not significant. Most other studies have not investigated if women of low gravidity have an increased risk of ovarian cancer because of reduced fertility or because of voluntary limitation of family size, although Joly *et al.* (1974), McGowan *et al.* (1979) and Nasca *et al.* (1984) found a higher risk in women who had tried to conceive but had failed. Our findings also suggest that infertility is a risk factor for ovarian cancer. Women who had not conceived but had been sexually active for more than 10 years without using contraception had about six times the risk of all other women. Approximately half these women had undergone investigations for infertility. For only five cases and one control was the cause of their infertility determined. Thus, it is not possible to assess whether this high risk group had normal or impaired ovarian function. Subfertility may also be associated with ovarian cancer. Gravid women reporting over 10 years of unprotected intercourse had a 50% higher risk than other gravid women, but this increase was not statistically significant.

Age at first pregnancy was not found to be associated with ovarian cancer risk although women having a first pregnancy after the age of 35 years had a higher risk compared to women having a first pregnancy at earlier ages and to nulligravid women. Since subfertility might be a risk factor for ovarian cancer, the relative risks associated with age at first pregnancy were also adjusted for duration of unprotected intercourse. The raised risk for women with a first pregnancy after 35 years persisted (RR = 3.9, 95% CI 1.1–14.2). Results from other studies regarding the risk for women having a first child at relatively older ages are inconclusive, some finding no association (Newhouse *et al.*, 1977; Casagrande *et al.*, 1979; Cramer *et al.*, 1983; Lesher *et al.*, 1985), others an increased risk (Joly *et al.*, 1974; McGowan *et al.*, 1979; Hildreth *et al.*, 1981; Franceschi *et al.*, 1982). Only Franceschi *et al.* (1982) found the increased risk to be statistically significant and independent of parity.

Overall, there was no association between use of any contraception and ovarian cancer. Of the specific methods studied, female sterilisation and use of oral contraception were associated with a significant reduction in risk and no method was associated with a significant increase in risk. The associations remained after adjusting for gravidity and duration of unprotected intercourse, the measure used to indicate infertility. While few studies have examined the association between female sterilisation and ovarian cancer, the relation between oral contraceptives and ovarian cancer has been demonstrated in many studies (Booth & Beral, 1985). Like others, we demonstrated that the longer oral contraceptives had been used, the lower the risk. Our findings also suggested that the earlier the age at first use the lower the risk and that the protective effect of oral contraceptives persists after their use is stopped.

It has been suggested that inhibition of ovulation, as induced by pregnancy and oral contraceptives, is the factor which protects against ovarian cancer (Fathalla, 1971). If so, postpartum anovulation associated with lactation might also be expected to be protective. We found no evidence that the longer a woman had breastfed the lower her risk of ovarian cancer. Indeed, the highest risk was found in those who had breastfed longest. Results from other studies are contradic-

tory (Cramer *et al.*, 1983; Mori *et al.*, 1984; Risch *et al.*, 1983; Cancer and Steroid Hormone Study, 1987).

Our finding that age at menarche was not associated with risk is consistent with results from most other studies (Casagrande *et al.*, 1979; McGowan *et al.*, 1979; Hildreth *et al.*, 1981; Franceschi *et al.*, 1982). Age at natural menopause, however, was strongly related to risk. While Hildreth *et al.* (1981), Franceschi *et al.* (1982) and Tzonou *et al.* (1984) also demonstrated that the later the age at menopause the greater the risk, other studies have found no association (West, 1966; Newhouse *et al.*, 1977; Annegers *et al.*, 1979; McGowan *et al.*, 1979; Cramer *et al.*, 1983).

A lower frequency of hysterectomy, of unilateral oophorectomy, or of both among cases compared to controls has also been reported from several other studies (Wynder *et al.*, 1969; Joly *et al.*, 1974; Annegers *et al.*, 1979; McGowan *et al.*, 1979; Franceschi *et al.*, 1982; Cramer *et al.*, 1983). As these studies were case–control in design, there may have been some misclassification of controls who, rather than having a hysterectomy with ovarian conservation, actually had a hysterectomy with bilateral oophorectomy. Another explanation for the findings might be that if at hysterectomy a woman's ovaries look diseased, it is likely that they are removed. If the diseased ovaries were precancerous, those women who might otherwise have developed ovarian cancer do not. Following hysterectomy with ovarian conservation, reduced ovarian function or ovarian failure occurs in a proportion of women, due possibly to the blood supply to the ovaries being compromised (Beavis *et al.*, 1969; Ellsworth *et al.*, 1983). Female sterilisation was also associated with a low risk of ovarian cancer. Neil *et al.* (1975) have suggested that the menstrual disturbance that many women experience after sterilisation may reflect changed ovarian function due to damage to the vascular supply to the ovaries. If both hysterectomy and female sterilisation can indirectly affect ovarian function then both procedures could also influence the risk of ovarian cancer.

Recent investigators have shown that the longer a woman ovulates the greater her risk of ovarian cancer (Casagrande *et al.*, 1979; Hildreth *et al.*, 1981; Franceschi *et al.*, 1982; Wu *et al.*, 1988). We also found that risk increased the longer the duration of ovulation. Duration of ovulation is, however, highly correlated with the 'anovulatory' factors used to estimate the exposure. In an attempt to determine whether duration of ovulation had an effect over and above that expressed by its relation with these factors, the risks and trends were adjusted for duration of anovulation due to pregnancy and oral contraceptive use and, where appropriate, for age at menopause. The significance of the effect disappeared. We conclude that it is not possible to determine from these data whether it is the above factors which inhibit ovulation that prevent ovarian cancer, whether repeated ovulations promote it, or whether a combination of both is acting.

Our finding of no overall relationship between hormone replacement therapy and ovarian cancer supports those of other investigators (Newhouse *et al.*, 1977; Hildreth *et al.*, 1981; Weiss *et al.*, 1982). An increased risk associated with the therapy was found among women who reported hysterectomy, but the finding was based on very few cases and may have been due to chance. We did not find an increased risk associated with oestrogen therapy for any particular tumour types as suggested by Cramer *et al.* (1981) and Weiss *et al.* (1982).

The evidence linking talc with ovarian cancer is controversial (Anonymous, 1977; Roe, 1979; Longo & Young 1979; Cramer *et al.*, 1982; Hartge *et al.*, 1983). In this study, women who reported talc use in the genital area more than once a week or daily had higher risks of ovarian cancer than women who used talc less frequently. The women were not asked how long they had been using talc. It is possible that because of their symptoms or disease-related pelvic examinations, the frequency of current talc use by the cases may not have reflected their frequency of past use. Since these and other results (Cramer *et al.*, 1982; Hartge *et al.*, 1983) are insufficient to reject an association, further work is needed on the relation between genital use of talc and ovarian cancer.

We would like to thank the Imperial Cancer Research Fund for financing the study, Professor Sir Richard Doll for helpful advice, Professor Christopher Hudson and Dr Marigold Curling for reviewing the histology, Dr Eve Wiltshaw, the consultants, nurses and other staff of the participating hospitals for their co-operation and support of the study, Ann Bateman, Kate Rodriques, Gillian Saunders and Rosalie Thomson for their skilful interviewing, and Nina Saroi for typing the manuscript. Margaret Booth is funded by a grant from the Medical Research Council.

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REGULATORY TOXICOLOGY AND PHARMACOLOGY 21, 211–215 (1995)

Talc: Consumer Uses and Health Perspectives[1]

Bethesda, Maryland, January 31–February 1, 1994

Co-Sponsored by:

The International Society of Regulatory Toxicology & Pharmacology
and The United States Food and Drug Administration

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[1] Presented, in part, at the International Society of Regulatory Toxicology and Pharmacology/U.S. FDA Workshop on Talc: Consumer Uses and Health Perspectives, NIH, Bethesda, MD, January 31–February 1, 1994.

0273-2300/95 $6.00

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EXECUTIVE SUMMARY

This issue of the journal is largely dedicated to report on a January 31–February 1, 1994 workshop on talc, organized under joint sponsorship of the U.S. Food and Drug Administration (FDA), the Cosmetics, Toiletries, and Fragrances Association (CTFA), and the International Society of Regulatory Toxicology and Pharmacology (ISRTP). Although not all papers given at the meeting were made available for publication, this offers a general overview of the substance of the presentations and discussions.

The workshop was to provide a forum for an updated discussion of the origins, manufacture, characterization, toxicology, and epidemiology of talc. Principal focus of the meeting was on the latest toxicologic and epidemiologic studies, as they reflect on the safe uses of talc in consumer products. The characteristics of cosmetic-grade talc, the history of its uses in a variety of products, and the current quality-control measures to ensure safety were followed by a review of the regulatory history of talc and by an appraisal of recent National Toxicology Program (NTP) studies of chronic pulmonary exposure of rodents to talc.

Of special interest was the relevance of these studies to human risk assessment, in view of reported technical problems of lung overload for these bioassays, and of the standing concerns about the physiologic, anatomic, genetic, and other differences of rodents and man. Experimental data were then evaluated against the contrasting evidence emerging from epidemiologic studies of human talc exposure. A critical panel of invited experts and speakers completed each session. Faculty and panelists included talc mineralogists, geologists, toxicologists, epidemiologists, pathologists, food/drug/cosmetic/medical device manufacturers, qualified regulatory specialists, and consumer representatives.

In brief opening remarks, Dr. John E. Bailey (FDA) reminded participants that the workshop was not part of a formal rule-making process and could not be a forum to reach a consensus for regulatory decisions. Emphasis was to be on a free interaction between participants to explore and possibly reach some conclusion on the validity and significance of the existing knowledge regarding the safety of cosmetic talc.

A first and essential presentation by Dr. Gettings (CTFA) addressed conclusively the nature of cosmetic talc, the specific form of talc under study now recognized in the 1990 U.S. Pharmacopoeia, and other official standards. Unique physical and chemical properties—inertness, hygroscopicity, self-aggregation, lubrication, tactile sensation, translucency, and color—make powdered talc desirable, useful, and virtually indispensable in cosmetic applications. Cosmetic talc is a negligible fraction of the nearly 50,000 tons mined for industrial use in the United States and today stringent safety and quality-control measures ensure the absence of asbestos fibers formerly considered as a potential hazard, albeit not a defined one.

Dr. Gilbertson (FDA) reviewed the harmonization of international standards and regulations for cosmetic talc and its consumer applications. In their joint evaluation, talc has proven to be among the safest of all consumer products.

In addressing fundamental aspects of inhalation toxicology, Dr. Oberdörster (Rochester University) noted that any inhaled dust—and most substances for that matter—may cause inflammation and cell proliferation in the lung, possibly leading to tumor formation if the challenge is sufficiently strong and persistent to overcome the natural defenses of the animals under test. This condition implies the presence of a no-effect threshold at levels below which natural defenses remain functional to prevent tumor formation. He noted that the NTP inhalation bioassays of talc invariably created lung overloads, thus making the interpretation of results quite problematic. In his overall evaluation of the toxicologic literature on talc, there is no reason for concern for low-level uses of cosmetic talc.

As a staff scientist of the National Toxicology Program, Dr. Boorman emphasized that NTP protocols call for maximum tolerated doses (MTDs) in an attempt to identify any hazards. Negative findings may receive little or no further attention, but positive ones call for detailed mechanistic studies to determine their relevancy for human health. In a detailed description of the talc inhalation bioassays at NTP, their protocol, and pathology findings, he concurred with evidence of dose overloads in most of the animals that ended up developing tumors. He explained the lack of an ovarian effect of lifetime exposure in F344/14 rats and B6C3F1 mice. In his summary report he notes the many factors that complicate interpretation of these rodent studies.

The results of the NTP inhalation bioassays on talc were first evaluated as customary in 1992 by the Technical Reports Review Subcommittee of the NTP Board of Scientific Counselors. Dr. Goodman (Michigan State

of epidemiologic studies more straightforward and direct?

Unfortunately—Dr. Gori suggested—epidemiology presents a new set of impediments. The epidemiology we are facing does not offer the same direct cause and effect associations typical of infectious diseases. Human cancers are multifactorial diseases arising from a combination of simultaneous exposures to many potential etiologic determinants. To extricate the significance of any one of these factors from the integrated effects of all others is a challenging task. If one adds the technical problems in the execution of these studies, we soon find that the epidemiologic fog is just as difficult to penetrate as the one generated by animal bioassays. Kenneth Rothman (1986), a leading theoretician of American epidemiology, has reviewed extensively these difficulties of interpretation of causal inferences and writes:

> Despite philosophic injunctions concerning inductive inference, criteria have commonly been used to make such inferences. The justification offered has been that the exigencies of public health problems demand action and that despite imperfect knowledge causal inferences must be made.

This definition is widely accepted by mainstream epidemiologists today. On this basis however—just as we could ask whether extrapolation from animal bioassays qualifies as a scientific exercise—we are justified in asking the same question of human epidemiology.

In a general overview of ovarian cancer epidemiology, Dr. Austin (Emory University) noted the many factors that may confound the putative association of perineal talc exposure and ovarian cancer. Following a presentation by Dr. Brown (University of Wisconsin), the discussion made it clear that available histologic and physiologic studies provide no basis to conclude that talc can migrate to the ovaries from the perineal region.

Dr. Hartge (National Cancer Institute) and Dr. Harlow (Harvard University) presented a review of epidemiologic studies—including their own original studies—pertaining to perineal talc exposure and ovarian cancer risk. The studies reviewed brought to light the many interpretive difficulties of epidemiology as an observational science and are detailed in the papers by Drs. Hartge and Harlow appearing in this issue of the journal. From the unique perspective of his long and distinguished experience in the epidemiology of multifactorial diseases, Dr. Wynder (American Health Foundation) reviewed the history of the evolutionary processes that provided natural defense systems against disease. He stressed the specific problems in the epidemiology of weak associations: biases of respondents and investigators, known and unknown confounding factors, and the irresistible urge to interpret results as if only a reduced set of variables of interest was operant, without acknowledging and controlling for a more complex multifactorial reality. Following the many issues raised by all presenters, the ensuing discussion generally agreed that while some weak association between talc exposure and ovarian tumors has been reported, it was not sufficient warning for concern.

A final panel included most speakers and other experts and was able to reach an unanimous assessment of the workshop. In regard to the NTP talc bioassay in rodents, it found that because of the extreme doses and the unrealistic particle sizes of the talc employed, because of the negative results in mice and male rats, because of the lack of tumor excess at the low doses, and because of the clear biochemical and cytological markers of excessive toxicity in female rats, the positive talc bioassay results in female F344/N rats are the likely experimental artifact and nonspecific generic response of dust overload of the lungs and not a reflection of a direct activity of talc. Given the gross differences of rodent and human lungs, the lung clearance capabilities of humans, and the possible conditions of customary human exposures, the NTP bioassay results in F344/N female rats cannot be considered as relevant predictors of human risk.

In regard to the proposed association of talc exposure and ovarian cancer, the panel found that epidemiologic data are conflicting and remain equivocal. Although it is theoretically possible that talc could reach the ovaries, the actual access to or the presence of talc in ovarian tissue is not documented. Diet, parity, contraceptive use, ovulatory frequency, familial predisposition, age to menarche and menopause, and other factors associate strongly and plausibly with ovarian cancer incidence. These possible confounders and control selection biases, publication biases, interviewer and interviewee biases, and other factors may well explain the conflicting results that have appeared in the literature.

The possibility of an association of talc exposure and ovarian cancer is an important hypothesis of potential public health importance. However, this association remains a research hypothesis whose verification or falsification needs additional study. Experimental studies may be needed to determine whether talc could access the ovaries under field conditions and, if so, whether it could be embedded in ovarian tissue and produce pathologic effects. For epidemiology, further refinements may be possible in the selection and characterization of control subjects and in the accounting of possible confounders and biases. However, epidemiologic studies have provided weak and conflicting risk signals for this association, and it is unlikely that further studies may prove adequate to raise concern at a level sufficient to warrant regulatory or public health measures.

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Ovarian Cancer and Talc

A Case-Control Study

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Opportunities for genital exposure to talc were assessed in 215 white females with epithelial ovarian cancers and in 215 control women from the general population matched by age, race, and residence. Ninety-two (42.8%) cases regularly used talc either as a dusting powder on the perineum or on sanitary napkins compared with 61 (28.4%) controls. Adjusted for parity and menopausal status, this difference yielded a relative risk of 1.92 ($P < 0.003$) for ovarian cancer associated with these practices. Women who had regularly engaged in both practices had an adjusted relative risk of 3.28 ($P < 0.001$) compared to women with neither exposure. This provides some support for an association between talc and ovarian cancer hypothesized because of the similarity of ovarian cancer to mesotheliomas and the chemical relation of talc to asbestos, a known cause of mesotheliomas. The authors also investigated opportunities for potential talc exposure from rubber products such as condoms or diaphragms or from pelvic surgery. No significant differences were noted between cases and controls in these exposures, although the intensity of talc exposure from these sources was likely affected by variables not assessed in this study.
***Cancer* 50:372–376, 1982.**

THE POSSIBILITY that ovarian cancer may be caused by exposure to certain hydrous magnesium silicates such as talc and asbestos has been raised by several researchers.[1-3] The lack of epidemiologic studies regarding this hypothesis prompted us to investigate talc exposure in a case-control study of ovarian cancer.

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Supported by Grant Number 5-RO1 CA24209, awarded by the National Institutes of Health, DHEW.

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This study could not have occurred without the generous participation of many clinicians and institutions in the greater Boston area including: Dr. Emanuel Friedman of the Beth Israel Hospital, Drs. Robert Knapp and Thomas Griffiths of the Brigham and Women's Hospital and Sidney Farber Cancer Institute, Dr. Arthur Hassett of the Brockton Hospital, Dr. Joel Rankin of the Framingham Union Hospital, Dr. Edward Copenhaver of the Lahey Clinic Foundation, Dr. James Nelson of the Massachusetts General Hospital, Dr. Clement Yahia of the New England Deaconess Hospital, Dr. Lalita Gandbhir of the Pondville Hospital, Dr. James Whelton of Saint Elizabeth's Hospital, Dr. Stephen Alpert of the Salem Hospital, Dr. Richard Hunter of the University of Massachusetts Medical School. The superb clerical and technical assistance of Ms. Eileen McManus, Ms. Sally Cassells, and Ms. Christine Peters is also gratefully acknowledged.

Accepted for publication December 29, 1981.

Methods

The cases studied were women with ovarian cancer, diagnosed between November 1978 and September 1981 and identified through the pathology logs or tumor boards of twelve participating hospitals in the Greater Boston area. The study was restricted to English-speaking residents of Massachusetts ranging in age from 18 to 80 years. During the study period, 297 eligible cases were identified. Physicians denied permission to contact their patients in 13 instances. Fourteen patients declined to participate, and 14 other patients had died or moved before they could be contacted.

For each of the 256 interviewed cases, slides of the surgical specimens were reviewed by two authors (W.R.W. or R.E.S). Eighteen cases were excluded as nonovarian primaries. Each ovarian tumor was classified according to the Histological Classification of Ovarian Tumors of the World Health Organization.[4] The present analysis was restricted to 215 white women with epithelial cancers, including 39 with tumors of borderline malignancy and their matched controls.

Control cases were identified through the Massachusetts Town Books, annual publications that list residents by name, age, and address. Controls were selected randomly from those women who matched cases by precinct of residence, race, and age within two years. Additionally, it was required that a subject be excluded

0008-543X/82/0715/0372 $0.75

as a control if she had had a bilateral salpingo-oophorectomy, but subjects were not excluded because of prior hysterectomy or other types of pelvic operations. Women who had had pelvic operations were generally confident in their knowledge of whether their ovaries had been removed, but the nature of the operations could not be verified by hospital records in each instance. Women whose statements could not be verified were included or excluded on the basis of their recollection of the surgery. The 215 controls in this study were eventually obtained from a total of 475 potential controls identified through the Town Books. Fifty-six (12%) of the total could not be reached because they had moved, died, or had disconnected or unlisted phones. Twenty-nine (6%) of the total were ineligible because of a history of bilateral salpingo-oophorectomy, while 20 (4%) were of the wrong age or race or did not speak English. Of the total potential controls, 155 (33%) refused to participate. If the 215 cases are characterized as to ease of matching, 121 (56%) cases were matched with no refusals, 58 (27%) were matched after one refusal, and 36 (17%) were matched only after two or more refusals.

Interviews were conducted personally to assess a number of factors from the menstrual and reproductive history, medical and family history, and environmental exposures. This report will deal only with the results of several questions related to potential or definite talc exposure by way of contraceptive practices, operations, or perineal hygiene. Subjects were stratified by potential confounders described below, and adjusted relative risks associated with these exposures were calculated by the Mantel-Haenszel procedure as adapted by Rothman and Boice.[5] To accommodate other confounders as well as the matched design in the data collection, logistic analysis for matched data as described by Breslow *et al.*[6] was also employed.

Results

The average age (and standard error of the mean, SEM) for cases was 53.2 (1.0) years and for controls, 53.5 (1.0) years. Table 1 shows other characteristics of subjects. Controls were comparable to cases in educational level and religion. Cases and controls differed significantly in marital status and parity with parity being the more important discriminator between them. Sixty-four percent of the cases were postmenopausal at the time of diagnosis, whereas 60% of controls were postmenopausal. Of these, 15 cases and 20 controls had had an artificial menopause. Parity and menopausal status were considered important potential confounders in this analysis and were adjusted for as described above.

TABLE 1. Characteristics of Cases and Controls

Characteristic	Cases (Total = 215) No.	Cases (Total = 215) %	Controls (Total = 215) No.	Controls (Total = 215) %
Educational level (completed college)	48	22.3	49	22.8
Religion (Roman Catholic)	126	58.6	128	59.5
Marital status (never married)	46	21.4	24	11.2
Nulliparous	78	36.3	39	18.1
Menopausal status (postmenopausal*)	137	63.7	129	60.0

* Postmenopausal at time of diagnosis for cases or for interview for controls.

Relative risks associated with potential talc exposure from contamination on rubber products are explored in Table 2. Although surgical gloves of recent vintage are dusted with starch, talc contamination may still be found.[7] Thus, a history of pelvic operations (appendectomy, cesarean section, hysterectomy, and other operations on internal genital organs other than bilateral salpingo-oophorectomy) was determined in cases and controls. Excluding operations associated with the diagnosis or treatment of the ovarian cancer among the cases, no excess in the occurrence of pelvic operations was noted. The greatest opportunity for talc exposure from surgery occurred before 1950, when talc was the

TABLE 2. Relative Risks (RR) for Common Epithelial Ovarian Cancers Associated with Potential Talc Exposure from Contamination on Rubber Products

Exposure	Cases Total	Cases No. (%) with exposure	Controls Total	Controls No. (%) with exposure	Crude RR	Adjusted RR*	95% Confidence limits
Pelvic surgery	215	78 (36.3)	215	75 (34.9)	1.06	1.17	(0.76–1.79)
Pelvic surgery (prior to 1950)	215	51 (23.7)	215	48 (22.3)	1.08	1.12	(0.69–1.82)
Use of condoms†	169	19 (11.2)	191	30 (15.7)	0.68	0.77	(0.41–1.44)
Use of diaphragm†	169	37 (21.9)	191	35 (18.3)	1.24	1.19	(0.69–2.05)

* Adjusted for parity (nulliparous, parous) and menopausal status (pre- and postmenopausal).

† Restricted to subjects who had ever been married.

TABLE 3. Relative Risks (RR) Associated with Using Talc for Storage Among Diaphragm Users* by Duration of Use of Diaphragm

Duration of diaphragm use	Cases: Total	Cases: No. (%) who used talc on diaphragm	Controls: Total	Controls: No. (%) who used talc on diaphragm	Crude RR	Adjusted RR†	95% Confidence limits
Total diaphragm use less than five years	13	6 (46.2)	21	8 (38.1)	1.39	1.82	(0.42-8.00)
Total diaphragm use five or more years	27	16 (59.3)	19	11 (57.9)	1.06	1.23	(0.36-4.17)
All users	40	22 (55.0)	40	19 (47.5)	1.35	1.56	(0.62-3.88)

* Includes all women who used diaphragm regardless of marital status.

† Adjusted for parity and menopausal status.

predominantly used dusting powder for surgical gloves. However, no significant excess of pelvic operations prior to 1950 was observed for cases.

The patients (cases) who, at sometime, had been married, chose condoms less frequently and diaphragms more frequently for contraception than the control group, but neither difference was statistically significant. Condom use is not necessarily associated with talc exposure. Not all brands of condoms are dusted with talc, and lubricants could affect the shedding of talc from the condom. Unfortunately, details on specific brands of condoms were not obtained. Similarly, talc exposure is not a necessary consequence of diaphragm use. We inquired specifically about the practice of dusting the diaphragm with talc for storage after use (Table 3). Among all subjects who had used a diaphragm, there was no significant excess of cases who regularly stored their diaphragm using talc, nor was any greater risk associated with this practice observed among women who had used the diaphragm for longer durations. Before the risk from this exposure can be adequately assessed, greater detail is needed including frequency of use and whether the powder was washed off prior to use. Furthermore, contraceptive jellies used with the diaphragm could affect the transport of talc in the genital tract.

Hygienic practices involving talc were also studied. Specifically, we inquired whether women had regularly used talc as a dusting powder on the perineum or regularly dusted sanitary napkins with talc (Table 4). Ninety-two (42.8%) of the cases had talc exposure by either or both of these routes compared with 61 (28.4%) of the controls. The adjusted relative risk was 1.92 ($P < 0.003$) with 95% confidence limits of 1.27–2.89 compared to subjects who had neither exposure. Sixty (27.9%) cases and 48 (22.3%) controls had either used talc for dusting or on napkins but not both. This difference yielded an adjusted relative risk of 1.55, which was of borderline significance ($P = 0.06$). The greatest risk occurred in women who had both exposures (use on the perineum and on napkins) compared to women who had neither exposure. Thirty-two (14.9%) of cases were in this category compared with 13 (6.0%) controls, for an adjusted relative risk of 3.28 ($P < .001$) and 95% confidence limits of 1.68–6.42. The histologic characteristics of tumors developing in women with perineal exposure to talc did not differ significantly from those in women without perineal exposure to talc (Table 5). In addition, the proportion of cases with tumors of borderline malignancy was identical among those with and without perineal exposure to talc. Twenty-two (18%) of 123 cases without the exposure had tumors of bor-

TABLE 4. Relative Risks (RR) for Common Epithelial Ovarian Cancers Associated with Talc Exposure in Perineal Hygiene

	No perineal exposure	Any perineal exposure	Types of perineal exposure: As dusting powder but not on napkins	Types of perineal exposure: On napkins but not as dusting powder	Types of perineal exposure: Both on napkins and as dusting powder
Cases (Total = 215)	123 (57.2%)	92 (42.8%)	43 (20.0%)	17 (7.9%)	32 (14.9%)
Controls (Total = 215)	154 (71.6%)	61 (28.4%)	34 (15.8%)	14 (6.5%)	13 (6.0%)
Crude rr	1	1.89	1.58	1.52	3.08
Adjusted RR*	—	1.92	1.55		3.28
95% confidence limits	—	(1.27-2.89)	(0.98-2.47)		(1.68-6.42)

* Adjusted for parity and menopausal status.

derline malignancy compared to 17 (18%) of 92 with the talc exposure.

Discussion

The argument linking talc and ovarian cancer includes four elements: the chemical relationship between talc and asbestos, asbestos as a cause of pleural and peritoneal mesotheliomas, the possible relation between epithelial ovarian cancers and mesotheliomas, and the ability of talc to enter the pelvic cavity. The mineral talc is a specific hydrous magnesium silicate chemically related to several asbestos group minerals and occurring in nature with them. Generic "talc" is seldom pure and may be contaminated with asbestos, particularly in powders formulated prior to 1976.[8,9]

Epidemiologic studies have clearly linked lung cancer and pleural and peritoneal mesotheliomas with asbestos exposure.[10] An excess of similar pulmonary lesions has been reported in talc workers and seems to be correlated with the amount of asbestos contamination in the talc deposits worked.[11] Graham and Graham[1] were able to induce ovarian neoplasms in guinea pigs with asbestos and suggested that ovarian cancer could be related to asbestos exposure, noting the similarity between mesotheliomas and ovarian cancers. Parmley and Woodruff[12] further emphasized this similarity and popularized the pelvic contamination theory, which proposed that environmental carcinogens might enter the pelvic cavity via the genital tract. Years earlier it had been observed that inert carbon particles placed in the vagina immediately prior to hysterectomy could be recovered from the fallopian tubes.[13] Although greeted with skepticism, the finding of talc particles embedded in normal and abnormal ovaries suggests that talc is a substance that can enter the pelvic cavity via the vagina.[2]

Although no consensus concerning the risks of talc has emerged from letters, editorial and articles,[3,14-16] participants in the discussion have agreed upon the need for epidemiologic studies of ovarian cancer and talc exposure. In this case-control study of ovarian cancer of the epithelial variety, we investigated several sources of potential talc exposure. Among these, the only significant finding was an association between ovarian cancer and hygienic practices involving the use of talc on the perineum. It is especially notable that women who regularly had both dusted their perineum with talc and had used it on sanitary napkins had more than a threefold increase in risk compared to women with neither exposure. Several potential biases must be considered in interpreting this association.

The observation by Wynder *et al.*[17] that menstrual characteristics may differ between women with ovarian cancer and controls might suggest that such differences may confound the association between perineal use of talc and ovarian cancer. We found that menstrual characteristics of cases and controls were virtually identical in this study. Fifty-three (24.7%) cases complained of moderate or severe dysmenorrhea compared to 56 (26.0%) controls. Twenty-five (11.6%) cases complained of irregular periods compared to 32 (14.9%) controls. The average numbers (and SEM) of days of flow and cycle length were, respectively, 4.9 (0.1) and 28.9 (0.3) days for cases and 4.9 (0.1) and 29.6 (0.3) days for controls.

TABLE 5. Characteristics of Ovarian Cancer in Women with and without Perineal Exposure to Talc

	No perineal use of talc	Any perineal use of talc
	No. (%)	No. (%)
Serous	66 (53.7)	45 (48.9)
Mucinous	16 (13.0)	14 (15.2)
Endometrioid and clear cell	32 (26.0)	24 (26.1)
Other and undifferentiated	9 (7.3)	9 (9.8)
Total	123 (100)	92 (100)

Since entry of talc into the pelvic cavity is prevented by hysterectomy or tubal ligation, it might also be argued that the inclusion of subjects with pelvic surgery in the analysis may obviate any association between talc and ovarian cancer. It should be noted that such surgery generally occurred near the end of reproductive life for both cases and controls, probably after most significant talc exposure had already occurred. The exclusion of such subjects from the analysis did not substantially alter the observed associations. For example, the adjusted relative risk for the use of talc both on the perineum and sanitary napkins was 2.79 ($P < 0.003$) in the group without pelvic surgery compared to 3.28 observed for the entire group.

In terms of other confounders, the association persisted after adjustment for menopausal status and parity. We also applied multivariate logistic regression for paired observations.[6] The maximum likelihood estimate of relative risk associated with any perineal use of talc was 1.61 ($P = 0.03$) with 95% confidence limits of 1.04–2.49 after simultaneous adjustment for religion, marital status, educational level, ponderal index, age at menarche, exact parity, oral contraceptive or menopausal hormone use, and smoking.

Our sample of cases represents more than 50% of ovarian cancer cases diagnosed in Boston residents in the study period. Therefore, it is difficult to conceive of a plausible bias in the selection of cases that would yield this excess use of talc. There is reason for concern that the high refusal rate among the controls may have introduced a selection bias among the controls. Pat-

when we restricted the analysis to the 121 cases who were matched without a control refusal, we again found a significant association between talc use and ovarian cancer. For women who had used talc both in dusting and on the perineum we found an adjusted relative risk of 2.44 ($P < 0.05$). Interviewer bias is also unlikely to explain the association. Of the 18 women who were initially interviewed as ovarian cancer cases but later excluded as having metastatic tumors to the ovary, only one (5.6%) had both perineal and napkin exposure as compared with 15% in cases and 6% in controls.

Experimental data which might bear on the carcinogenicity of talc come primarily from models using pleural implantation of various minerals in rats.[18] These data suggest that carcinogenicity is dependent primarily upon the shape of the particles with long thin fibers such as those occurring in crocidolite asbestos being most carcinogenic. Talc consists primarily of plates but may contain fibers, although voluntary guidelines to limit the content of asbestisform fibers in consumer talcums were proposed by the cosmetics industry in 1976.[19]

If talc is involved in the etiology of ovarian cancer, it is not clear whether this derives from the asbestos content of talc or from the uniqueness of the ovary which might make it susceptible to carcinogenesis from both talc and other particulates. With ovulation entrapment of the surface epithelium of the ovary into the ovarian stroma occurs. If present, talc or other particulates might be incorporated into these inclusion cysts. Apparently implantation of foreign bodies into the lumens of epithelial lined organs provides a favorable environment for carcinogenesis.[20] Alternatively, talc might serve to stimulate entrapment of the surface epithelium and act in the same way that "incessant ovulation" has been proposed as an etiologic factor for ovarian cancer.[21] Given the histologic and clinical diversity of ovarian cancer, talc exposure is unlikely to be the only cause. Undoubtedly, reproductive experiences such as pregnancies and, perhaps, oral contraceptive use play a role in its etiology.[21-23] The possibility that talc exposure interacts with these variables deserves further investigation.

It is hoped that this report will stimulate further study of talc exposure in relation to ovarian cancer. Animal studies would be helpful to determine whether and under what circumstances ovarian tumors may be induced by various talc preparations. Epidemiologic studies should focus on opportunities for excessive vaginal contamination with talc such as when it is repeatedly used in perineal dusting powders or sprays and in or on tampons, sanitary napkins, or other products intended for intravaginal use. More precise details on the exact nature and frequency of the exposure and the amount and specific brand of powder used are essential. Opportunities for talc exposure are widespread and pervasive,[24] but that should not discourage epidemiologists from studying this potentially important exposure in relation to ovarian cancer.

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Talc File
12/28/93

Original Contribution

Tubal Ligation, Hysterectomy, and Risk of Ovarian Cancer

A Prospective Study

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Objective.—To assess whether tubal ligation and hysterectomy affect subsequent risk of ovarian cancer.

Design.—Prospective cohort study with 12 years of follow-up.

Setting.—United States, multistate.

Participants.—A total of 121 700 female registered nurses who were 30 to 55 years of age in 1976; the follow-up rate was 90% as of 1988.

Main Outcome Measure.—Ovarian cancer of epithelial origin confirmed by medical record review.

Results.—We observed a strong inverse association between tubal ligation and ovarian cancer, which persisted after adjustment for age, oral contraceptive use, parity, and other ovarian cancer risk factors (multivariate relative risk [RR], 0.33; 95% confidence interval [CI], 0.16 to 0.64). The association was similar when we assessed tubal ligation status at the baseline questionnaire and excluded cases in the first 4 years to eliminate any possible short-term decrease in risk due to screening of the ovaries during ligation surgery. We noted a weaker inverse association between simple hysterectomy and ovarian cancer (RR, 0.67; 95% CI, 0.45 to 1.00). Neither vasectomy nor condom use by a partner was associated with risk of ovarian cancer.

Conclusions.—These data indicate that tubal ligation, and perhaps hysterectomy, may substantially reduce risk of epithelial ovarian cancer.

(*JAMA.* 1993;270:2813-2818)

OVARIAN cancer is the fourth leading cause of cancer death in American women, accounting for approximately 12 000 deaths per year.[1] This disease will affect 1% to 2% of women in their lifetime,[2] of whom fewer than 40% will survive 5 years from diagnosis.[1] The poor prognosis reinforces the need to find modifiable risk factors.

For editorial comment see p 2855.

Both tubal ligation and hysterectomy are commonly performed in the United States. From 1970 to 1980 in the United States, approximately 5.5 million women between the ages of 15 and 44 years underwent a tubal sterilization procedure.[3,4] Similarly, over 400 000 hysterectomies with the removal of neither or only one ovary are performed each year.[5]

An inverse association between tubal ligation and ovarian cancer risk has been reported in past case-control studies,[6-12] although the magnitude of the odds ratios varied substantially (range, 0.2 to 0.9). In a small retrospective cohort study,[13] a nonsignificant increased risk of ovarian cancer was noted. An inverse association between hysterectomy and ovarian cancer has also been reported.[12,14-17] For both of these procedures, the observed inverse association may be due to a bias resulting from screening the ovaries at the time of surgery, rather than a biologic effect[7,8,18]; this issue remains unresolved.

In this article, we provide the first prospective assessment of the relationships of tubal ligation and hysterectomy with risk of ovarian cancer during 12 years of follow-up in a large cohort of registered nurses.

METHODS

The Nurses' Health Study is a prospective cohort study of 121 700 married, female registered nurses who were 30 to 55 years of age in 1976 when the study began. Participants have received questionnaires biennially since that time to update information on exposure status and newly diagnosed illnesses. Through June 1, 1988, the follow-up rate among living participants was 90%.

From the Channing Laboratory (Drs Hankinson, Hunter, Colditz, Willett, Stampfer, Rosner, and Speizer) and the Division of Preventive Medicine (Dr Hennekens), Department of Medicine, Brigham and Women's Hospital; the Department of Ambulatory Care and Prevention (Dr Hennekens), Harvard Medical School; and the Departments of Epidemiology (Drs Hankinson, Hunter, Colditz, Willett, and Stampfer) and Nutrition (Dr Willett), Harvard School of Public Health, Boston, Mass.

Reprint requests to Channing Laboratory, 180 Longwood Ave, Boston, MA 02115 (Dr Hankinson).

Table 1.—Age-Standardized Prevalence (%) of Potential Ovarian Cancer Risk Factors According to Tubal Ligation and Hysterectomy Status in 1976*

Risk Factor	Tubal Ligation†		Hysterectomy‡	
	Yes	No	Yes	No
Parity§				
0	2	8	5	7
1	3	9	5	8
2	24	30	28	28
≥3	70	52	61	55
Age at menarche, y				
<11	23	23	25	22
≥13	51	50	49	51
Oral contraceptive use				
Yes	60	54	52	49
Use for ≥5 y	17	10	13	11
Smoking§				
Never	43	44	45	43
Past	23	24	22	23
Current	33	32	33	33
Quetelet's index, kg/m²				
<21	27	28	26	26
≥29	10	10	11	10

*Directly standardized to 1976 age distribution of all premenopausal (for tubal ligation) or all premenopausal and postmenopausal women combined (for hysterectomy); numbers indicate percentages of subjects with potential risk factor.
†Population includes 77 544 premenopausal women.
‡Population includes 107 868 premenopausal and postmenopausal women.
§Numbers do not always add up to 100% because of missing data.

Ascertainment of Exposures

In 1976, participants were asked whether they currently used any method of contraception and, if so, to specify which of the following methods they used: oral contraceptives, rhythm, diaphragm, condom, intrauterine device, foam or jelly, tubal ligation, or husband's vasectomy. Women who had ever used oral contraceptives were asked to specify intervals of use starting from first use and continuing up to 1976.

Participants were also asked if their menstrual periods had ceased and, if so, at what age this occurred, for what reason (natural menopause or menopause due to surgery or radiation) and, if surgical menopause, whether their ovaries had been removed. We have also collected data on height, weight, smoking status, age at menarche, and parity. Parity was defined as the number of pregnancies lasting 6 months or more. Data on menopausal status, smoking, and weight have been updated every 2 years; contraceptive use was ascertained every 2 years until 1982 (by then use was uncommon) and parity was asked biennially until 1984. In 1982, we asked participants if they had ever commonly used talc, baby powder, or deodorizing powder to apply to their perineum or on sanitary napkins.

The reproducibility and validity of self-reported menopausal status has been assessed among Nurses' Health Study participants who answered questionnaires in 1976 and 1978.[19] Reporting of age at natural menopause, assessed among over 31 000 postmenopausal women, was reproducible (within 1 year) 82% of the time; reproducibility increased with decreasing time since menopause (range, 1 to ≥25 years). To determine the validity of self-reported surgical menopause, we examined medical records from a sample of incident menopausal women. For 65 women who reported having had a hysterectomy only, there was complete agreement with the medical record. Among 66 women who reported hysterectomy plus excision of one ovary, there was 97% agreement with the medical record, and for 69 women who reported hysterectomy plus bilateral oophorectomy, self-reports were confirmed in all but one woman.

To determine the distribution of types of tubal ligation procedures performed in this cohort, we requested permission to obtain medical records from 100 randomly chosen women who had reported having a tubal ligation procedure.

Study Population

In all analyses, participants who reported a diagnosis of cancer (except for nonmelanoma skin cancer) or who reported having one or both ovaries removed were excluded, leaving 107 868 women in our baseline population in 1976. These exclusions were updated every 2 years. From 1976 to 1988, the cohort accrued a total of 1 191 524 person-years of follow-up. We limited analyses of tubal ligation to women who were premenopausal in 1976 because (except for oral contraceptive use) women had been asked to indicate the contraceptive method currently used, and thus we had no information on tubal ligation among postmenopausal women in 1976. This additional exclusion resulted in the 1976 baseline population of 77 544 women and, from 1976 to 1988, a total of 859 791 person-years of follow-up. In all analyses, women contributed person-time in each 2-year interval until the report of a unilateral or bilateral oophorectomy, cancer, or death, or until June 1, 1988.

Case Ascertainment

In 1976 we asked participants to indicate if they had ever been diagnosed with cancer, and if yes, to indicate the type. On each subsequent questionnaire, we specifically asked if ovarian cancer had been diagnosed in the previous 2 years. In addition, we searched the National Death Index to identify women who may have died of cancer. Mortality follow-up has been estimated to be 98% complete in this cohort.[20] For each reported case of ovarian cancer, we requested permission from the participant (or from the next of kin for decedents) to obtain medical records; the medical records were reviewed by physicians without knowledge of exposure status. Only women confirmed to have incident epithelial ovarian cancer (either borderline or malignant) on medical record review were included as cases in this analysis. The results were unchanged when the very small number of cases with borderline tumors (n=17) were excluded, so we present results that include both borderline and malignant tumors.

Data Analysis

For each participant, follow-up time, equal to the number of months between the return of the 1976 questionnaire and the return of the 1978 questionnaire, was assigned according to each covariate by its status in 1976. Similarly, for each subsequent 2-year interval, we assigned additional months of follow-up according to the updated exposures at the beginning of the interval.

Hysterectomy status was updated every 2 years. Because women reporting oophorectomy were censored at the time of this report, hysterectomy, as an exposure in this analysis, is by definition a simple hysterectomy. In defining age at natural menopause, women who were premenopausal when they had a hysterectomy had all subsequent person-time assigned to the "unknown" category. Tubal ligation and vasectomy status was updated every 2 years until 1982 (the last time the question was asked); the response in 1982 was then maintained through 1988. Condom use was not updated, as substantially fewer

women reported use at each subsequent questionnaire; thus, the response in 1976 was judged the best estimate of a woman's long-term exposure.

Incidence rates were calculated for each category of an exposure by dividing the number of ovarian cancer cases for that category by the person-time of follow-up. Relative risks (RRs) were used as the measure of association and were calculated as the ratio of incidence rates of the exposed to the unexposed. To control simultaneously for other potential risk factors for ovarian cancer, we used proportional hazards models.[21,22] We calculated 95% confidence intervals (CIs).[23]

RESULTS

From 1976 to 1988, we documented 260 incident cases of histologically confirmed ovarian cancer; these cases are included in the hysterectomy analyses. A subset of 157 cases occurred among 77 544 women who were premenopausal in 1976 and are included in the tubal ligation analyses.

At baseline in 1976, 10 818 (14%) of 77 544 premenopausal women reported having had a tubal ligation; from 1976 to 1988, 20% of the person-years were among women who reported a tubal ligation. As shown in Table 1, compared with women who had not had this procedure, those who had a tubal ligation were of higher parity and were more likely to have used oral contraceptives, especially for longer durations.

A substantial reduction in risk of ovarian cancer was noted among women who reported having a tubal ligation (age-adjusted RR, 0.29; 95% CI, 0.15 to 0.55) (Table 2). Results were essentially unchanged after further adjustment for parity, duration of oral contraceptive use, smoking, Quetelet's index, age at menarche, age at natural menopause, and hysterectomy status (RR, 0.33; 95% CI, 0.16 to 0.64). Control for parity in single birth categories (up to six or more) also did not alter the results. When analyses were repeated among only women reporting current contraceptive use, results were similar (age-adjusted RR, 0.30). Among parous women only, the age-adjusted RR for those who reported having a tubal ligation was also decreased (parity = 1 or 2: RR, 0.28 [95% CI, 0.09 to 0.86]; parity = 3 or more: RR, 0.35 [95% CI, 0.11 to 0.68]). Similarly, among women undergoing tubal ligation who never used oral contraceptives, the RR was 0.30 (95% CI, 0.14 to 0.67).

Because women may have had their ovaries screened at the time of the sterilization procedure (and thus would have the procedure performed only if no malignant or premalignant condition was found), we wished to determine whether detection bias might account for the inverse association. We were unable to assess ovarian cancer risk by years since tubal ligation because, for women who reported a tubal ligation on the 1976 questionnaire, we did not know when the procedure had been performed. Therefore, we assessed the association between tubal ligation status as reported in 1976 and ovarian cancer risk from 1980 to 1988, thus allowing a minimum of a 4-year lag from the time of the procedure to the beginning of the period at risk. Of note, women who reported having a tubal ligation between 1976 and 1978 had the procedure, on average, 8 years after their last pregnancy. Women reporting a tubal ligation as of 1976 had their last pregnancy an average of 10 years before; thus, the actual average lag was probably about 6 years ([10 years − 8 years] + 4-year lag). The age-adjusted RR was 0.23 (95% CI, 0.09 to 0.58); multivariate results were similar (Table 2). In addition, we reviewed all medical records of ovarian cancer cases to determine how many were detected at tubal ligation. Only 51 of the 157 women with ovarian cancer in the tubal ligation analysis were 46 years of age or younger at diagnosis (and presumably at risk of tubal ligation), and of these, 38 records indicated how the cancer had been detected. Ovarian cancer was detected at tubal ligation in just one case.

The observation that ovarian cancer incidence is reduced among both oral contraceptive users[24] and women with a tubal ligation suggested that contraceptors in general may be at lower risk. We therefore assessed the relationship between several other contraceptive methods and ovarian cancer, using the same baseline population as in the tubal ligation analyses, but excluding those who reported a tubal ligation in 1976. Neither vasectomy in a partner nor condom use (the two most common contraceptive methods after oral contraceptive use and tubal ligation) was significantly associated with risk of ovarian cancer (Table 2).

In all, 11 017 (10%) of women reported having had a hysterectomy in 1976; from 1976 to 1988, 15% of the person-years were among women who reported a hysterectomy. Women who had a hysterectomy were slightly more likely to be of higher parity and to have used oral contraceptives for longer duration than women who did not have a hysterectomy (Table 1).

The age-adjusted RR of ovarian cancer associated with hysterectomy was 0.66 (95% CI, 0.45 to 0.99); the multivariate RR was essentially unchanged (Table 3). Among parous women, the age-adjusted RR for those who had a hysterectomy was similar (parity = 1 or 2: RR, 0.61 [95% CI, 0.31 to 1.21]; parity = 3 or more: RR, 0.77 [95% CI, 0.45 to 1.31]). The RR of ovarian cancer associated with hysterectomy was somewhat lower among women who ever used oral contraceptives (RR, 0.45; 95% CI, 0.20 to 1.01) than among never users (RR, 0.77; 95% CI, 0.48 to 1.22), but the CIs were wide. We also assessed risk by time since hysterectomy to determine if the inverse association was maintained over time. We observed a statistically significant inverse association in the 5- to 9-year category and a nonsignificant decrease in risk 15 or more years after

Table 2.—Relative Risk (RR) of Ovarian Cancer by Tubal Ligation Status and Use of Other Contraceptives

	Cases	Person-Years	Age-Adjusted RR (95% Confidence Interval)	Multivariate RR (95% Confidence Interval)*
Tubal ligation: risk period 1976-1988				
No	148	690 508	1.0	1.0
Yes	9	169 283	0.29 (0.15-0.55)	0.33 (0.16-0.64)
Tubal ligation in 1976: risk period 1980-1988				
No	116	477 772	1.0	1.0
Yes	4	77 481	0.23 (0.09-0.58)	0.26 (0.10-0.70)
Tubal ligation in non–talc users: risk period 1982-1988				
No	55	211 323	1.0	1.0
Yes	3	58 482	0.22 (0.07-0.66)	0.26 (0.08-0.85)
Vasectomy: risk period 1976-1988†				
No	113	560 621	1.0	1.0
Yes	37	179 150	1.11 (0.76-1.61)	1.25 (0.85-1.84)
Condom use: risk period 1976-1988†				
No	129	614 340	1.0	1.0
Yes	21	125 431	0.78 (0.49-1.23)	0.76 (0.48-1.21)

*Controlling for age (in 5-year categories); parity (0, 1, 2, 3, ≥4); duration of oral contraceptive use (never, current use, duration of past use, mo: 1 to 11, 12 to 35, 36 to 59, ≥60), age at menarche, y (<12, 12, ≥13); age at natural menopause, y (premenopausal, <50, 50 to 54, ≥55), hysterectomy (yes/no); smoking status (never, current, past); and Quetelet's index in kg/m² (<21, 21 to <23, 23 to <25, 25 to <29, ≥29).

†Assessed among women without tubal ligation in 1976.

the procedure, although the number of cases in each category was small. Multivariate estimates were similar. Finally, we assessed the association between hysterectomy and ovarian cancer according to age at which the procedure was performed. Women who had a hysterectomy before 45 years of age were at a somewhat, but not statistically significant, lower risk than women who had the procedure at or after 45 years of age.

Tubal ligation and hysterectomy have been hypothesized to decrease ovarian cancer risk by preventing vaginally introduced talc, a possible risk factor for ovarian cancer, from reaching the ovaries.[25] We did not find a statistically significant association between talc use and ovarian cancer; however, because the question was not asked until 1982, there were relatively few cases and the CIs were wide. Therefore, we assessed the relationship of tubal ligation and hysterectomy with ovarian cancer among women who reported that they never used talc on their perineum or on sanitary pads. The inverse association with tubal ligation was unchanged (RR, 0.26; 95% CI, 0.08 to 0.85) (Table 2) and the association with hysterectomy was somewhat weakened (RR, 0.80; 95% CI, 0.43 to 1.49).

We obtained medical records for 61 of 100 women who had reported a tubal ligation (10 nonrespondents, one refusal, and 28 medical records unavailable primarily because the procedure had been performed so long ago). The types of procedures were distributed as follows: 33 ligations (includes Pomeroy and Irving procedures, 54% of total), 19 coagulations/fulgurations (31%), seven salpingectomies (11%), one banding (2%), and one fimbriectomy (2%).

COMMENT

In this large prospective study, we found a strong inverse association between tubal ligation and ovarian cancer. The association remained significant after controlling for a number of potential ovarian cancer risk factors and did not appear to result simply from screening the ovaries at the time of the procedure. We also noted an inverse, yet weaker, association between hysterectomy and ovarian cancer.

Our study has several strengths. Its prospective design eliminates the potential for selection bias. Additionally, because exposure information is collected prior to disease diagnosis, recall bias is eliminated. We also accounted for most known or suspected ovarian cancer risk factors in our analysis.

The study also has several potential limitations. Some premenopausal women may not have reported their tubal ligation because they were not sexually active at the time the questionnaire was completed, but when analyses were repeated among women reporting current contraceptive use, results were unchanged.

Because we asked about contraceptive use only until 1982, a number of women in our unexposed category probably subsequently had a tubal ligation. However, by 1982 the number of new reports of tubal ligation was rapidly declining, so that the shift in person-years from nonexposed to exposed would be quite small and because these misclassifed women tend to be both of higher parity and oral contraceptive users (Table 1), this error would tend to bias our RR toward 1.0.

We were unable to assess fully ovarian cancer risk by time since tubal ligation to determine whether the protective effect waned over time. However, when we assessed ovarian cancer risk from 1980 to 1988 by tubal ligation status in 1976, the RR was unchanged. Also, among cases 46 years of age or younger at diagnosis, we found that only one case had been detected at tubal ligation. Some women may have had a malignancy detected during a tubal ligation performed before 1976 who might otherwise have been diagnosed during our study period; however, given the above results, this bias is also likely to

Table 3.—Relative Risk (RR) of Ovarian Cancer From 1976 Through 1988 by Hysterectomy Status

	Cases	Person-Years	Age-Adjusted RR (95% Confidence Interval)	Multivariate RR* (95% Confidence Interval)
Hysterectomy†				
No	192	855 827	1.0	1.0
Yes	28	144 879	0.66 (0.45-0.99)	0.67 (0.45-1.00)
Time since hysterectomy, y				
1-4	5	28 555	0.96 (0.39-2.33)	1.03 (0.42-2.51)
5-9	3	42 136	0.29 (0.10-0.85)	0.29 (0.10-0.88)
10-14	11	37 708	0.92 (0.50-1.70)	0.92 (0.50-1.69)
≥15	9	36 479	0.62 (0.31-1.21)	0.62 (0.31-1.22)
Age at hysterectomy, y				
<45	6	64 141	0.51 (0.23-1.15)	0.48 (0.21-1.08)
≥45	22	80 739	0.72 (0.46-1.13)	0.76 (0.48-1.19)

*Controlling for age (in 5-year categories); parity (0, 1, 2, 3, ≥4); duration of oral contraceptive use (never, current use, duration of past use, mo: 1 to 11, 12 to 35, 36 to 59, ≥60); age at menarche, y (<12, 12, ≥13); tubal ligation status (yes/no); smoking status (never, current, past); and Quetelet's index in kg/m² (<21, 21 to <23, 23 to <25, 25 to <29, ≥29).

†Data on hysterectomy status was missing for 40 cases and 190 817 person-years.

Table 4.—Studies of Tubal Ligation and Ovarian Cancer and Summary of Relative Risks (RRs)

Source, y	Design	Size*	Covariates Controlled	RR (95% Confidence Interval)
Koch et al,[13] 1984	Retrospective cohort	4/22 095	Age, parity	2.4 (0.9-6.7)†
Koch et al,[11] 1988	Case-control	200/211	None	0.8 (0.5-1.3)‡
Mori et al,[6] 1988	Case-control	110/220	Age, parity, marital status, number of induced abortions	0.5 (0.25-1.0)†
Booth et al,[9] 1989	Case-control	213/420	Age, social class, gravidity, unprotected intercourse	0.2 (0.1-0.6)
Shu et al,[10] 1989	Case-control	172/172	Age, education, parity, age at menarche, ovarian cyst	0.8 (0.4-1.6)
Whittemore et al,[12] 1992§				
Hospital controls		517/1970	Age, study, parity, oral contraceptive use	0.59 (0.38-0.93)
Population controls		766/4089	Age, study, parity, oral contraceptive use	0.87 (0.62-1.2)
Hankinson et al, 1993	Prospective cohort	157/859 791	Age, parity, oral contraceptive use, age at menarche and menopause, smoking, hysterectomy, Quetelet's index	0.33 (0.16-0.64)
Summary RR‖				0.63 (0.44-0.91)

*Cases/controls for case-control studies; cases/person-years for cohort studies.

†Confidence intervals calculated from *P* value provided in article.

‡Crude relative risk and 95% confidence intervals calculated using Cornfield's method from data provided in article.

§Pooled analysis of eight studies: four with hosptal controls and four with population controls. Previously published findings from Whittemore et al[7] (1988) and Irwin et al[8] (1991) were included in the pooled estimate.

‖Calculated using a random effects model (see text); estimate includes all seven previous studies plus the current study.

be modest. Finally, it is possible that we failed to control for other ovarian cancer risk factors (eg, family history of ovarian cancer) that might have distorted our findings. However, we controlled for most known risk factors and the association with tubal ligation was strong, thus minimizing this possibility.

Because the fallopian tubes are ligated or occluded at both tubal ligation and hysterectomy, similar effects on ovarian cancer risk might be expected; yet, in our data the reduction in risk appeared greater for women having a tubal ligation. To determine whether this effect was due to the different ages when these two procedures are usually performed, we assessed cancer risk by age at hysterectomy. Our data suggested a slightly larger protective effect in the women who were younger at the time of hysterectomy although only six cases had surgery before 45 years of age. Similar results were reported in a pooled analysis of 12 case-control studies.[12] The effects of tubal ligation and hysterectomy on risk of ovarian cancer also may vary; even the techniques used for performing tubal sterilizations (eg, coagulation vs use of a band or ring) may vary in their subsequent effect on ovarian function, perhaps because of differing degrees of tissue destruction.[4]

Several mechanisms of ovarian cancer etiology have been proposed. Fathalla[26] suggested that "incessant ovulation" increases the risk of ovarian cancer perhaps by exposing the epithelium to steroid-rich follicular fluid or by subjecting it to increased cellular proliferation.[27] In some,[28,29] although not all,[30] studies, tubal ligation was associated with an increased frequency of abnormal menstrual cycles and therefore the procedure might also decrease the number of ovulations. Although we were unable to address this issue specifically, age at natural menopause does not vary by tubal ligation status in our data.

Tubal ligation and hysterectomy might also serve to prevent talc exposure, a hypothesized ovarian cancer risk factor.[25,31,32] However, we found that tubal ligation was also highly protective in women who reported never using talc. In contrast, the association between hysterectomy and ovarian cancer was somewhat weaker when assessed in non–talc users, although the CIs overlapped. If other contaminants apart from talc influence risk of ovarian cancer, tubal ligation and hysterectomy might serve to prevent such exposures.

High circulating levels of pituitary gonadotropins also have been hypothesized to play a role in ovarian cancer.[33] High gonadotropin levels have been associated with stromal ovarian tumors in animal studies[33] and gonadotropins stimulate the growth of cell lines derived from human ovarian cancers.[34] If tubal ligation influenced ovarian circulation,[35] plasma hormone levels and ovarian function might be affected. Lower preovulatory luteinizing hormone levels have been reported among women with tubal ligation in one study,[36] but not in a second study.[37] Plasma gonadotropin levels were unchanged when assessed before and after tubal sterilization[38]; however, the blood samples were drawn only 3 months after the procedure and the assay coefficient of variation was 25%, thus the ability to detect long-term hormonal changes was limited. Lower estrogen[35,36,39] and progesterone levels[37,40,41] have also been noted in women who had a tubal sterilization relative to women who were not sterilized, although others have noted no association.[36,39,42,43] In none of these studies were other possible determinants of hormone levels, such as weight or dietary intake, considered in the analysis.

The data assessing possible hormonal changes after hysterectomy are even more sparse. Estradiol and progesterone levels were decreased 3 to 4 weeks after hysterectomy[44] and a reduced ovarian blood flow was noted immediately after hysterectomy.[45] However, hysterectomy does not result in immediate cessation of ovulation as evidenced by ovulatory increases in progesterone.[46]

In previous studies, including a recent pooled analysis of case-control studies, either a statistically significant[9,12] or a nonsignificant[6,10,11,12] inverse association between tubal ligation and ovarian cancer has been reported (Table 4). In a retrospective cohort study, a nonsignificant increased risk of ovarian cancer was found; however, the study was small and control for parity was limited.[13] To summarize these findings, we calculated a weighted average of the RR using a random effects model that allows for heterogeneity across studies.[47] Overall, among past studies, a significant 31% reduction in ovarian cancer risk was noted and, when including the current study, the reduction is 37%.

If the inverse association between tubal ligation and ovarian cancer simply resulted from screening the ovaries at the time of the procedure, the greatest reduction in risk would be expected in the first several years after the procedure. Whittemore et al[7] reported a greater decrease in RR among women who had a tubal ligation within the previous 10 years than among women who had the procedure more than 10 years before, although these differences were not statistically significant. More recently, the inverse association between tubal ligation and ovarian cancer was present at least 15 years after the procedure.[8] Our data also suggest that the inverse association is unlikely to be simply an effect of screening.

In a pooled analysis, hysterectomy was associated with either a statistically significant (RR, 0.69 for six hospital-based studies) or nonsignificant (RR, 0.88 for six studies with population controls) reduction in ovarian cancer risk.[12] Although the CIs were wide, the risk appeared lowest among women having a hysterectomy early in life, similar to our findings. In addition, the reduced risk appeared to be present at least 10 to 19 years after hysterectomy; our findings also tend to support a longer-term protective effect.

Our data indicate a strong inverse association between tubal ligation and subsequent risk of ovarian cancer. A modest protective effect of hysterectomy on ovarian cancer risk is also suggested. It may be appropriate to consider the reduced risk of ovarian cancer after tubal ligation, now seen in several studies, when choosing among alternative methods of contraception. Further research is needed to define biologic mechanisms and to assess effects of different tubal ligation procedures.

This study was supported by research grant CA 40356 from the National Institutes of Health. Dr Hankinson was supported in part by Research Service Award 5T32CA09001 from the National Institutes of Health. Dr Colditz is supported in part by American Cancer Society Faculty Research Award FRA-398. We are indebted to the registered nurses in the study for their continuing cooperation and to Mark Schneyder, Barbara Egan, Gary Chase, Maureen Ireland, Lisa Dunn, Lori Egan, Karen Corsano, MA, and Kate Saunders for their expert and unfailing assistance.

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AMERICAN JOURNAL OF EPIDEMIOLOGY
Vol. 130, No. 2
Printed in U.S.A.

Brief Original Contributions

A CASE-CONTROL STUDY OF BORDERLINE OVARIAN TUMORS: THE INFLUENCE OF PERINEAL EXPOSURE TO TALC

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Harlow, B. L. (Harvard Medical School, Brigham and Women's Hospital, Boston, MA 02115), and N. S. Weiss. A case-control study of borderline ovarian tumors: the influence of perineal exposure to talc. *Am J Epidemiol* 1989;130:390–4.

The authors interviewed 116 female residents of western Washington State with serous and mucinous borderline ovarian tumors diagnosed between 1980 and 1985 and questioned them on their use of hygienic powders. A sample of 158 control women from the same counties were identified through random digit dialing and were interviewed as well. Neither the perineal application of baby powder nor the perineal application of cornstarch was associated with an appreciably altered risk of borderline ovarian tumors. However, women who used deodorizing powders alone or in combination with other talc-containing powders had 2.8 times the risk (95% confidence interval 1.1–11.7) of women who had not had perineal exposure to powder. These results suggest that future studies of ovarian tumors in relation to the application of talc-containing powders should consider ascertaining the specific type(s) of powder used.

ovarian neoplasms; talc

In light of the marked differences in age-specific incidence and patient survival between borderline and malignant epithelial ovarian tumors (1), we conducted a case-control study of borderline ovarian tumors to determine whether etiologic differences between these low-grade tumors and their malignant counterparts exist as well. As part of this study, we sought to investigate the possible etiologic role of perineal exposure to talc.

Interest in talc as a potential ovarian carcinogen has grown from reports of occupational asbestos exposure and ovarian cancer (2–4). Mineral talc, similar in chemical composition to various asbestos minerals, is the common base for most dusting powders that women may apply to the perineum, sanitary napkins, or diaphragms prior to storage (5). Presently, three epidemiologic studies have examined the association between talc exposure and ovarian cancer (6–8).

MATERIALS AND METHODS

The Seattle-Puget Sound Cancer Surveillance System classifies borderline ovarian tumors according to the World Health Organization *International Classification of Diseases for Oncology* (ICD-O) (9). Female residents of three urban counties of western Washington State diagnosed as having a serous or mucinous borderline ovarian tumor (ICD-O codes 8,440–8,481) were identified from the files of this population-based cancer reporting system. Included were white women aged 20–79 years whose tumors were diagnosed during the years

Received for publication August 4, 1988, and in final form February 28, 1989.

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The authors thank Dr. Daniel W. Cramer for his helpful advice and comments.

1980–1985. Among those tumors subject to an independent pathology review (73 per cent), 83 of 88 (94 per cent) were confirmed as borderline ovarian tumors. Given the high degree of histologic agreement, we chose to include the additional 33 cases whose tumors had not been reviewed. Through random digit dialing, we identified a control group of white women who were similar to the cases with respect to age and county of residence. Controls who had undergone bilateral oophorectomy were excluded from the analysis. Further details of the study methods are described elsewhere (10).

Reproductive, sexual, and medical histories and information on perineal exposure to talc were obtained during an in-person interview. An open-ended question asked women to specify the type(s), but not the brand name(s), of powder they had used for perineal application after bathing, on sanitary napkins, and for diaphragm storage prior to diagnosis (or a similar date for controls). Affirmative responses were categorized either as one or more of three talc-containing powders (baby powder, deodorizing powder, and other or unspecified talcum or "dusting" powders) or as cornstarch.

We were successful in obtaining interviews from 116 cases (68 per cent of those eligible) and 158 controls (74 per cent of those eligible). A detailed discussion of response rates can be found elsewhere (10). Since previous studies (including ours) have reported an association of ovarian cancer risk in relation to reproductive history and exogenous female hormones, we controlled for age, parity, and the use of oral contraceptives during the analysis, by means of stratification (11).

Results

Women who reported any perineal use of dusting powders—either after bathing, on sanitary napkins, or for diaphragm storage—had an adjusted relative risk of 1.1 for developing a borderline ovarian tumor (95 per cent confidence interval (CI) 0.7–2.1) (table 1). We further examined this association according to both the specific method of exposure to dusting powders and the type of powder used. The analysis by method of use indicates that a smaller proportion of cases than controls used talc-containing powder or cornstarch for diaphragm storage. The risk associated with the use of talc-containing powders or cornstarch after bathing was 1.2 (95 per cent CI 0.6–2.6). Women who reported any use of talc-containing powder or cornstarch on sanitary napkins had a risk about double (relative risk (RR) = 2.2, 95 per cent CI 0.8–19.8) that of women who reported no talc use. This risk was the same for women who reported applying powder both after bathing and to sanitary napkins. No increase in risk was present among short- and long-term diaphragm users, the risk was not modified by the use of cornstarch versus other talc-containing powders, and there was no variation in risk with increasing number of days of use (not shown).

When we compared cases and controls by the type of powder used, there was no excess risk of borderline tumors among women who applied cornstarch, baby powder, or unspecified talcum powder alone or in combination to the perineum. However, women who applied deodorizing powders with or without baby powder (only baby powder was reported as a second powder in women who used deodorizing powders) had nearly three times the risk of developing a borderline ovarian tumor compared with women who reported no perineal use of powder (RR = 2.8, 95 per cent CI 1.1–11.7).

When we examined the type of powder used according to the method of application, the excess risk due to the use of deodorizing powders was present regardless of whether it was applied after bathing or to sanitary napkins. No subjects reported any use of deodorizing powders for diaphragm storage.

Discussion

Our results of perineal exposure to talc—no association among women who applied talcum powder to diaphragms, but a modest increase in risk among women who applied

TABLE 1

Perineal use of talc-containing powder and cornstarch among women with borderline ovarian tumors and their matched controls, by method of use and by type of powder used, western Washington State, 1980–1985

	Cases (n = 116)	Controls (n = 158)	Crude RR*	Adjusted RR†	95% CI*
No perineal exposure to powder	67	94	1.0‡		
Any perineal exposure to powder	49	64	1.1	1.1	0.7–2.1
Method of use					
Diaphragm storage only	8	21	0.5	0.5	0.2–1.4
Diaphragm storage only or with other methods	11	27	0.6	0.5	0.2–1.3
After bathing only	24	30	1.1	1.2	0.6–2.6
After bathing only or with other methods	34	37	1.3	1.3	0.8–2.7
Sanitary napkins only	7	4	2.5	2.2	0.8–19.8
Sanitary napkins only or with other methods	14	10	2.0	1.9	0.9–6.9
After bathing and on sanitary napkins	7	4	2.5	2.2	0.9–19.8
Type of powder used					
Cornstarch only (no combined use)	4	7	0.8	0.8	0.2–3.8
Baby powder only	18	31	0.8	0.8	0.4–1.9
Baby powder only or combined use	22	34	0.9	0.9	0.5–2.0
Talc, unspecified (no combined use)	13	19	1.0	1.0	0.4–2.4
Deodorizing powder only	10	4	3.5	3.5	1.2–28.7
Deodorizing powder only or combined use	14	7	2.8	2.8	1.1–11.7
Method and type of powder used					
Any powder use after bathing					
Any use of deodorizing powder	10	5	2.8	3.1	0.8–10.9
No use of deodorizing powder	24	32	1.1	1.1	0.5–2.4
Any powder use on sanitary napkins					
Any use of deodorizing powder	8	4	2.8	2.6	0.9–22.4
No use of deodorizing powder	6	6	1.4	1.5	0.4–6.5

* RR, relative risk; CI, confidence interval.

† Adjusted for age (20–39, 40–59, or 60–79 years), parity (nulliparous or parous), and use of oral contraceptives (ever or never).

‡ Reference group.

talc-containing powders to the perineum or to sanitary napkins—are consistent with those previously reported in studies of malignant ovarian tumors. Cramer et al. (6) observed a 50 per cent excess risk among women who used dusting powders or who applied talc-containing powders to sanitary napkins, and a relative risk of 3.3 among women who applied both. No association was found with use of talcum powder for diaphragm storage. Hartge et al. (7) also found no excess risk among users of talc for diaphragm storage, but they did report an association with perineal application (seven cases, three controls; RR = 2.5, 95 per cent CI 0.7–10.0). Whittemore et al. (8) reported a 40 per cent excess in risk of ovarian cancer associated with perineal exposure only and a modest increase in risk with increasing numbers of applications per month.

An association between talc use and ovarian neoplasms seems biologically plausible, since particulates contaminating the vaginal area may migrate into the pelvic cavity and since particles of talc have been observed within ovarian tissue (12–15). It is also conceivable that the excess risk as-

:n tumors and their ate. 1980–1985

ed	95% CI*
	0.7–2.1
	0.2–1.4
	0.2–1.3
	0.6–2.6
	0.8–2.7
	0.8–19.8
	0.9–6.9
	0.9–19.8
	0.2–3.8
	0.4–1.9
	0.5–2.0
	0.4–2.4
	1.2–28.7
	1.1–11.7
	0.8–10.9
	0.5–2.4
	0.9–22.4
	0.4–6.5

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sociated with application of talc to the perineum and to sanitary napkins that was seen in the three prior studies. none of which inquired about the type of powder, could have been due to a strong association restricted to the use of deodorizing powders. The lack of an increased risk among women who used talc-containing powder on diaphragms (both in our study and in the previous studies) supports this hypothesis, since deodorizing powder was infrequently used for diaphragm storage. Furthermore, differential asbestos contamination among different types of cosmetic talcum powders cannot be ruled out. Until 1975, US-manufactured cosmetic talcum powders were required to contain at least 90 per cent mineral talc, but until 1968, some products marked as cosmetic talcum powders did not conform to these guidelines (16, 17). In 1976, a study of 21 consumer talcum powders labeled as baby powders, facial powders, or body powders obtained from retail stores in New York City between 1971 and 1975 reported that 10 contained concentrations of asbestiform tremolite and anthophyllite ranging from 0.2 per cent to 14 per cent (4).

Although it is difficult to explain the lack of association among women who used baby powder exclusively, according to the product labels baby powder is reported to contain only talc and no other minerals or deodorizing substances. The product labels from deodorizing powders, body powders, and perfumed dusting powders, on the other hand, indicate that they contain deodorizing substances and a variety of other free and bonded silicas (potentially high in asbestiform fibers (18)) in addition to talc.

We suggest caution when interpreting the results of this study. The elevated risk among women who specifically used *deodorizing* powders could have been due to chance or applicable only to borderline, not malignant, ovarian tumors. We believe the latter possibility to be unlikely, since the risk associated with the use of any talc-containing powder was similar to that reported in previous studies of women with malignant ovarian tumors. In addition, because of refusals and other reasons for nonparticipation, we were unable to include approximately 30 per cent of potentially eligible cases and controls. Since nonparticipants were similar to participants with respect to certain characteristics such as age and county of residence, we have no reason to believe that there was any dissimilarity in their use of talc-containing powders.

Given the clues provided by this study regarding the possible importance of deodorizing powders, it would be advisable for future studies to elicit information on the brand names of talc-containing powders and the timing and duration of use of each type of talc-containing powder. Although these data need replication, they raise the possibility that the risk of ovarian tumors in women who apply deodorizing powder to the perineum may not relate to talc per se but rather to asbestos contamination and/or a substance or substances used specifically for deodorization.

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A CASE

GUY R. NEWELL.

The incidence of p
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diabetologists and in major medical centers where funduscopic examination is done routinely and competently. However, in the office of the primary care physicians, where most diabetics in this country receive much of their care, annual examination of the fundi through *dilated* pupils regrettably is performed infrequently if at all. Given that circumstance, an abnormal tourniquet test result demands a competent funduscopic examination to rule out proliferative retinopathy, often by referral to an ophthalmologist. I wish to emphasize that I am not advocating that the tourniquet test replace regular funduscopic examination.

If Drs Aaby and Zegarra have a cost-effective strategy to ensure adequate annual examination of the 11 million diabetics in the United States "by a physician who can recognize early proliferative diabetic retinopathy," I would happily endorse it and discard the tourniquet test; until then, the tourniquet test will identify nine of every ten patients with diabetic retinopathy who need to be referred to such a physician. Many of these patients' conditions are currently undiagnosed until loss of vision occurs.

Decrease in capillary fragility with improved diabetic control noted in several patients was not meant to imply regression of diabetic retinopathy. Histological study, however, may confirm that the tourniquet test does accurately reflect the progression or regression of diabetic dermal microangiopathy. At present, the vascular or platelet abnormality causing capillary fragility in diabetes is unknown. I am currently involved in a study correlating the tourniquet test with fluorescein retinal angiography in those patients who do not have identifiable diabetic retinopathy on ophthalmoscopic examination.

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Talc and Ovarian Cancer

To the Editor.—Cramer and co-workers' recently reported observing an association between talc use and risk of ovarian cancer. We therefore examined data on talc use that two of us (L.M. and L.P.L.) had collected as part of a case-control interview study of epithelial ovarian cancer conducted from 1974 to 1977 in the Washington, DC, area.[2] The cases were 197 women with pathologically confirmed primary epithelial ovarian cancers treated in participating hospitals. The controls were 197 women treated at the same hospitals for conditions other than gynecologic, psychiatric, or malignant diseases or pregnancy. The controls were frequency matched to the cases on age, race, and hospital. The interviewers asked questions about reproductive and sexual history, medical history, drug use, and other exposures. Questions about talc use were added to the questionnaire after the study began, so 135 cases and 171 controls were asked about talc exposure.

Estimated Relative Risk of Ovarian Cancer, According to Reported Use of Talc

	Cases	Controls	Estimated Relative Risk	95% Confidence Interval
No talc mentioned	62	61	1.0	. . .
Any talc mentioned	67	100	0.7	0.4-1.1
No diaphragm used	92	118	1.0	. . .
Diaphragm used, no talc	14	11	1.6	0.7-3.7
Diaphragm, with talc	25	41	0.8	0.4-1.4
No body talc	77	84	1.0	. . .
Some body talc	54	78	0.8	0.5-1.2
"All over"	37	57	0.7	0.4-1.2
Genital*	7	3	2.5	0.7-10.0
Legs only	1	0	. . .	. . .
Not genital	6	8	0.8	0.3-2.5
Unknown where	3	10	0.3	0.1-1.2

*On genitals, sanitary napkins, or underwear.

The reported talc use among cases and controls is given in the Table. We estimated the relative risk to talc users as 0.7 (95% confidence interval [CI]=0.4 to 1.1). The estimate was unaffected by adjustment for race, age, and gravidity. Neither women who used talc on their diaphragms nor those who used it as body powder seemed to be at excess risk. Women who used talc as a body powder were asked how they used it. Among the ten who specifically mentioned use on sanitary napkins, underwear, or the genital area, the relative risk was estimated as 2.5, but the small number of exposed women yielded an unreliable estimate (95% CI=0.7 to 10.0).

Our data thus indicate no overall association between talc use and risk of ovarian cancer. Although a small group of women who specifically reported genital use of body talcum powders showed an excess relative risk, use of talc on a diaphragm, which would be the closest exposure to the ovaries, did not seem to elevate risk.

Chance, bias in selection or observation, or confounding may have influenced these estimates. One important potential bias to consider in this and Cramer's study is a difference between cases and controls in recollecting or reporting talcum powder use, especially in the genital area. Talc exposure was not a major focus of this study, and few data are available to assess the likelihood of recall bias. Such a bias could stem from cases' heightened awareness or from the fact that controls were interviewed in the hospital while most cases were interviewed at home. On the other hand, the questions about talc use were rather simple and unambiguous. Also, we noted that cases and controls were equally likely to report douching. Since reporting of use of douches might be subject to the same recall biases as talc use, this observation suggests that little recall bias operated. Another possible interpretation of our findings of no apparent effect of using talc on the diaphragm but some effect of perineal use of powder is that talc itself does not increase risk of ovarian cancer but that patients with ovarian cancer have or perceive a greater need for using body powder in the genital area, for reasons related either to the biology of the disease or to life-style. We agree with Cramer and co-workers that other epidemiologic data will be useful.

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Perineal Exposure to Talc and Ovarian Cancer Risk

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Objective: We sought to determine whether the use of talc in genital hygiene increases the risk for epithelial ovarian cancer.

Methods: We interviewed 235 white women diagnosed with epithelial ovarian cancer between 1984–1987 at ten Boston metropolitan area hospitals and 239 population-based controls of similar race, age, and residence.

Results: Overall, 49% of cases and 39% of controls reported exposure to talc, via direct application to the perineum or to undergarments, sanitary napkins, or diaphragms, which yielded a 1.5 odds ratio (OR) for ovarian cancer (95% confidence interval [CI] 1.0–2.1). Among women with perineal exposure to talc, the risk was significantly elevated in the subgroups of women who applied it: 1) directly as a body powder (OR 1.7, 95% CI 1.1–2.7), 2) on a daily basis (OR 1.8, 95% CI 1.1–3.0), and 3) for more than 10 years (OR 1.6, 95% CI 1.0–2.7). The greatest ovarian cancer risk associated with perineal talc use was observed in the subgroup of women estimated to have made more than 10,000 applications during years when they were ovulating and had an intact genital tract (OR 2.8, 95% CI 1.4–5.4); however, this exposure was found in only 14% of the women with ovarian cancer.

Conclusions: These data support the concept that a lifetime pattern of perineal talc use may increase the risk for epithelial ovarian cancer but is unlikely to be the etiology for the majority of epithelial ovarian cancers. (*Obstet Gynecol 1992;80:19–26*)

The theory that human ovarian cancer may be mesotheliomas that originate from asbestos exposure was first proposed by Graham and Graham.[1] Later, Parmley and Woodruff[2] suggested that effluences that may arise from the vagina, uterus, or tubes might enter the pelvic cavity and interact with ovarian surface epithelium to induce such mesotheliomas. Longo and Young[3] further speculated that cosmetic talc might act in this manner because of its chemical similarity to asbestos. Although there is little doubt that asbestos may induce mesotheliomas, the link between genital exposure to talc and ovarian cancer is less clear.

The few epidemiologic studies that have examined the association between talc and ovarian cancer reported only modest elevations of risk, but data on all potential sources of genital talc exposure were limited.[4–7] The purpose of this report was to present findings from a new case-control study of ovarian cancer conducted in the Boston metropolitan area, in which we considered a variety of modes for perineal talc exposure, the frequency and duration of use, and various reproductive characteristics that might influence the ability of talc to enter the pelvic cavity and affect the ovaries.

Materials and Methods

Between July 1984 and September 1987, we identified 394 women between 18–76 years of age diagnosed with borderline or malignant epithelial ovarian cancer at one of ten participating hospitals in the Boston metropolitan area. Permission to contact each patient was obtained in advance from the physician of record. An in-person interview was conducted with 272 (69%) of the 394 cases identified. Thirty-one percent were not interviewed because of physician and/or patient refusal, patient death, or relocation. The final sample for analysis was further restricted to the 235 white women confirmed as having an epithelial ovarian tumor based on an independent pathology review conducted by two of the authors (DAB and WRW).

Controls were selected from the Massachusetts Town Books, annual publications that list residents by name, age, and address according to voter precincts. For each new ovarian cancer case interviewed, a ran-

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Supported by grant R01 CA 42008 from the National Cancer Institute.

Table 1. Influence of Any Perineal Talc Exposure* on Ovarian Cancer Risk by Characteristics of Study Participants, Boston Metropolitan Area, 1984–1987

	Cases		Controls			
	Total	Talc exposure	Total	Talc exposure	Crude OR	95% CI
All subjects	235	114 (48.5%)	239	94 (39.3%)	1.5	1.0–2.1
Age (y)						
<50	96	41 (42.7%)	101	28 (28.0%)	1.9	1.2–3.4
≥50	139	73 (52.5%)	138	66 (47.8%)	1.2	0.8–2.1
Education (y)						
≤12	93	51 (54.8%)	115	48 (41.7%)	1.7	1.1–3.0
>12	142	63 (44.4%)	124	46 (37.1%)	1.4	0.9–2.4
Marital status						
Never married	40	14 (35.0%)	24	6 (25.0%)	1.6	0.5–5.7
Ever married	195	100 (51.3%)	215	88 (40.9%)	1.5	1.0–2.7
Religion						
Jewish	35	21 (60.0%)	21	12 (57.1%)	1.1	0.4–3.9
Non-Jewish	200	93 (46.5%)	218	82 (37.6%)	1.4	0.9–2.5
Weight (lb)						
<140	123	53 (43.1%)	125	49 (39.2%)	1.2	0.7–1.9
≥140	112	61 (54.5%)	114	45 (39.2%)	1.8	1.1–3.1
Use of OCs (mo)						
≥3	66	31 (47.0%)	82	27 (32.9%)	1.8	0.8–4.5
<3 or never	169	83 (49.1%)	157	67 (42.7%)	1.3	0.8–2.3
No. of live-born children						
0	79	30 (38.0%)	43	12 (27.9%)	1.6	0.7–4.0
1	31	21 (67.7%)	27	5 (18.5%)	9.2	2.9–46.2
2	40	24 (60.0%)	63	26 (41.3%)	2.1	0.9–3.3
≥3	85	39 (45.9%)	106	51 (48.1%)	0.9	0.6–1.6

OR = odds ratio; CI = confidence interval; OCs = oral contraceptives.

* Sources of perineal talc exposure include: dusting of underwear, diaphragms, or sanitary napkins; use by partner on his perineal area; use as a body powder.

dom number generator selected one page from the town book corresponding to the case's precinct of residence. By working forward in the town book, we selected the first five female subjects within 2 years of age of the case as potential controls. Of these five, the first subject of the same race as the case without a history of a bilateral oophorectomy was asked to participate in the study. Of the 526 controls contacted, 239 interviews were conducted (25% could not be reached, 10% reported a history of bilateral oophorectomy, and 19% declined to participate). Further details of the study methods can be found elsewhere.[6]

The in-person interview focused on the following: demographic and occupational history; medical and reproductive histories, including pregnancies, hormones used, and gynecologic operations; dietary history; cigarette smoking; and hygienic practices. The hygienic practices included information regarding the use of douches, type of sanitary protection used, and perineal exposure to talc. Queried sources of perineal talc exposure included dusting of underwear, sanitary napkins, and diaphragms; exposure via husband's use of talc; and more direct exposure to the perineum as a body powder. No reliable information on talc exposure during infancy with diapering could be obtained, and women using talc as a body powder on areas other than the perineum were considered nonexposed. For each exposure, we inquired about brands used, age at first use, total years of use, and frequency of use per month, to enable us to estimate the total lifetime number of applications from all sources of exposure.

Differences between cases and controls in the distribution of these various exposures to talc were examined both qualitatively and quantitatively. The influence of confounders and effect modifiers was assessed first through stratification and then using unconditional logistic regression.[9] The primary matching variable, age, was retained in each logistic model. The χ^2 test for linear trend was calculated based on the change in deviance in models with and without continuous exposure variables.[9]

Results

Table 1 shows the proportion of cases and controls with any reported perineal talc exposure, and the associated crude exposure odds ratio (OR), by certain demographic and reproductive subgroups. Overall, a

Table 2. History of Talc Exposure by Types of Application, Brand of Powders, Years and Frequency of Use, and Era of Use

	Cases	Controls	Adjusted OR*	95% CI
No genital talc application	121 (51.5%)	145 (60.7%)	1.0	
Any genital talc application	114 (48.5%)	94 (39.3%)	1.5	1.0–2.1
Type of application				
Only via sanitary napkins and/or underwear	9 (3.8%)	12 (5.0%)	1.1	0.4–2.8
Via partner or applications to diaphragm†	20 (8.5%)	21 (8.8%)	1.2	0.6–2.4
Via dusting powder to perineum†	85 (36.2%)	61 (25.5%)	1.7	1.1–2.7
Applications of talc per month				
<5	32 (13.6%)	28 (11.7%)	1.5	0.8–2.7
5–29	24 (10.2%)	25 (10.5%)	1.2	0.6–2.2
≥30	58 (24.7%)	41 (16.7%)	1.8	1.1–3.0
Years of talc use				
<10	14 (6.0%)	15 (6.3%)	1.2	0.5–2.6
10–29	49 (20.9%)	39 (16.3%)	1.6	1.0–2.7
≥30	51 (21.7%)	40 (16.3%)	1.6	1.0–2.7
Age (y) at first talc use				
<20	66 (28.1%)	50 (20.9%)	1.7	1.1–2.7
20–25	27 (11.5%)	26 (10.9%)	1.2	0.6–2.2
>25	21 (8.9%)	18 (7.5%)	1.6	0.8–3.2
Years since last talc use				
Within last 6 mo	48 (20.4%)	27 (11.3%)	2.3	1.3–4.0
Between 6 mo–10 y	36 (15.3%)	39 (16.3%)	1.1	0.7–1.9
10 y or more	30 (12.8%)	28 (11.7%)	1.4	0.8–2.6
Era of use‡				
Exclusive use after 1960	29 (12.3%)	30 (12.6%)	1.1	0.6–2.1
Any use before 1960	75 (31.9%)	57 (23.9%)	1.7	1.1–2.7
Brand of application§				
Brand or generic baby powder	91 (38.7%)	72 (30.1%)	1.6	1.1–2.5
Deodorizing or other scented powders	16 (6.8%)	17 (7.2%)	1.2	0.6–2.5

OR = odds ratio; CI = confidence interval.

* Adjusted for parity (0, 1–2, >2), education (<12 years, >12 years), marital status (never married, ever married), religion (Jewish, non-Jewish), use of sanitary napkins (no, yes), douching (no, yes), age (continuous), and weight (<140 lb, ≥140 lb).

† Includes combinations with sanitary napkins or underwear.

‡ Restricted to women older than 10 years in 1960; 100 cases and 118 controls were unexposed and used as the referent group.

§ Excludes seven cases and five controls with unknown powders. Seven cases and four controls reported combinations of more than one brand and were classified according to the brand used most frequently and for the longest period. Specific brands mentioned by cases were: Johnson and Johnson, 71; generic baby powder, 20; Shower to Shower, four; other scented, 12. Specific brands mentioned by controls were: Johnson and Johnson, 54; generic baby powder, 18; Shower to Shower, three; other scented powder, 14.

greater percentage of cases (48.5%) than controls (39.3%) reported any perineal exposure to talc-containing powders. Subgroups of controls in which exposure to talc appeared to be more common were women older than 50 years ($P = .002$), ever married ($P = .12$), Jewish ($P = .08$), and parous ($P = .05$). Controls who reported no oral contraceptive (OC) use also reported greater talc use. However, this interaction may be explained by the older age distribution among controls who reported perineal application of talc. We observed stronger associations between talc and ovarian cancer risk in the subgroups of cases and controls younger than age 50, less well educated, heavier than 140 lb 5 years before diagnosis, and reporting a history of one or two live births. The number of live births was the only factor that produced statistically significant heterogeneity in the ORs for the talc and ovarian cancer association. Age, education, marital status, religion, weight, and parity were considered confounders and were included as covariates in subsequent multivariate models. Use of OCs did not confound the talc-ovarian cancer association.

Table 2 examines the association between talc use and ovarian cancer by variables related to the specific types and kinds of applications, and measures of duration including length, frequency, and period of use. Compared with women with no genital talc exposure, women exposed to talc only through use as a dusting powder on sanitary napkins or underwear had no appreciable increased risk for ovarian cancer. There was also no substantial increase in risk among women exposed to talc only through a husband's use or use in the storage of diaphragms, or in combination with applications to sanitary napkins or underwear. The most frequent method of perineal talc exposure was use as a dusting powder directly to the perineum,

Table 3. Estimated Total Lifetime Perineal Applications of Talc-Containing Powders in Cases and Controls

Applications	Cases	Controls	Adjusted OR*	95% CI
Total applications				
None	121	145	1.0	
<1000	18	19	1.3	0.7–2.7
1000–10,000	54	44	1.5	0.9–2.4
>10,000	42	31	1.8	1.0–3.0
χ^2 1*df* test for linear trend = 2.85, P = .094†				
Applications excluding use after hysterectomy or tubal ligation				
None	121	145	1.0	
<1000	19	19	1.4	0.7–2.9
1000–10,000	57	46	1.5	0.9–2.4
>10,000	38	29	1.7	1.0–3.0
χ^2 1*df* test for linear trend = 3.19, P = .077†				
Applications excluding use after hysterectomy or tubal ligation, and use during nonovulatory months‡				
None	124	149	1.0	
<1000	24	23	1.5	0.8–2.9
1000–10,000	55	51	1.3	0.8–2.0
>10,000	32	16	2.8	1.4–5.4
χ^2 1*df* test for linear trend = 6.15, P = .015†				

Abbreviations as in Table 2.

* Adjusted for parity (0, 1–2, >2), education (<12 years, >12 years), marital status (never married, ever married), religion (Jewish, non-Jewish), use of sanitary napkins (no, yes), douching (no, yes), age (continuous), and weight (<140 lb, ≥140 lb).

† Trend test based on actual applications as a continuous variable.

‡ Excludes exposures while taking oral contraceptives, while pregnant or breast-feeding, or occurring after menopause. There were three cases and four controls who moved from "exposed" to "nonexposed" in this category, as all of the exposure occurred during oral contraceptive use, pregnancies, or after menopause.

alone or in combination with either a partner's use or use in the storage of diaphragms. This exposure occurred in 85 cases (36.2%) and 61 controls (25.5%) (P = .01). Of the application modes studied, direct perineal application produced the greatest risk (OR 1.7, 95% confidence interval [CI] 1.1–2.7).

We also examined the talc-ovarian cancer association by frequency and years of use (Table 2). When monthly frequency was considered as a continuous variable in the logistic model, the χ^2 linear test of trend was 4.06 (P = .046), indicating that the risk for ovarian cancer increased significantly with increasing frequency of applications per month. The categorical analysis showed that relative to non-users, the risk was greatest in women who applied talc at least once per day. When years of use was included as a continuous variable, the test for linear trend was 3.32 (P = .07). The categorical analysis showed that relative to non-users, women who applied talc for more than 10 years were at 60% greater risk for ovarian cancer. Likewise, perineal applications of talc early in life (before age 20) or applications within 6 months of diagnosis (reference age for controls) produced the stronger ORs.

To assess whether the risk of ovarian cancer with perineal exposure to talc was affected by the time when talc-containing products were manufactured, we examined the association separately in women who only used talcum powder after 1960 and in women who reported any use of talcum powder before 1960 (Table 2). After restricting the population to women older than age 10 in 1960 and adjusting for age, parity, and a number of other demographic characteristics, we found that the association of talc and ovarian cancer was greater in women using talc products before 1960 (P = .025).

The last entry in Table 2 shows the risks by brand of powder used. No subjects could recall exclusive use of starch-based powders. Brand or generic "baby powder" was used most frequently and was the category associated with a statistically significant risk for ovarian cancer. With respect to other powders, four cases and three controls reported primary use of deodorizing powders, and 12 cases and 14 controls reported primary use of other scented powders. It should be appreciated that, because the period of exposure often occurred over decades, verification of brands was not possible.

Table 3 examines the ovarian cancer risk associated with the total number of applications to the perineum, estimated by cumulating frequency and years of use for the various kinds of exposures. An 80% excess risk was associated with an estimated exposure of more than 10,000 applications (equivalent to daily use for 30

Table 4. Adjusted Odds Ratios and 95% Confidence Intervals for Ovarian Cancer by Any Perineal Exposure to Talc* and Indicators of Ovulation and Tubal Occlusion

	Cases		Controls			
	Total	Talc exposure	Total	Talc exposure	Adjusted OR†	95% CI
All subjects	235	114 (48.5%)	239	94 (39.3%)	1.5	1.0–2.1
Mid-cycle pain						
No	181	88 (48.6%)	184	77 (41.9%)	1.4	0.9–2.2
Yes	54	26 (48.2%)	55	17 (30.9%)	2.0	0.8–5.2
Regular period						
No	26	12 (46.2%)	34	16 (47.1%)	1.1	0.4–3.4
Yes	209	102 (48.8%)	205	78 (38.1%)	1.7	1.1–2.5
PID or ectopic pregnancy						
No	226	113 (50.0%)	230	92 (40.0%)	1.6	1.1–2.4
Yes	9	1 (11.1%)	9	2 (22.2%)	0.1	0.01–7.0

PID = pelvic inflammatory disease; other abbreviations as in Table 2.

* Sources of perineal talc exposure include: dusting of underwear, diaphragms, or sanitary napkins; use by partner on his perineal area; use as a body powder.

† Adjusted for parity (0, 1–2, >2), education (<12 years, >12 years), marital status (never married, ever married), religion (Jewish, non-Jewish), use of sanitary napkins (no, yes), douching (no, yes), age (continuous), and weight (<140 lb, ≥140 lb).

years) as compared with non-users. When considered as a continuous variable in the logistic model, the χ^2 linear test of trend was 2.85 ($P = .094$). The remaining entries in Table 3 show how conditions that either close the upper genital tract or are associated with anovulation affect the dose response of number of applications on ovarian cancer risk. The second entry shows the effect of censoring applications that occurred after tubal ligation or hysterectomy. No appreciable change in the ORs or the dose response was noted. The third entry shows the effect of censoring applications after hysterectomy and tubal ligation and use during presumed nonovulatory periods. Excluded were exposures occurring while taking OCs, while pregnant or breast-feeding, and after menopause. The risk associated with fewer than 10,000 applications was not substantially altered. However, the risk associated with more than 10,000 applications was nearly threefold and statistically significant. The χ^2 test for a linear trend of risk, by number of applications as a continuous variable, increased to 6.15 ($P = .015$).

Table 4 shows the association of talc exposure and ovarian cancer based on other clinical factors that may predict either ovulation or tubal occlusion. The ORs were greater in women with a history of mid-cycle pain or regular periods—potential clinical predictors of ovulatory cycles. An association between talc and ovarian cancer was absent in women with a history of either pelvic inflammatory disease or ectopic pregnancy—potential markers of a closed genital tract. However, only nine cases and nine controls reported a history of pelvic inflammatory disease or ectopic pregnancy, and the CI on the exposure OR was correspondingly wide.

Table 5 shows the relative risk for any perineal use of talc when restricted to specific histologic type and grade of ovarian tumors. The greatest association was found in women diagnosed with an endometrioid tumor or borderline ovarian tumor.

Discussion

Animal and epidemiologic studies have addressed the plausibility of an association between talc and ovarian cancer. Intraperitoneal injection of talc in rodents produced papillary changes in the surface epithelium not inconsistent with the first stage in the development of surface papillary epithelial neoplasms.[10] However, because the ovaries of small rodents are surrounded by a

Table 5. History of Talc Use by Histologic Type and Grade

Histologic type	Any use of talc	No use of talc	Adjusted OR*	95% CI
Controls	94	145	1.0	
Histologic type				
Serous	60	64	1.4	0.9–2.2
Mucinous	17	25	1.2	0.6–2.5
Endometrioid	18	11	2.8	1.2–6.4
Other	19	21	1.6	0.8–3.3
Histologic grade				
Borderline	32	30	2.4	1.2–4.5
Grade 1	6	11	1.0	0.3–2.8
Grade 2	21	22	1.5	0.7–3.0
Grade 3	27	24	1.5	0.8–2.8
Undifferentiated	28	34	1.2	0.7–2.2

Abbreviations as in Table 2.

* Adjusted for parity (0, 1–2, >2), education (<12 years, >12 years), marital status (never married, ever married), religion (Jewish, non-Jewish), age (continuous), and weight (<140 lb, ≥140 lb).

Table 6. Odds Ratios With 95% Confidence Intervals of Ovarian Cancer in Relation to Any Perineal Exposure to Talc as Reported in Previous Epidemiologic Studies

Author(s) (year)	Cases		Controls		Crude OR	95% CI
	Total	Talc exposure	Total	Talc exposure		
Cramer et al[4] (1982)	215	92 (42.8%)	215	61 (28.4%)	1.9	1.3–2.9
Hartge et al (1983)*	135	67 (49.6%)	171	100 (58.5%)	0.7	0.4–1.1
Whittemore et al[5] (1988)	188	98 (52.1%)	539	248 (46.0%)	1.4	0.9–2.0
Harlow and Weiss[6] (1989)†	116	49 (42.2%)	158	64 (40.5%)	1.1	0.7–2.1
Booth et al[7] (1989)	217	141 (65.0%)	434	256 (59.0%)	1.3	0.9–1.9
Harlow et al (1992) (current study)	235	114 (48.5%)	239	94 (39.3%)	1.5	0.9–1.8
All studies‡	1106	561 (50.7%)	1756	823 (46.9%)	1.3	1.1–1.6

Abbreviations as in Table 2.

* Hartge P, Hoover R, Lesher LP, et al. Talc and ovarian cancer (letter). JAMA 1983;250:1844. Odds ratio may include nonperineal talc exposure as well.

† Restricted to borderline ovarian tumors.

‡ Meta-analysis.[19]

peritoneal bursa which often becomes occluded and distended with follicular fluid after intraperitoneal injection of foreign bodies,[1] it is difficult to distinguish whether the papillary changes are the effects of foreign-body exposure or bursal distention. It would be worthwhile to repeat the talc experiments in guinea pigs or rabbits, the animals used in the original research of Graham and Graham.[1]

Is there evidence to suggest that talc can translocate from the vagina to the peritoneal cavity? It is known that red cells and endometrial tissue are capable of retrograde flow from the fallopian tubes.[11] Experiments in rats confirmed the presence of talc in the ovaries after the introduction of a talc suspension into the vagina and cervical os.[12] In humans, two studies observed the migration of inert carbon particles[13] and radioactively labeled human albumin microspheres[14] from the vagina to the fallopian tubes. In addition, several investigators have observed birefringent crystals embedded in ovarian tissue.[15–17] However, critics have argued that these findings resulted from poorly designed studies or lack of precision in measuring particulates, due to leaching of radionucleotide markers from the test materials or the introduction of contaminants during tissue processing.[18] In six multiparous cynomolgus monkeys separately caged, no translocation of talc was observed after 30 consecutive days of douching with a suspension of neutron-activated talc coupled with weekly injections of oxytocin.[18] However, this study was not able to address the effects of long-term use or coitus, which might facilitate talc translocation.

Several epidemiologic studies[4–7] have addressed the genital talc-ovarian cancer association (Table 6). Each study has consistently reported little or no association with the use of talc-dusted diaphragms, and stronger associations with direct perineal application. Dose response of perineal talc exposure from all sources by frequency or years of use was not available in the studies by Hartge et al (Hartge P, Hoover R, Lesher LP, et al. Talc and ovarian cancer [letter]. JAMA 1983;250:1844), Harlow and Weiss,[6] or Cramer et al.[4] Whittemore et al[5] reported no significant dose response by years or frequency of use, whereas Booth et al[7] reported a marginally significant trend with frequency of use. Using the techniques of meta-analysis, in which ORs from multiple studies are weighted by their variances,[19] we calculated a statistically significant OR of 1.3 for any perineal talc exposure and ovarian cancer risk (95% CI 1.1–1.6) from the various studies. We therefore conclude that there is an association, albeit modest, between ovarian cancer and perineal talc use. However, insufficient detail has been available to rule out a stronger association in certain subgroups of users.

Our study included greater detail on genital talc use, including methods, frequency, and years of use (Table 2). Talc applied as a dusting powder directly to the perineum carried a greater risk than less direct exposure via a partner's use or the dusting of undergarments, sanitary napkins, or diaphragms. Current use of talc was associated with a greater ovarian cancer risk than past use. Daily versus less than daily talc use, and talc use for more than 10 years versus less than 10 years, were associated with greater risk for ovarian cancer.

Most subjects reported use of "baby powder." We were unable to confirm a previous finding that powders with "deodorizing" agents were associated with particular risk.[6] Thus, this study failed to answer a key issue in the talc-ovarian cancer association: whether the risk pertains to all cosmetic talcs or only to certain

preparations likely to be contaminated by asbestos. Cralley et al[20] and Rohl et al[21] found considerable variation in the purity of cosmetic talcs, with fiberform contents varying from less than 1 to 30%. Most of these products were manufactured before 1970 and it is likely that the asbestiform content has decreased since 1976, when manufacturers instituted voluntary guidelines on asbestos contamination. Our finding of a lower ovarian cancer risk in women with exclusive use of talc after 1960 may support this theory. Because of the difficulty in obtaining a complete and detailed history of powders used, the issue of whether risk pertains only to asbestos-contaminated powders may need to be settled by animal experiments.

In our analysis, we first calculated all genital applications of talc based upon frequency and years of use. As a continuous variable in a multivariate model, no significant dose response was observed between total genital applications of talc and ovarian cancer risk. Because the "translocation" theory assumes an open genital tract, we then excluded application after tubal ligation or hysterectomy, but observed no appreciable change in the dose response. Further restricting talc exposure to months when the women were likely to be ovulatory, we observed a significant dose response, such that women with an intact genital tract and more than 10,000 applications during ovulatory cycles had nearly a threefold increase in risk for ovarian cancer. Some additional evidence that might support an interaction with ovulation includes stronger associations in women with regular periods and mid-cycle pain. Cramer et al[4] suggested that talc contamination around the time of ovulation might lead to the incorporation of talc particulates into inclusion cysts that may form with ovulation. Experiments on foreign-body tumorigenesis have shown that implantation of foreign bodies into the lumens of epithelial-lined organs provides a favorable environment for carcinogenesis.[22] Alternatively, Mostafa et al[16] speculated that foreign-body exposure might produce cortical "granulomas" whose link to stromal hyperactivity (and hormonally related cancers) is argued in older literature.[23]

We observed that the talc association was strongest in women with endometrioid or borderline ovarian tumors. However, an earlier study by Cramer et al[4] reported no such variation in risk by histologic subtype. It was noted that a greater proportion of women with endometrioid tumors than with other histologic types of ovarian cancer reported more than 10,000 lifetime applications of talc during ovulatory cycles while having an intact genital tract (34 versus 16%). Although this may explain in part the strong talc-ovarian cancer association noted in women with endometrioid tumors, it does not explain the strong association noted in women with borderline ovarian tumors, of whom only 13% reported long-term talc exposure. This variation in risk among histologic subtypes may reflect a chance finding or a need to examine endometrioid and borderline tumors more carefully for evidence of a foreign-body effect.

An unusual observation was the strong association between talc use and ovarian cancer in the subgroup of women with one or two pregnancies but a lower risk in women with either no children or three or more children. Although chance may be the most likely explanation for this finding, we wonder whether this peculiar interaction might reflect, in part, an effect of pregnancy on the degree of openness of the cervical os and its ability to allow translocation of vaginal particulates. The parous cervix has a larger os than the nulliparous cervix, and this could explain the greater risk in women with one child compared with women with no live births. At the other extreme, multiple pregnancies may offer other protective mechanisms (such as reduced ovulation as discussed above) that could outweigh this effect. Clearly, this is a very speculative hypothesis but one that might be tested in experimental studies (ie, repeating the vaginal talc experiments in nulliparous and parous mice or primates).

Noncausal explanations are possible in any epidemiologic research. We cannot rule out the possibility of differential over- or under-reporting of talc exposure in our cases and controls, especially in those with reproductive events that enhance ORs. In addition, though we were successful in interviewing 69% of eligible ovarian cancer cases and 81% of eligible controls contacted, we cannot assess whether the cases and controls not interviewed could have selectively differed in their reproductive characteristics or in their use of talc-containing powders. Because our associations are based upon responses from participating cases and controls, the validity of our results depends upon the assumption that respondents and non-respondents were similar with respect to talc and other relevant exposures, or that the magnitude of any respondent-non-respondent difference was similar for cases and controls. Because the interview provided the only source of "exposure" information, we were unable to assess the likelihood of this assumption. The extent of this bias, however, is likely to be small because the reproductive characteristics and history of talc exposure in participating cases and controls are, for the most part, reasonably consistent with earlier epidemiologic studies of ovarian cancer. Finally, in our attempt to present the most accurate ORs, we made a variety of adjustments to account for the confounding influence of factors associated with both ovarian cancer risk and

talc exposure. Nevertheless, we cannot rule out the presence of other unknown factors that might have influenced, in part, our observed associations.

Because the overall association between genital use of talc and ovarian cancer remains weak, it is unlikely that this exposure-disease pathway is the principal one involved in ovarian cancer etiology. We have previously discussed the role of dietary and metabolic factors in a model for ovarian cancer involving gonadotropin stimulation of failing ovaries.[8] Even if an etiologic association were to pertain in the subgroups of daily users or users with more than 10,000 applications during ovulatory months, we calculate that by applying these ORs to the exposure rate among cases,[24] the proportion of ovarian cancer incidence attributable to this level of talc exposure is about 10%. Nevertheless, given the poor prognosis for ovarian cancer, any potentially harmful exposures should be avoided, particularly those with limited benefits. For this reason, we discourage the use of talc in genital hygiene, particularly as a daily habit.

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Received January 6, 1992.
Received in revised form March 23, 1992.
Accepted March 23, 1992.

TALC

GYNECOLOGIC ONCOLOGY 45, 20–25 (1992)

Mineral Fiber Exposure and the Development of Ovarian Cancer

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Received July 10, 1991

A hospital-based case–control study of the association between fiber exposure and the development of epithelial ovarian cancer was performed at the Johns Hopkins Hospital in Baltimore, Maryland. Genital and respiratory fiber exposures were ascertained from incident cases ($N = 77$) and age–race matched controls ($N = 46$) using a structured questionnaire. Cases were ascertained between 1981 and 1985. An increased risk was observed for exposure to talc on sanitary napkins (OR = 4.79, 95% CI, = 1.29–17.79), genital fiber exposure from different sources for a long (cumulative exposure ≥37.4 years) length of time (OR = 2.35, 95% CI = 0.95–5.80), and occupational fiber exposure in relatives (OR = 2.81, 95% CI = 0.90–8.75). A negative association was observed for antecedent tubal ligation (OR = 0.15, 95% CI = 0.027–0.88). Findings from this study should be confirmed in larger investigations.

INTRODUCTION

Occupational exposure to asbestos has been identified as a risk factor for ovarian cancer in several studies [1–3]. Similarly, fiber-containing substances, such as talc, have also been implicated as risk factors [4–9]. A matched case–control study of epithelial ovarian cancer was conducted, in which the role of both genital and respiratory sources of fiber was assessed, to confirm these findings.

STUDY POPULATION

Cases and controls were ascertained from the Johns Hopkins Hospital between 1981 and 1985. This analysis is restricted to the 77 cases who were matched to 46 hospital controls and treated for conditions other than gynecologic or malignant diseases. Originally 140 newly diagnosed cases of epithelial ovarian cancer who met the eligibility criteria were ascertained from the Johns Hopkins Hospital. One hundred eight (77.1%) of these cases were successfully interviewed. These cases were diagnosed within 6 months of admission, were pathologically confirmed by examination of the ovaries, were admitted as in-patients for treatment or diagnosis, and were residents of the United States. Controls were in-patient females without gynecologic or malignant conditions who were initially matched to cases by age (within 5 years), race, and date of diagnostic admission (within 1 year). Since it was difficult to find controls meeting all of the matching criteria, a control could not be found for all cases. Unmatched cases were therefore matched a posteriori to controls, to form matched triplets of 2 cases and 1 control. A posteriori matching was performed within the same 5-year interval, by race and by closest date of diagnostic admission. The matching procedure allowed for the inclusion of 77 cases in the study. There were 46 matched sets, of which 31 consisted of 2 cases and 1 control. No matched control could be found for 13 cases, which were excluded from the analysis.

METHODS

Data were ascertained primarily from a questionnaire that was administered to participants both by telephone and in the hospital. Information on previous abdominal and gynecologic operations was also ascertained from medical records.

The questionnaire elicited information on the presence and length of genital fiber and respiratory fiber exposure, reproductive factors, estrogen use, family history of cancer in first-degree relatives, and other contraceptive use. Descriptions of the questions used to ascertain fiber exposure are listed in Appendix 1. Fiber exposure was defined as exposure to asbestos, talc (which may contain asbestos), and fiberglass.

An attempt was made to estimate the overall effect of

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0090-8258/92 $1.50

"dose" or length of genital and respiratory fiber exposure. This was accomplished by adding the number of years of each type of genital or respiratory exposure from all sources. Since many of these exposures occurred at the same time, these variables should be considered as crude measures of dose rather than the true length of exposure.

Conditional logistic regression [10] was used to determine the strength of the association. Odds ratios were calculated to describe the relationship of genital and respiratory fiber exposure to ovarian cancer. The odds ratio is an estimate of the relative risk for relatively rare diseases such as ovarian cancer and, in this paper, is referred to as the relative risk estimate (RR).

The following variables were assessed as potential confounders:

Tobacco use
Number of cigarettes per day
Ovulatory time period
Number of pregnancies
Cancer in mother or father
Obesity 1 year prior to diagnosis
Obesity 20 years prior to diagnosis
Obesity at highest weight during the 20 years prior to diagnosis
Obesity according to average weight during the 20 years prior to diagnosis
Husband's and subject's education
Previous cancer
Marital status
Religion
Use of oral contraceptives
Use of contraceptive foams, creams, and jellies by themselves
Use of an IUD

On the basis of the strength of their relative risk estimates and the difference in frequency with which they were observed in cases and controls, the following variables were identified as potential confounders: measures of obesity, socioeconomic status (subject's education; RR = 0.5, 95% CI = 0.2–1.1), and religion (Jewish, RR = 2.9, 95% CI = 0.6–13.8; Catholic, RR = 0.5, 95% CI = 0.2–1.5), reproductive status (total live births, 1–2, RR = 0.7, 95% CI = 0.2–1.9; live births >2, RR = 0.4, 95% CI = 0.2–1.3), and oral contraceptive use (OR = 0.9, 95% CI = 0.3–23.0).

Obesity 1 year prior to diagnosis (RR = 2.3, 95% CI = 0.9–6.1), obesity 20 years prior to diagnosis (RR = 2.1, 95% CI = 0.7–6.9), and obesity according to highest weight during the 20 years prior to diagnosis (RR = 1.5, 95% CI = 0.7–3.2) were also found to be potential confounders. Obesity was defined as a height/weight index greater than that exhibited by women in the 85th percentile [11] of the population.

TABLE 1
Distribution of Matched Cases and Controls

	Cases		Controls	
	N	%	*N*	%
Age (years)				
Less than 30	3	3.9	2	4.4
30–39	1	1.3	1	2.2
40–49	11	14.3	8	17.4
50–59	21	27.3	10	21.7
60–69	35	45.4	21	45.6
70–79	5	6.5	3	6.5
80 or higher	1	1.3	1	2.2
Total	77		46	
Race				
White	70	90.9	41	89.1
Black	7	9.1	5	10.9
Total	77		46	

If a potential confounder changed the relative risk estimate associated with a fiber exposure by more than 15%, it was retained in the multivariate model. Confounders were sequentially added to multivariate models, depending on the level with which they changed the odds ratio of a fiber related exposure.

Interaction between fiber exposures and potential confounders was evaluated by comparing the deviance of models with and without the interaction term [12].

RESULTS

Age and Racial Distribution

Table 1 lists the age and racial distribution of cases and controls. Most cases and controls were in the age groups 40 to 69. It was difficult to obtain controls that did not have a chronic disease, resulting in a lower than expected number of controls.

Diagnoses of Controls

Controls were selected so that they did not have their primary diagnostic condition for more than 1 year. Table 2 lists the primary diagnosis of the controls included in the study.

Genital Fiber Exposure

Different sources of genital fiber exposure were examined (Table 3). Exposure from any of the potential sources of genital fiber was highly prevalent (91.1% in controls) and not found to be related to ovarian cancer (RR = 1.0, 95% CI = 0.2–4.0). Since it was felt that this index did not accurately characterize cumulative exposure, the median length of exposure from all genital sources (with the time since tubal ligation subtracted) was

TABLE 2
Distribution of Matched Controls by Primary Diagnosis

Diagnosis of restricted controls	N	%
Infectious and parasitic diseases	1	2.2
Diseases of the digestive system	6	13.0
Opthamologic disorders	15	32.6
Diseases of the circulatory system	11	23.9
Symptoms, signs, and ill-defined conditions	8	17.4
Diseases of musculoskeletal and connective tissue	1	2.2
Diseases of the endocrine system or metabolic or immunologic disorders	1	2.2
Diseases of the respiratory system	1	2.2
Benign neoplasms	1	2.2
Injury and poisoning	1	2.2
Total	46	100.00

used to categorize the dose of genital fiber exposure. A relative risk estimate of borderline significance was seen for exposure above the median length of time (37.4 years, RR = 2.4, 95% CI = 1.0–5.8). A significant negative association was observed for previous tubal ligation (RR = 0.2, 95% CI = 0.03–0.9).

A history of several gynecologic and abdominal operations, given by responses to the questionnaire, was examined to determine if exposure to talc from surgeon's gloves increased risk [13]. No statistically significant relationships were detected but, with the exception of ovarian biopsies, relative risk estimates were generally below 1. An attempt was made to combine information from the questionnaire and medical records, with no important changes in the relative risk estimates.

Moderately elevated relative risk estimates, which were not statistically significant, were observed with use of condoms, diaphragms (when powder was used), and genital bath talc. The level of association observed with exposure to talc on sanitary napkins (RR = 4.8, 95% CI = 1.3–18.0) was significantly greater than unity.

Respiratory Fiber Exposure

Numerous sources of respiratory fiber exposure were evaluated (Table 4): the only such source showing an important effect was occupational fiber exposure in relatives (RR = 2.8, 95% CI = 0.9–8.8). This relative risk estimate was calculated after three cases who were both exposed to asbestos themselves and received asbestos exposure from their relatives were excluded.

DISCUSSION

Genital talc exposure has been proposed as an etiologic agent of ovarian cancer [14] because talc was observed more frequently in cancerous ovaries [15] than in noncancerous ovaries, occupational studies of talc-containing asbestos showed an increased risk of lung cancer [16], asbestos has been observed in cosmetic face powders [17–19], and papillary growth has been observed after implantation of asbestos [20] and talc [21] into the peritoneal cavity.

We found an increased relative risk (4.8) for talc use on sanitary napkins with a smaller effect for genital bath talc exposure (RR = 1.7). This is in accordance with the original finding of a significant increased risk for perineal talc exposure (RR = 1.9, 95% CI = 1.3–2.9) by Cramer *et al.* [4]. Preliminary findings from a Chinese study also suggest that perineal application of talc-containing dusting powder increased the risk of epithelial ovarian cancer (RR = 3.9, 95% CI = 1.1–13.8) [5]. A nonsignificant effect for genital talc exposure (on genitals, sanitary napkins, or underwear) was detected in the study of Hartge *et al.* [6] (RR = 2.5, 95% CI = 0.7–10.0). Whittemore *et al.* [7] detected an increased risk (RR = 1.4, $P = 0.06$) for perineal exposure. In a study of borderline ovarian tumors, an increased risk was also observed with talc exposure from use on sanitary napkins (RR = 1.9, 95% CI = 0.9–6.9, 8).

We also investigated the relationship with cumulative duration of all forms of genital fiber exposure (with the time since tubal ligation subtracted). The positive association (RR = 7.35) in our study for exposure longer than the median length of time contrasts with the lack of association with duration observed by Whittemore *et al.* [7]. Whittemore *et al.* [7], however, did observe a positive dose–response relationship with frequency of exposure (1–20 times per month, RR = 1.3, 95% CI = 0.8–2.0; >20 times per month, RR = 1.4, 95% CI = 0.9–2.2). Although Booth *et al.*, [9] did not observe a significant ($P = 0.05$) trend of increasing risk with more frequent use, weekly genital talc use (RR = 2.0, 95% CI = 1.3–3.4) was associated with an increased risk of ovarian cancer.

The results of our study and others suggest that genital fiber exposure may be associated with an adverse effect [4–8] but further study is needed to determine if this relationship is causal in nature.

The present study also showed a significant negative association with tubal ligation (RR = 0.15). Harlow [22] (RR = 0.3, 95% CI = 0.3–1.1), Booth *et al.* [9] (RR = 0.2, 95% CI = 0.1–0.6), Mori *et al.* [23] (RR = 0.4, 95% CI = 0.2–1.0), Whittemore *et al.* [7] (RR = 0.6, $P = 0.07$), and Irwin *et al.* [24] (RR = 0.7, 95% CI = 0.5–1.0) also observed negative associations. Although the findings of our study differ from the positive association observed by Koch *et al.* [25] the comparison rates [26] used in Koch's cohort study may have been underestimated.

Tubal ligation may protect against ovarian cancer by inhibiting the carcinogenic action of talc through blockage

TABLE 3
Number and Frequency Distribution of Cases and Controls with Odds Ratios for Genital Fiber Exposure and Other Related Variables

Exposure interval	Attribute	Cases N	Cases %	Controls N	Controls %	Odds ratio	95% confidence interval
Genital fiber use	Yes	67	87.0	40	88.0	1.0	0.2–4.0[b]
	No	10	13.0	5	11.1		
	Missing	0		1			
Length of use of genital fiber[a] (years)	≥37.4	39	55.7	16	39.0	2.4	1.0–5.8[c]
	<37.4	31	44.3	25	61.0		
	Missing	7		5			
(Median of cases and controls)	Median		41.9		24.0		
Ovarian biopsies	Yes	6	7.8	3	6.5	1.1	0.3 4.4
	No	71	92.2	43	93.5		
Unilateral oophorectomy	Yes	8	10.4	5	10.9	0.8	0.2–2.5[d]
	No	69	89.6	41	89.1		
Tubal ligation	Yes	4	5.2	6	13.0	0.2	0.3–0.9[d,e]
	No	73	94.8	40	87.0		
Hysterectomy	Yes	19	24.7	12	26.1	0.7	0.3 1.7[d]
	No	58	75.3	34	73.9		
Condom use	Yes	35	49.3	22	51.2	1.6	0.6–3.9[b,d]
	No	37	50.7	21	48.8		
	Missing	5		3			
Diaphragm use with powder	Yes	14	18.9	5	11.4	3.0	0.8–10.8[b,d]
	No	60	81.1	39	88.6		
	Missing	3		2			
Genital bath talc	Yes	22	28.9	8	18.6	1.7	0.7–3.9
	No	54	71.0	35	81.4		
	Missing	1		3			
Sanitary napkin with talc exposure	Yes	21	30.0	6	13.6	4.8	1.3–17.8[f]
	No	49	70.0	38	86.4		
	Missing	7		2			

[a] After subtraction of the time since tubal ligation, for those who had ligation.
[b] Adjusted for number of live births.
[c] Adjusted for religion.
[d] Adjusted for years of education on subject.
[e] Adjusted for highest weight 20 years prior to diagnosis.
[f] Adjusted for highest weight 1 year prior to diagnosis.

of the fallopian tube or through a "screening" effect [27]. The effects of antecedent tubal ligation should be evaluated in future studies of ovarian cancer to determine if a negative association is consistently observed and to determine the reason for this.

Several cohort studies of women with respiratory exposure to asbestos detected an increased relative risk for ovarian cancer [1–3]. We elicited information about employment by relatives in occupations with asbestos or fiberglass exposure [28] and the relative risk was found to be elevated (RR = 2.8). In a previous case–control study of ovarian cancer, the relative risk for occupational asbestos exposure in relatives was not elevated [29], although the authors did not describe how the asbestos exposure was ascertained. Our initially suggestive findings regarding asbestos or fiberglass exposure in relatives should be evaluated further in additional studies.

In summary, our study shows that the development of ovarian cancer may be associated with genital fiber exposure (especially talc on sanitary napkins) and occupational exposure to fibers in relatives. Given its small sample size and the potential selection bias stemming from inclusion of patients from only one hospital, further research needs to be performed in order to confirm our findings.

APPENDIX 1

Questions Asked to Ascertain Fiber Exposures

Genital Fiber Exposure

1. Have you had any of the following operations prior to your hospitalization in ____?
   —Biopsy or removal of part of an ovary
   —Removal of one ovary
   —Removal of uterus (hysterectomy)

TABLE 4
Number and Frequency Distribution of Cases and Controls with Odds Ratios for Selected Characteristics Related to Respiratory Fiber Exposure

Exposure interval	Attribute	Cases N	Cases %	Controls N	Controls %	Odds ratio	95% confidence interval
Respiratory fiber exposure	Yes	69	89.6	41	89.1	1.3	0.3–3.6[a]
	No	8	10.4	5	10.4		
Cosmetic face powder use	Yes	38	50.7	24	54.5	1.1	0.4–2.7[b]
	No	37	49.3	20	45.4		
	Missing	2		2			
Insulation installed at residence	Yes	30	39.5	17	37.8	1.2	0.3 4.6[c]
	No	46	60.5	28	62.2		
	Missing	1		1			
Living in the vicinity of a fiber-emitting industrial establishment	Yes	7	9.2	4	8.9	1.0	0.3–3.4
	No	69	90.8	41	91.1		
	Missing	1		1			
Applied bath talc to body (respiratory exposure)	Yes	47	61.8	24	55.8	1.6	0.6–2.7[b]
	No	29	38.2	19	44.2		
	Missing	1		3			
Fiber exposure in relatives	Yes	18[d]	24.3	5	10.9	2.8	0.9–8.8
	No	56	75.7	41	89.1		
Use of spackling and taping compounds	Yes	20	29.0	14	31.1	1.6	0.5–3.4[e,f]
	No	49	71.0	31	68.9		
	Missing	8		1			

[a] Adjusted for highest weight 1 year prior to diagnosis.
[b] Adjusted for years of education on subject.
[c] Adjusted for number of live births.
[d] Three cases were excluded because they had been directly exposed to fibers and had a household member who had been occupationally exposed to fibers. Fiber exposure included asbestos, talc, or fiberglass exposure.
[e] Adjusted for religion.

—Tubal ligation
—Any other abdominal operation
(These items were confirmed by examination of medical records)

2. Did you use a diaphragm prior to your hospitalization in ____?

If yes: Did you use a powder to dust and dry the diaphragm?

If yes: Was it talc?

3. Did you and your sexual partner ever use a condom prior to your hospitalization in ____?

4. Have you regularly applied talcum powder to your body after bathing or for other reasons prior to your hospitalization in ____?

Did you commonly apply the talcum powder to your genital area?

5. Have you used talc on sanitary napkins or any other sanitary products used during your menstrual period prior to your hospitalization in ____?

Respiratory Fiber Exposure

1. Prior to your hospitalization in ____ had you ever regularly used cosmetic face powder?

2. Have you ever had insulation installed in a place that you lived in prior to your hospitalization in ____?

3. Have you ever lived in the vicinity of a shipyard, an asbestos or talc mine, or an asbestos, talc, or fiberglass processing plant prior to your hospitalization in ____?

4. Have you regularly applied talcum powder to your body after bathing or for other reasons prior to your hospitalization in ____?

Did you commonly apply the talcum powder to your face, upper torso, or legs and feet?

5. Have you or anyone who has ever lived in your household (including your husband) been employed in any of the following industries prior to your hospitalization in ____?

(a) Installation or removal of insulation materials
(b) Brake lining manufacture
(c) Automobile repair involving brake repair
(d) Roofing using asbestos materials
(e) Asbestos milling or mining
(f) Asbestos textile or paper manufacture
(g) Building construction
(h) Other industries where asbestos is used
(i) Talc mining and milling
(j) Other industries where talc is used
(k) Fiberglass or mineral wool manufacture
(l) Other industries where fiberglass or mineral wool was used

6. Did you ever use spackling and taping compounds?

If the respondent answered yes to any of these questions, they were asked when the exposure started and stopped. If they could not answer this question, they were asked how long the exposure occurred.

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AMERICAN JOURNAL OF EPIDEMIOLOGY
Vol. 128, No. 6
Printed in U.S.A.

PERSONAL AND ENVIRONMENTAL CHARACTERISTICS RELATED TO EPITHELIAL OVARIAN CANCER

II. EXPOSURES TO TALCUM POWDER, TOBACCO, ALCOHOL, AND COFFEE

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Vaginal exposures to talc and other particulates may play an etiologic role in epithelial ovarian cancer. Surgical sterilization may protect against ovarian cancer by blocking entry of such particulates into the peritoneal cavity. The authors assessed histories of talcum powder use, tubal sterilization, and hysterectomy with ovarian conservation in 188 women in the San Francisco Bay Area with epithelial ovarian cancers diagnosed in 1983–1985 and in 539 control women. To investigate the roles of blood-borne environmental exposures on ovarian cancer risk, they assessed lifetime consumption of coffee, tobacco, and alcohol in these women. Of the 539 controls, 280 were hospitalized women without overt cancer, and 259 were chosen from the general population by random digit telephone dialing. Ninety-seven (52%) of the cancer patients habitually used talcum powder on the perineum, compared with 247 (46%) of the controls. Adjusted for parity, the relative risk (RR) = 1.40, $p = 0.06$. There were no statistically significant trends with increasing frequency or duration of talc use, and patients did not differ from controls in use of talc on sanitary pads and/or contraceptive diaphragms. Fewer ovarian cancer patients (7%) than controls (13%) reported prior fallopian tube ligation (RR, adjusted for parity, = 0.56, $p = 0.06$), and fewer patients (20%) than controls (28%) reported prior hysterectomy (RR = 0.66, $p = 0.05$). The protective effect of hysterectomy was confined to those who underwent this surgery 10 or more years prior to interview and to those who had not undergone prior tubal sterilization. Consumption of cigarettes and alcohol did not differ between cases and controls. By contrast, 11 (6%) cases never regularly consumed coffee, compared with 31 (11%) hospital controls and 26 (10%) population controls (RR, adjusted for smoking, = 2.2, $p = 0.03$, for the comparison using all controls). Overall, ovarian cancer risk among women who had drunk coffee for more than 40 years was 3.4 times that of women who had never regularly consumed coffee ($p < 0.01$). However, the data exhibited no clear trends in risk with increasing consumption. Although risk ratios relating duration of coffee drinking to ovarian cancer were unaffected by adjustment for several characteristics, further study is needed to exclude potential confounding by other unmeasured characteristics.

alcohol drinking; coffee; environmental exposure; hysterectomy; ovarian neoplasms; talc; tobacco; sterilization, tubal

Received for publication May 29, 1987, and in final form April 11, 1988.

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Several investigators have hypothesized a carcinogenic role for exposures of the ovarian epithelium to environmental agents that enter the pelvic cavity through the vaginal canal (1–7). Attention has focused on hydrous magnesium silicates such as talc and asbestos because of the similarity between epithelial ovarian cancers and mesotheliomas, which are caused by exposure to asbestos. The hypothesis predicts that talcum powder use on the perineum, on sanitary napkins, and on contraceptive devices increases ovarian cancer risk. It also predicts that tubal sterilization and hysterectomy without bilateral oophorectomy protect against the disease by preventing environmental carcinogens from contacting the ovarian epithelium.

There are few data regarding these predicted consequences of the hypothesis. Cramer et al. (8) reported that ovarian cancer patients were significantly more likely than nonhospitalized control women to use talcum powder on the perineum or on sanitary napkins. However, Hartge et al. (9) found no statistically significant differences in prior talc use between cases and a series of hospital controls. A cohort study (10) of women who had undergone tubal ligation noted four ovarian cancers versus 1.45 expected, after 22,000 person-years of follow-up ($p = 0.06$). The four published case-control studies that have examined the effects of hysterectomy without bilateral oophorectomy found cases to have a lower prevalence of hysterectomy than controls, indicating that hysterectomy is associated with decreased risk of ovarian cancer (11–14). Weiss and Harlow (15) suggested that this association may be due to screening for malignant or premalignant ovarian conditions at hysterectomy by physicians who do not routinely remove the ovaries. According to this hypothesis, women who pass such screening would have reduced subsequent ovarian cancer risk when compared with women who were not subjected to hysterectomy.

Data relating ovarian cancer to consumption of coffee, tobacco, and alcohol also are sparse and conflicting. One case-control study has shown a statistically significant association between coffee consumption and increased risk of epithelial ovarian cancer (16). This study compared cases with hospitalized control women, whose current coffee consumption may not represent that of women in the general population. Four other studies using hospitalized controls (17–20) and one using nonhospitalized controls (21) found weak, nonsignificant positive associations between risk of this disease and amount of usual coffee consumption at interview. It is important to determine to what extent these conflicting findings may be due to differences in recent coffee consumption between hospitalized and population-based control groups or to other sources of bias.

Cigarette smoking has been associated with increased ovarian cancer risk in one prospective study (22) but was unassociated with it in several case-control studies (16, 17, 21). Indeed, the case-control studies have found small (nonsignificant) reductions in risk among smokers. Generally, such studies have found no relation between alcohol consumption and ovarian cancer, although a recent large study found a reduction in risk associated with heavy drinking (23).

We present the results of a case-control study of histologically verified epithelial ovarian carcinoma in which cases' prior histories of talc use, tubal ligation, hysterectomy without bilateral oophorectomy, and consumption of coffee, tobacco, and alcohol were compared with those of women from the general population, as well as with those of hospitalized control women. All subjects reported lifetime habits of talc use, coffee drinking, cigarette smoking, and alcohol consumption. Sum-

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This work was supported by NIH Grant CA 35067.

mary measures of lifetime exposures are evaluated in relation to ovarian cancer risk.

Materials and methods

Study subjects

Cases were residents of northern California aged 18 to 74 years who were diagnosed during the period January 1983 to December 1985 at one of the seven hospitals in Santa Clara County or at the University of California, San Francisco, Medical Center. The present report is restricted to 188 women with primary epithelial ovarian cancer.

Two groups of control women were selected. The first group consisted of women who were hospitalized in one of the hospitals to which cases were admitted. The second group was selected from the general population using random digit dialing telephone contacts. Both groups of women were matched to cases on age (within five-year intervals), race (white, black, oriental), and additional criteria described in the accompanying paper (24). A total of 188 ovarian cancer patients, 280 hospital controls, and 259 population-based controls participated.

Exposure data and statistical analysis

Study subjects participated in structured interviews in their homes conducted by trained interviewers to assess menstrual and reproductive history, medical and family history, and environmental exposures. Subjects were asked whether they had ever used talcum powder on the perineum, on sanitary pads, or on diaphragms. Subjects who responded affirmatively to any of these questions were asked about frequency and duration of use. Subjects also were asked whether they had ever drunk more than 10 cups of coffee in any one year. Subjects who responded affirmatively reported the ages when coffee drinking started and stopped, the total number of years of coffee drinking, and the number of cups usually consumed per day or per week either currently or prior to stopping. Similar questions were asked about cigarette smoking, provided that the subject had smoked at least 100 cigarettes during her life, and about consumption of alcoholic beverages, provided that she had ever consumed more than 10 such beverages in any one year.

Eligibility criteria, participation rates, and details of the statistical analysis are provided in the accompanying paper (24). Odds ratios are called relative risks, and all p values are two-tailed.

Results

Characteristics of the cases are compared with those of the two control groups in table 1. Population controls were somewhat younger, better educated, and more likely to be premenopausal than were cases and hospital controls. Furthermore, cases were less likely to have used oral contraceptives and had an earlier age at menarche and

Table 1

Characteristics of study participants, San Francisco Bay Area, 1983–1985

Characteristic	Cases (n = 188) (%)	Controls	
		Hospital (n = 280) (%)	Population (n = 259) (%)
Age (years)			
<40	10	10	15
40–49	30	29	29
50–59	26	28	27
60+	35	33	29
Race			
White	95	97	94
Education (years)			
>12	59	59	67
No. of term pregnancies*			
0	21	17	10
1–3	60	59	67
4+	19	24	23
Age (years) at menarche			
12 or less	48	44	46
Menopausal status			
Premenopausal	34	32	41
Natural menopause	45	33	38
Surgical menopause	22	35	21
Oral contraceptives			
Ever used	46	50	58

* 20 or more weeks gestation.

fewer term pregnancies than either control group. The latter findings are reported in detail in the companion paper (24).

Relative risks associated with talc use, tubal ligation, and hysterectomy were similar when cases were compared with hospital controls and with population controls. Therefore, for these variables, we report results only for cases versus the combined group of all controls.

Talc use

A greater proportion of cases (52 per cent) than controls (46 per cent) reported prior use of talcum powder on the perineum (relative risk (RR) = 1.40, $p = 0.06$). However, there was little difference between cases and controls in use of talc on sanitary pads and diaphragms, with relative risks of 0.93 ($p = 0.76$) and 0.95 ($p = 0.86$), respectively. Table 2 shows the distributions of cases and controls and relative risks for talc use directly on the perineum, on sanitary pads, and on contraceptive diaphragms, singly and in combination. The table provides no evidence of elevated risk associated with more than one form of talc use. None of the women in the study reported prior occupational exposures to talc or asbestos fibers.

We next examined whether risk increased with increased duration or frequency of use of talcum powder on the perineum. Since tubal ligation and hysterectomy prevent contact between the ovarian epithelium and exogenous agents in the vaginal canal, we excluded any perineal talc use after the date of tubal ligation or hysterectomy in calculating duration of use. As seen in table 3, 55 per cent of cases versus 59 per cent of controls reported such use for less than one year. The risk for talc use between one and nine years, relative to that among users of shorter duration, was 1.60 ($p = 0.05$). However, an increasing dose-response pattern was not apparent, with risk among long-term users of 10 or more years only 1.11 times that of the nonusers or users of less than one year ($p = 0.61$). According to the logistic model fit to the data, the overall increase in risk for any 10-year increase in duration of use was 1.01 ($p = 0.56$).

TABLE 3

Ovarian cancer risk by length of talcum powder use on the perineum, San Francisco Bay Area, 1983–1985*

Years of talc use*	Cases		Controls		Relative risk†	95% confidence interval
	n	%	*n*	%		
None	103	55	320	59	1.00	
1–9	34	18	72	13	1.60	1.00–2.57
10+	50	27	147	27	1.11	0.74–1.65
Unknown	1	1	0	0		
Total	188	100	539	100		

* Prior to tubal ligation or hysterectomy.
† Adjusted for parity.

TABLE 2

Ovarian cancer risk by type of talcum powder use, San Francisco Bay Area, 1983–1985

Type of talc use	Cases		Controls		Relative risk*	95% confidence interval
	n	%	*n*	%		
None	75	40	230	43	1.00	
Perineum only	22	12	55	10	1.45	0.81–2.60
Sanitary pads only	5	3	28	5	0.62	0.21–1.80
Diaphragm only	9	5	19	4	1.50	0.63–3.58
Any two of perineum, pads, and diaphragm	67	36	168	31	1.36	0.91–2.04
All three of perineum, pads, and diaphragm	1	1	9	2	0.35	0.04–2.94
Incomplete data	9	5	30	6		
Total	188	100	539	100		

* Adjusted for parity and oral contraceptive use.

The relation between ovarian cancer risk and usual frequency of talc use on the perineum is shown in table 4. Women who used talc an average of one to 20 times per month were not at significantly altered risk from those who used it less frequently (RR = 1.27, $p = 0.29$). Those who customarily used talc on the perineum 20 or more times per month were at 1.45 times the risk of women in the lowest use category ($p = 0.09$). The overall increase in risk associated with 30 applications per month was 1.30 ($p = 0.19$).

Tubal ligation and hysterectomy

To investigate further the hypothesis of a role for vaginal exposure to environmental carcinogens in the etiology of ovarian cancer, we examined the effect of tubal ligation and hysterectomy on risk for the disease. Seven per cent of cases and 13 per cent of controls reported a history of tubal ligation (RR = 0.53, $p = 0.05$). Relative risks for tubal ligation were less than one among nulliparous, uniparous, and multiparous women, indicating that the protective effect of such surgery on ovarian cancer risk cannot be explained by confounding due to its greater prevalence among parous women who are at reduced risk of the disease. The overall reduction in risk associated with tubal ligation, adjusted for parity, was 0.56 ($p = 0.07$).

Cases with tubal ligation tended to undergo this surgery at younger ages (mean age (± standard error) = 31.9 years (±0.5)) than did controls (34.2 years (±0.1)). This difference does not support the hypothesis that early tubal ligation confers greater protection to the ovaries by early termination of exposure from the vaginal canal. Relative to women without this surgery, the risk for women with tubal ligation within 10 years of interview was 0.35 (95 per cent confidence interval (CI) 0.12–1.02), while the risk for women who underwent the surgery more than 10 years before interview was 0.69 (95 per cent CI 0.32–1.50).

Table 5 shows that hysterectomized women experienced 0.66 times the risk of those without such surgery ($p = 0.05$), but it does not show any trend of decreasing protection with time since hysterectomy. Such a trend would be expected if the protective effect of hysterectomy reflected merely the removal of high-risk women from the hysterectomized population via selective oophorectomy as suggested by Weiss and Harlow (15). On the contrary, table 5 shows that protection is limited to women hysterectomized 10 or more years prior to interview. Overall, cases and controls did not differ significantly in the ages at which hysterectomy was performed. The mean age at hysterectomy among cases with such prior surgery was 40.1 years (± 0.3), while the corresponding age for controls was 39.7 years (±0.1).

The hypothesis that hysterectomy protects against ovarian cancer by blocking

TABLE 4

Ovarian cancer risk by usual frequency of talcum powder use on the perineum, San Francisco Bay Area, 1983–1985*

Applications of talc per month	Cases		Controls		Relative risk*	95% confidence interval
	n	%	*n*	%		
None	97	52	312	58	1.00	
1–20	41	22	114	21	1.27	0.82–1.96
20+	44	23	101	19	1.45	0.94–2.22
Unknown	6	3	12	2		
Total	188	100	539	100		
Overall trend for 30 uses per month					1.30	0.88–1.92

* Adjusted for parity.

exogenous agents' access to the ovaries predicts that such surgery confers no benefit on women whose ovaries are already protected by prior tubal ligation. Table 5 shows relative risks associated with hysterectomy among women with and without prior tubal ligation. As predicted, hysterectomy failed to protect women who had undergone prior tubal ligation (RR = 2.56, $p = 0.45$), but it did protect those who had not (RR = 0.57, $p = 0.02$).

Table 6 shows the effects of perineal talc use separately among women with and without prior tubal ligation or hysterectomy. The highest risk was experienced by talc users without such surgery. The risk in

TABLE 5

Ovarian cancer risk after hysterectomy without bilateral oophorectomy, by time between hysterectomy and interview and by absence or presence of prior tubal ligation, San Francisco Bay Area, 1983–1985

	Cases		Controls		Relative risk	95% confidence interval
	n	%	*n*	%		
No hysterectomy	151	80	389	72	1.00	
Hysterectomy	37	20	150*	28	0.66	0.43–1.00
Time (years) between hysterectomy and interview						
1–9	15	8	42	8	1.01	0.54–1.89
10–19	11	6	66	12	0.47	0.24–0.92
20+	11	6	41	8	0.63	0.31–1.29
No prior tubal ligation						
No hysterectomy	141	81†	333	71†	1.00	
Hysterectomy	33	19	135	29	0.57	0.36–0.90
Prior tubal ligation						
No hysterectomy	10	71‡	56	79‡	1.00	
Hysterectomy	4	29	15	21	2.56	0.23–29.12

* Date of hysterectomy was unknown for one woman.
† Per cent of cases or controls with no prior tubal ligation.
‡ Per cent of cases or controls with prior tubal ligation.

TABLE 6

Ovarian cancer risk by perineal talc use and by history of surgical sterilization,† San Francisco Bay Area, 1983–1985

Talc use	Surgical sterilization†	Cases		Controls		Relative risk‡	95% confidence interval
		n	%	*n*	%		
No	No	70	37	182	34	1.00	
Yes	No	71	38	151	28	1.33	0.88–2.01
No	Yes	21	11	110	20	0.50*	0.29–0.88
Yes	Yes	26	14	96	18	0.75	0.43–1.29
No	Total	91	48	292	54	1.00	
Yes	Total	97	52	247	46	1.37§	0.97–1.95
Total	No	141	75	333	62	1.00	
Total	Yes	47	25	206	38	0.53*‖	0.36–0.79

* $p < 0.01$.
† Tubal ligation or hysterectomy.
‡ Adjusted for parity.
§ Adjusted for parity and surgical sterilization.
‖ Adjusted for parity and talc use.

this group was 1.33 times that of women with neither history of talc use nor history of surgery ($p = 0.18$). By contrast, risk was lowest among women with a history of tubal ligation or hysterectomy who never regularly applied talc to the perineum (RR = 0.50, $p = 0.02$).

Table 6 shows that, regardless of talc use, women who underwent either tubal ligation or hysterectomy without bilateral oophorectomy experienced a risk of 0.53 ($p = 0.002$) compared with women without such surgery.

Cases and controls did not differ significantly in the prevalence of barrier contraceptive use, devices that prevent entry of semen into the peritoneal cavity. Risks for women who had used diaphragms and condoms were 0.81 ($p = 0.22$) and 0.91 ($p = 0.59$), respectively, relative to risk among nonusers. There was no trend in risk with exposure of the ovaries to semen, defined as sexually active time before tubal ligation or hysterectomy, minus duration of use of condoms and diaphragms.

Coffee, tobacco, and alcohol

Table 7 shows the distribution of cases and controls and relative risks by coffee consumption status (ever vs. never). The relative risk for any coffee consumption, adjusted for cigarette smoking, was 2.21 for both control groups combined ($p = 0.03$). The relative risk was 2.13 ($p = 0.06$) for cases versus hospital controls and 1.59 ($p = 0.24$) for cases versus population controls. The corresponding unadjusted relative risks were 2.03 for combined controls, 1.90 for hospital controls, and 1.51 for population controls (not shown).

Table 7 also shows that risk among coffee drinkers increases with increasing duration of coffee consumption. This trend is evident in both the comparison based on hospital controls and the one based on population controls. Overall, smoking-adjusted cancer rates among women who had consumed coffee for more than 40 years were 3.4 times those of women who had never consumed coffee ($p < 0.01$). Among coffee drinkers, each additional 10 years of coffee drinking conferred an 11 per cent increase in risk, according to the logistic function fit to the data ($p = 0.37$). These findings could be confounded by age despite the matching within five-year age groups. However, the results were similar when age was added to the regressions as a continuous variable.

Relations between ovarian cancer risk and amount of usual coffee consumption are shown in table 8. The table provides no evidence for a positive trend in risk with

TABLE 7

Ovarian cancer risk by years of coffee consumption, San Francisco Bay Area, 1983–1985

Years of coffee consumption	Cases		Controls				Relative risk†			95% confidence interval‡
			Hospital		Population		Cases vs. hospital controls	Cases vs. population controls	Cases vs. all controls	
	n	%	*n*	%	*n*	%				
None	11	6	31	11	26	10	1.00	1.00	1.00	
Any	177	94	249	89	233	90	2.13	1.59	2.21	1.10–4.41
1–14	18	10	27	10	34	13	1.57	0.65	1.45	0.59–3.57
15–24	32	17	43	15	36	14	1.81	1.69	2.18	1.00–4.79
25–39	62	33	105	38	98	38	2.36	1.70	2.26	1.06–4.85
40+	65	35	73	26	64	25	3.45*	2.54	3.41*	1.46–7.96
Unspecified	0	0	1	0	1	0				
Overall trend per 10 years among coffee drinkers							1.16	1.14	1.11	0.89–1.38

* $p < 0.01$.

† Adjusted for smoking (lifelong nonsmoker vs. ever smoker).

‡ Cases versus all controls.

frequency of coffee drinking, regardless of the control group used for comparison. Frequency was measured as usual number of cups consumed per day prior to disease onset for cases and hospital controls who were current coffee drinkers, and as usual number of cups per day prior to stopping for former coffee drinkers. The test for trend of increasing risk with increasing frequency among those who consumed coffee was not significant ($p = 0.91$).

Similarly, table 9 shows no trend in risk with total cups of coffee consumed prior to disease onset (cases and hospital controls) or prior to interview (population controls). We estimated total coffee consumption by multiplying each woman's reported duration of coffee consumption in years by her usual frequency of consumption in cups per day times 365. Among coffee drinkers, the test for trend in risk with increasing total consumption yielded a p value of 0.56.

Relative risks corresponding to the above measures of coffee consumption were unchanged by adjustment for other variables potentially associated with ovarian cancer including educational level, parity, oral contraceptive use, estimated years of ovu-

TABLE 8

Ovarian cancer risk by usual frequency of coffee consumption. San Francisco Bay Area, 1983–1985*

Cups/day	Cases		Controls				Relative risk†			95% confidence interval‡
			Hospital		Population		Cases vs. hospital controls	Cases vs. population controls	Cases vs. all controls	
	n	%	*n*	%	*n*	%				
0	11	6	31	11	26	10	1.00	1.00	1.00	
1	50	27	62	22	58	22	2.21	1.86	2.42	1.15–5.09
2–3	73	39	94	34	98	38	2.13	1.63	2.26	1.09–4.66
4+	54	29	93	33	77	30	2.02	1.56	2.07	0.97–4.38
Overall trend per cup/day among coffee drinkers							1.01	1.01	1.01	0.93–1.08

* Prior to stopping for ex-drinkers, and prior to hospitalization for current drinkers, among cases and hospital controls.

† Adjusted for smoking (lifelong nonsmoker vs. ever smoker).

‡ Cases versus all controls.

TABLE 9

Ovarian cancer risk by estimated total cups of coffee consumed, San Francisco Bay Area, 1983–1985

Cup-years* of coffee consumption	Cases		Controls				Relative risk†			95% confidence interval‡
			Hospital		Population		Cases vs. hospital controls	Cases vs. population controls	Cases vs. all controls	
	n	%	*n*	%	*n*	%				
0	11	6	31	11	26	10	1.00	1.00	1.00	
1–30	41	22	50	18	60	23	2.30	1.54	2.30	1.09–4.86
31–60	32	17	46	16	32	12	2.21	2.30	2.64	1.21–5.75
61–90	27	14	38	14	32	12	2.03	1.96	2.46	1.10–5.51
90+	77	41	114	41	108	42	2.42	1.60	2.28	1.08–4.78
Unknown	0	0	1	0	1	0				
Overall trend per 10 cup-years among coffee drinkers							1.01	1.01	1.01	0.99–1.03

* One cup-year equals 365 cups of coffee.

† Adjusted for smoking (lifelong nonsmoker vs. ever smoker).

‡ Cases versus all controls.

lation, and duration of contraceptive-free marriage.

The risk for cigarette smoking relative to lifelong nonsmoking, adjusted for coffee consumption, was 0.73 ($p = 0.08$) for the comparison based on combined control groups. Corresponding relative risks for the hospital and population comparisons were 0.65 ($p = 0.04$) and 0.84 ($p = 0.39$), respectively. Unadjusted relative risks and confidence intervals were similar. While the odds ratios associated with smoking were less than unity, few of them achieved statistical significance.

Cases did not differ from either control group in the prevalence of prior alcohol consumption. For the comparison based on all controls, the relative risk was 0.74 ($p = 0.14$). Furthermore, there was no evidence of a trend in risk with increasing duration or amount of alcohol consumption. Women who drank heavily (20 or more drinks per week) had a risk 0.66 times that of nondrinkers, but the reduction was not statistically significant ($p = 0.34$). None of these observations were altered by adjustment for cigarette smoking or coffee consumption.

Discussion

Genital exposures to talc and other agents

The rationale for suspecting talc as an ovarian carcinogen derives from its chemical relation to and natural occurrence with asbestos. Asbestos causes pleural and peritoneal mesotheliomas (25), which are histologically similar to epithelial ovarian carcinomas (6). Graham and Graham (2) showed that, in guinea pigs and rabbits, asbestos can induce ovarian epithelial hyperplasia similar to early epithelial tumors in women. Evidence suggesting a role for vaginal exposure to particulates in human ovarian carcinogenesis is twofold. First, Egli et al. (26) demonstrated that nonmotile inert carbon particles deposited in the vagina prior to hysterectomy can be recovered in the fallopian tubes. Second, Henderson and coworkers have found talc particles embedded in both normal and malignant ovarian tissue (27, 28). While these findings indicate that vaginal exposure to particulates can lead to deposition on the ovaries, they do not implicate such exposure in ovarian carcinogenesis, and data relating directly to this possibility are needed.

In a comparison of epithelial ovarian cancer cases and nonhospitalized controls in Boston, Cramer et al. (8) reported a relative risk of 1.92 ($p < 0.003$) for epithelial ovarian cancer associated with use of talcum powder on the perineum or on sanitary pads. By contrast, the results of a case-control study in Washington, DC (9) and those of the present study show neither a strong nor a consistent association between genital talcum powder exposure and ovarian cancer. In the present data, regular use of talc on the perineum was associated with only a marginally significant elevation in relative risk. Furthermore, there were no clear differences between cases and controls when other forms of genital talc exposure were considered, either singly or in combination. Although the data show a trend of increasing risk with increasing frequency of perineal exposure, the trend is not statistically significant, and there is no trend with duration of exposure. Thus, while these data do not exonerate talc as an ovarian carcinogen, neither do they provide strong evidence to implicate it.

Several sources of bias must be considered as possible explanations for the lack of strong findings related to talc, including the study's failure to interview all eligible ovarian cancer patients and a completely random sample of controls, as well as the potential pitfalls of combining the two control groups. Another source of bias is confounding by differential talc use among women with characteristics predictive of ovarian cancer. However, such confounding seems unlikely. For example, although Wynder et al. (14) reported that certain menstrual characteristics differ between women with ovarian cancer and controls,

we and others (8, 13) found no significant differences between cases and controls in any of several menstrual characteristics examined, including history of amenorrhea, irregular menstrual cycles, and midcycle pain.

An additional source of bias is random error in reported talc use, which tends to attenuate relative risk estimates. Some error seems likely. Nevertheless, there seems only a small probability that these data contain reporting errors large enough to obscure the twofold increase in risk noted by Cramer et al. (8) for talc use on the perineum and on sanitary pads. Further epidemiologic studies are needed to clarify the role of talc as carcinogen, cocarcinogen, or promoter of epithelial ovarian carcinogenesis.

Indirect evidence in support of an etiologic role for vaginal exposures to some exogenous substances derives from the reduced risk associated here with fallopian tube sterilization and hysterectomy, procedures that block entry to the pelvic area through the reproductive tract. Apart from talc and asbestos, vaginally introduced substances that may initiate or promote ovarian cancer include other particulates, semen, spermicidal foams or creams, and douche solutions. We are unaware of data implicating any of these substances.

The reduced risk associated in these data with prior tubal sterilization is inconsistent with the results of a historical prospective study of 666 women who had undergone tubal ligation (10). These women experienced a slight, marginally significant increase in ovarian cancer risk. However, the study involved only four cases of ovarian cancer.

Women tend to undergo tubal sterilization during the height of their reproductive years (on average, in the early fourth decade of life, for the present data). By contrast, hysterectomy is generally performed at the end of the reproductive years, probably after a large fraction of genital exposures have already occurred. It is therefore noteworthy that the protective effects of hysterectomy noted here were limited to surgery 10 or more years prior to interview. This finding fails to support the conjecture of Weiss and Harlow (15) that the protection of hysterectomy is an artifact due to selective removal of precancerous ovaries at the time of surgery. If such selection explained the association, one would expect to find, as those authors did, a decrease in the level of protection with increasing years since surgery. However, the present data show just the opposite trend. They also indicate that the benefits of hysterectomy are confined to women without prior tubal sterilization, a finding that supports an etiologic role for genital exposures to the ovaries.

Other explanations are possible for the protective effects of tubal ligation and hysterectomy, if the effects are not due to chance or bias. For example, these procedures may alter the levels and/or the cyclic variations of estrogen, progesterone, or the gonadotropins. Tubal sterilization has been shown to reduce subsequent serum and urinary estrogen levels, possibly by inducing localized hypertension at the ovary (29, 30). Some data (30, 31) suggest that hysterectomy with ovarian conservation also may reduce estrogen production. Elevated estrogen levels have been linked to breast cancer and could be involved in ovarian carcinogenesis, although there are few data to support this possibility (32, 33). Tubal sterilization and hysterectomy may protect by reducing ovarian estrogen exposure. Alternatively, some forms of tubal sterilization have been associated with subsequent increased frequency of abnormal or anovulatory cycles (34, 35). Since estimated number of ovulations has been associated with increased ovarian cancer risk (36, 37), tubal sterilization may protect by suppressing ovulation.

Coffee, tobacco, and alcohol

The present data provide some support for the hypothesis that coffee drinking in-

creases risk for epithelial ovarian cancer. Comparison of cases versus the combined group of both hospitalized and population-based controls suggests that current or former coffee drinkers experience double the risk of nondrinkers. The relative risk based on comparison of cases versus only the population-based controls was smaller (RR = 1.59) and did not achieve statistical significance. Nevertheless, its magnitude suggests that coffee may be implicated in increased risk for the disease.

The hypothesis is also supported by the dose-response relation noted between risk and duration of coffee drinking among those who had ever drunk coffee. Compared with risks for nondrinkers, smoking-adjusted risks increased from 1.45 for fewer than 15 years of coffee drinking to 3.41 for 40 or more years of consumption. Trends of increasing risk with increasing years of coffee drinking were evident in both hospital and population-based control comparisons and in both smoking-adjusted and unadjusted analyses.

Yet, no trends were evident with increasing amount of usual coffee consumption in cups per day, or with total lifetime consumption, estimated by multiplying reported years of consumption by reported amount of usual consumption prior to illness or cessation of coffee drinking. Instead, risk remained approximately constant at two to three times that of nondrinkers, regardless of amount consumed. This lack of dose response is consistent with the negative findings of other studies (17–20) for trend in risk with increasing coffee consumption at time of interview. The absence of trend noted here may be due to inaccuracies in reported usual consumption as a measure of average coffee drinking frequency. Such inaccuracies (if similar in magnitude between cases and controls) could mask evidence of a dose-response relation. The observed patterns of trend with duration of coffee drinking and lack of trend with frequency of consumption and total consumption would be consistent with a causal relation if women tended to report their duration of coffee drinking more accurately than they reported the amount they usually consumed.

The present observation of increased ovarian cancer risk among coffee drinkers could be due to several sources of bias. The first possibility is differences between cases and controls in some measured or unmeasured characteristics that are correlated with coffee drinking. Body size is an unlikely source of such confounding, because cases and controls were similar in several assessments of weight, height, and weight for height, as related to body size both at age 20 and in recent years. Relative risks for coffee consumption were not altered in multivariate analyses with other variables found to be predictive of ovarian cancer. These include parity, oral contraceptive use, and duration of contraceptive-free marriage.

Potential confounding by dietary factors must also be considered. Cramer et al. (21) found a statistically significant trend of increasing risk with increasing consumption of animal fat, after adjusting for body weight and parity. Absence of data on dietary fat consumption in the present study precludes examination of potential confounding by this factor.

Second, the stronger association noted when cases were compared with hospital controls could be explained by reduced coffee consumption among these controls after the onset of subclinical disease manifestations. However, a statistically significant trend in risk with years of coffee drinking was also noted when cases were compared with population controls. Furthermore, the two sets of controls had similar prevalences of prior regular coffee drinking. Therefore, the possibility of selection bias among hospital controls seems unlikely to explain the observations.

Third, cases may have overreported their coffee consumption relative to controls. However, this explanation also seems unlikely, in view of the absence of evidence

for such reporting bias in cases' reports of tobacco and alcohol consumption.

To date, seven case-control studies have examined the relation between coffee and ovarian cancer (16–21, and the present study). Of these, two (the present study and one in Italy (16)) found statistically significant elevations of risk among coffee drinkers, with relative risks ranging from 1.5 to 2.0. The remaining five studies, one in Greece (17) and four in the United States (18–21), found slightly and nonsignificantly elevated risks associated with coffee consumption. These disparities are not easily explained by differences in coffee constituents or in women among the different study areas.

It is difficult to propose biologically plausible causal mechanisms to explain the positive associations, if the associations are not due to bias or chance. Coffee consumption has been shown to increase urinary excretion of catecholamines (38–41), suggesting that coffee increases the activity of the adrenal medulla. Coffee consumption also may increase adrenal production of androstenedione. Since peripheral aromatization of androstenedione to estrone forms the primary source of estrogen in postmenopausal women (41), coffee consumption may alter ovarian cancer risk by increasing estrogen production after the menopause. Such a mechanism is speculative, however, in view of the sparsity of data implicating estrogens in ovarian carcinogenesis (32, 33).

Cigarette smoking was associated nonsignificantly with reduced ovarian cancer risk. This finding agrees with those of other case-control studies (16, 17, 21) but disagrees with an increased ovarian cancer risk found in a prospective study of women who smoke (22). The present data show no relation between ovarian cancer risk and duration of cigarette smoking.

The data also provide no evidence for any relation between alcohol consumption and ovarian cancer risk, which is consistent with results of other studies that have examined this issue (17, 20, 21). One large study (23) found that women who consumed 20 or more drinks per week had half the risk of women who did not drink. We also found reduced risk associated with such heavy drinking, but the association did not achieve statistical significance. The lack of significance may be due to small numbers in the present study; thus, the relation of alcohol consumption to ovarian cancer should be examined in larger series.

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American Journal of
EPIDEMIOLOGY

Volume 136
Number 10
November 15, 1992

Sponsored by the Society for Epidemiologic Research

ORIGINAL CONTRIBUTIONS

Characteristics Relating to Ovarian Cancer Risk: Collaborative Analysis of 12 US Case-Control Studies

I. Methods

Alice S. Whittemore,[1] Robin Harris,[1] Jacqueline Itnyre,[1] Jerry Halpern,[2] and the Collaborative Ovarian Cancer Group[3]

Data from 12 US case-control studies of ovarian cancer, conducted during the period 1956–1986 and representing some 3,000 cases and 10,000 controls, were pooled and reanalyzed. Separate analyses were conducted for four subgroups of the pooled data: invasive epithelial ovarian cancers in white women; epithelial ovarian cancers of low malignant potential in white women; epithelial ovarian cancers in black women; and nonepithelial ovarian cancers. This paper gives a brief description of the participating studies and describes the methods used in the collaborative analysis. *Am J Epidemiol* 1992;136:1175–83.

case-control studies; methods; ovarian neoplasms

This is the first in a series of articles describing a collaborative combined analysis of data from 12 case-control studies of ovarian cancer conducted in the United States

Received for publication August 21, 1991, and in final form July 23, 1992.

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TABLE 1. **Case-control studies of epithelial ovarian cancer among US white women**

Authors (reference no.)	Cases				Controls	
	Invasive	Low malignant potential	Year of diagnosis	Place of diagnosis	No.*	Source
Byers et al. (1)	196		1956–1963	Buffalo, NY	795	Case hospitals
Hildreth et al. (2)	59	3	1976–1979	Connecticut	1,008	Case hospitals
McGowan et al. (3)	133	33	1974–1977	Washington, DC	165	Case hospitals
Wu et al. (4)	111		1975–1977	San Francisco Bay Area, CA	482	Case hospitals
Rosenberg et al. (5)	115	8	1976–1980	Eastern US cities†	486	Case hospitals
Hartge et al. (6)	220	41	1978–1981	Washington, DC	288	Case hospitals
Casagrande et al. (7)	133		1973–1976	Los Angeles, CA	134	Case neighborhoods
Cramer et al. (8)	177	41	1978–1981	Boston, MA	229	Town directories
Nasca et al. (9)	314	27	1977–1980	New York State	694	Motor vehicle files
Weiss et al. (10)	269	23	1975–1979	Utah, Washington	700	Household and RDD‡ phone surveys
CASH‡ group (11)	303	107	1980–1982	8 SEER‡ areas§	3,542	RDD phone surveys
Whittemore et al. (12)	167	44	1983–1986	San Francisco Bay Area, CA	310	Group 1: RDD phone surveys Group 2: case hospitals
Total	2,197	327			8,893	

* Number included in the present analysis.
† Including Boston, Massachusetts; New York, New York; Philadelphia, Pennsylvania; and Baltimore, Maryland.
‡ RDD, random digit dialing; CASH, Cancer and Steroid Hormone Study; SEER, Surveillance, Epidemiology, and End Results program.
§ Atlanta, Georgia; Detroit, Michigan; Seattle, Washington; San Francisco Bay Area, California; Iowa; Connecticut; New Mexico; and Utah.

women who had or might have had a bilateral oophorectomy. Table 1 shows the numbers of white women with epithelial ovarian cancer (classified by tumor behavior) and white control women included in the analysis.

ANALYSIS

Analysis involved the following tasks: 1) constructing the basic variables (e.g., race and subtype of disease) needed to organize the data into subsets for separate analysis; 2) choosing the major hypotheses to be tested; 3) defining the variables needed to test these hypotheses; 4) reviewing the individual questionnaires and code books to determine the information available on the variables of interest; 5) using this information to identify a list of "working variables" to be used in all analyses; 6) identifying the positions on each of the data tapes of the information needed to assemble each variable and writing a separate program to extract this information from each tape; 7) preparing descriptive statistics for each variable and each study in search of outlying observations and outlying studies; 8) merging the extracted data into a composite file containing data for all variables and all studies; 9) extracting subsets of data for separate analyses; 10) assembling a list of regression models for each variable; 11) conducting study-specific and combined regression analyses; 12) documenting and cataloging output; and 13) discussing and interpreting results and planning further follow-up regressions. These steps are discussed below.

Data organization

We first copied to an IBM 3090 mainframe computer (IBM, Poughkeepsie, NY) original data files from the 12 studies. This represented data for some 13,600 subjects and comprised 3,900 variables, ranging from

amples of working variables include a woman's total number of term pregnancies, defined as pregnancies of at least 20 weeks gestation and coded as 0, 1, ..., 5, ≥6, and her total number of failed pregnancies, defined as miscarriages, induced abortions, ectopic pregnancies, and stillbirths, and coded similarly.

Once a working variable was identified, the individual questionnaires and code books were reviewed to determine a common definition and coding scheme. This sometimes involved difficult trade-offs between coding detail and study inclusion in order to obtain data from as many studies in as much detail as possible. For example, we defined failed pregnancies to include stillbirths (which also are term pregnancies) in order to use data from a study that had aggregated stillbirths with the other types of pregnancy failure. When the convention of using the coarsest definition resulted in unacceptable loss of specificity or detail for too many studies, we conducted additional, more detailed analyses using only those studies with appropriate data.

To produce the working variables, separate programs were written to extract the data from the individual study files. For complex variables, such as menopausal status or estimated age at last ovulation, flow charts (e.g., figure 1) helped to ensure that the different studies contributed comparable information. Once created, the working variables were edited for subtle interstudy incompatibilities. Descriptive statistics were used to identify outliers and other problems. Some of these were resolved locally; others required discussion with the collaborating investigators. Some investigators occasionally had to review their original data to confirm or recode questionable values or to provide further detail. More than 90 working variables were constructed.

Data processing and management

Once created and edited, the working variables were used to derive categorical and other variables for regressions. When analyzing a topic, we included only those studies that had data for all variables occurring in all relevant regressions. Further, women with unknown values of a variable were deleted from all regressions containing that variable. Thus, total case and control numbers vary across regressions, and numbers presented in the following parts vary across tables.

Statistical analysis

Several pitfalls may result from pooling data from separate studies with differing protocols and differing exposure prevalence. The variable "study" could represent both a strong confounding factor and an effect modifier. It is a potential confounder because both the case:control ratio and the prevalence for a particular exposure may vary from study to study. It is a potential effect modifier because odds ratios may vary from one study population to another. Therefore, an analysis that pools data across studies could yield seriously misleading results.

We used several strategies to address these pitfalls. First, we stratified all analyses jointly by study and reference age (<25, 25–29, ..., 75–79, and ≥80 years). Studies 10 and 11 were further stratified by their constituent study centers. We used conditional logistic regression (22), implemented on EGRET software (23), to estimate odds ratios and calculate (two-tailed) significance levels and confidence intervals. This approach has two advantages over an unconditional analysis containing dummy variables for the studies, the age groups, and their interactions: It avoids unwieldy output of regression coefficients for the resulting 200-plus age-study variables which are not of primary interest, and it allows inspection of numbers of subjects who failed to contribute to a given analysis because their age-study stratum lacked cases or controls or discordant exposures. However, either conditional or stratified unconditional regression could be used (22); we found that the two produced nearly identical estimates for odds ratios and their standard errors when performed on the same data. The standard errors and confidence intervals produced by these regressions do not reflect variance because of interstudy

one for parous women and zero for nulliparous women), while the expanded model contained PAR and five additional dummy variables of the form PAR × STUDY j, $j = 1, \ldots, 5$, where STUDY j is coded one if a woman participated in study j and 0 otherwise. The coefficient for PAR × STUDY j gives the proportional amount that the odds ratio for parity in study j differs from that of study 6, an arbitrarily chosen referent study. An overall test of the null hypothesis of no study heterogeneity is provided by the likelihood ratio statistic based on the maximized log-likelihoods of small and expanded models. Under the null hypothesis, this statistic has approximately a chi-squared distribution with degrees of freedom equal to one less than the number of studies being examined (22).

In addition to examining heterogeneity across studies of odds ratios for all major variables associated with risk of invasive epithelial ovarian cancer, we also evaluated such odds ratio heterogeneity with respect to parity, history of oral contraceptive use, and 10-year strata of reference age. Other effect modification was evaluated when motivated by a specific biological mechanism suggested by one or more of the collaborating investigators.

Third, we conducted two sets of combined analyses: one for the six studies that involved hospital controls (studies 1–6 in table 1, hereafter called hospital studies (1–6)) and one for the six studies that involved random digit dial or neighborhood controls (studies 7–12, hereafter called population studies (7–12)). Study 12 used both hospital and population controls; we omitted the hospital control data. Software limitations on the maximum numbers of study subjects and variables in a regression mandated the split into hospital and population studies, which fortuitously allowed us to assess the strength and consistency of an association by comparing two independent sets of odds ratio estimates. The split also provided an opportunity to compare odds ratio estimates and prevalence of characteristics between hospital and population controls. Overall, we found good agreement between odds ratios obtained by the two types of study; such agreement lends support to both study designs.

Finally, we included year of birth as a continuous variable in all regressions to control more precisely for reference age and to control for any case-control differences in year of interview that might bias comparison of temporal variables such as oral contraceptive use.

Limitations and strengths

Combined analyses such as this share some of the pitfalls of meta-analysis (i.e., the review and synthesis of published findings). Despite the common definitions used to recode variables and all efforts to ensure inter-study comparability, the data available to a combined analysis nevertheless derive from questions whose wording varied across studies and thus could have elicited different responses. Interpretation of the final results also is hampered by any defects in the original studies, including selection bias in the enrollment of cases and controls and confounding by unmeasured or imprecisely measured variables. Pooling data from several studies that have the same types of bias can produce relative risk estimates that are statistically highly significant but nevertheless unconvincing of an underlying causal relation. (See reference 26 for further discussion of the sources of bias in pooled analyses of epidemiologic data.)

On the other hand, a combined analysis offers several benefits. It provides large sample sizes for examining effects of rare exposures, interactions among established or suspected risk factors, consistency of associations previously suggested by some studies but not confirmed by others, and effects of risk factors by subtype of disease. For example, part IV (15) in this series describes the finding that odds ratios relating epithelial ovarian cancer risk to pregnancy and oral contraceptive use differ between younger and older women, a finding that has not emerged from any individual study and

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