REFERENCES

(FDA Docket No. 94P-0420/CP 1)

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REGULATORY TOXICOLOGY AND PHARMACOLOGY 21, 233–241 (1995)

The NTP Talc Inhalation Study: A Critical Appraisal Focused on Lung Particle Overload[1]

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Recently published results in a NTP report of a 2-year inhalation study with talc in rats and mice seem to fit the category of being associated with particle overload quite well: Exposure concentrations of 6 and 18 mg/m^3 induced pulmonary inflammation and fibrosis in male and female rats and induction of lung tumors (in female rats only) of the high exposure group; mice of either sex showed an inflammatory response but did not show pulmonary fibrosis or lung tumors. Analysis of the particle accumulation kinetics in lungs of both rats and mice indeed shows that lung overload had been reached at both exposure concentrations in both species resulting in increased talc accumulation of high lung burdens. This and the chronic inflammatory response indicate that the maximum tolerated dose (MTD) had been exceeded at both exposure levels. This result was predictable based on the outcome of a 4-week range-finding study prior to initiation of the chronic talc study; however, the short duration of the range-finding study may have been inadequate to give great confidence in the prediction and therefore may have accounted for the failure to include a concentration below the MTD in the chronic study. Further analysis of the results of the chronic talc study show that talc particles behave like other low-toxicity particles such as TiO_2 and toner with respect to effects on lung clearance and chronic pulmonary inflammation. The conclusion of the NTP report that there is clear evidence of pulmonary carcinogenicity of talc in female rats should, therefore, be qualified by a statement that this is a secondary effect due to the high pulmonary particle load and its associated chronic toxicity. Accordingly, the relevancy of the tumors observed in the female high-exposure group for occupational human exposures may be questionable.

INTRODUCTION

Conclusions in a recently published NTP report (1993) of a chronic inhalation study with talc were that there was clear evidence of carcinogenic activity of talc in female F344/N rats based on increased incidences of alveolar/bronchiolar adenomas and carcinomas of the lung, and that there was no evidence of carcinogenic activity of talc in male or female B6C3F$_1$ mice.[2]

In this study, male and female F344/N rats were exposed to aerosols of 0, 6, or 18 mg nonfibrous talc/m^3, free of SiO_2 and asbestiform minerals, for 113 and 122 weeks, respectively; male and female B6C3F$_1$ mice were exposed to the same talc concentrations for up to 104 weeks. This exposure resulted in concentration-related chronic inflammation, cell proliferation, and fibrosis in the lungs of both male and female rats, and 26% of the female rats of the high-exposure group (13 out of 50) developed lung tumors. The mice showed only limited chronic inflammation and no increased cell proliferative, fibrotic, or tumorigenic responses in their lungs. According to the report (NTP, 1993), the exposure concentrations for the chronic study had been selected based on results of a 4-week talc inhalation study which led to the following conclusion: Exposure concentrations greater than 18 mg/m^3 were expected to overwhelm lung clearance mechanisms and impair lung function.

It is precisely this aspect of overwhelming lung clearance mechanisms that is of critical importance for the interpretation of results from chronic inhalation studies with highly insoluble particles of low cytotoxicity. These particles were formerly categorized as "nuisance" dusts, but are now categorized as particles not otherwise classified (PNOC; ACGIH, 1994). A number of chronic inhalation studies in rats involving such types of particles have resulted in pulmonary fibrosis and even lung tumors at high-exposure concentrations which affect lung

[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.

[2] There was also some evidence of increased incidence of benign or malignant pheochromocytomas of the adrenal glands in rats. Yet, this aspect will not be considered further in this paper since these tumors are not likely to be a direct effect of talc particles on the adrenals inasmuch as talc particles are highly unlikely to reach these glands and background incidences of pheochromocytomas in control rats were already high.

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REGULATORY TOXICOLOGY AND PHARMACOLOGY 21, 244–249 (1995)

An Analysis of the National Toxicology Program's (NTP) Technical Report (NTP TR 421) on the Toxicology and Carcinogenesis Studies of Talc[1]

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The NTP toxicology and carcinogenicity studies of nonasbestiform, cosmetic-grade talc (the NTP Talc Report) were conducted by exposing male and female F344/N rats and B6C3F1 mice to target aerosol concentrations of 0, 6, and 18 mg/m^3 talc for 6 hr daily, 5 days per week. Based on results of the high dose, the Report concluded that talc caused lung tumors in female rats and pheochromocytomas in male and female rats, and there was no evidence of carcinogenic activity in mice. A thorough evaluation of lung toxicity revealed that talc-induced lung tumors occurred only in the group of animals that exhibited the most profound degree of chronic toxicity. However, these data were presented as empirical observations rather than discussed in a manner that would relate them to the risk assessment implications of the bioassay, i.e., relevant data were collected but not "used." In addition, the evaluation of the pheochromocytomas was inadequate because it failed to place sufficient emphasis on the spontaneous incidence of this tumor in rats. These deficiencies caused the author to vote against the conclusions presented in the Talc Report when it was reviewed by the NTP Board of Scientific Counselors. The appropriate conclusions are (1) the data do not indicate that the pheochromocytomas were treatment-related; (2) the maximum tolerated dose (MTD) was exceeded in the female rats exposed to the high dose; and (3) talc is not expected to cause lung tumors under conditions of exposure that fail to result in marked chronic lung toxicity.

INTRODUCTION

The NTP toxicology and carcinogenicity studies of nonasbestiform, cosmetic-grade talc (the NTP Talc Report) were conducted by exposing male and female F344/N rats and B6C3F1 mice to target aerosol concentrations of 0, 6, and 18 mg/m^3 talc for 6 hr daily, 5 days per week (NTP, 1993). A unique aspect of these studies was the inclusion of a very thorough evaluation of lung toxicity, in addition to the standard gross pathology and histopathology assessments. The NTP Talc Report was reviewed by the NTP Board of Scientific Counselors (The Board), Technical Reports Review Subcommittee on June 23–24, 1992. Jay I. Goodman participated in this review as a member of the Board and a member of the Board's Technical Reports Review Subcommittee. The Board concurred with the recommendations of the NTP staff and voted to approve (by a vote of seven yes votes to one no vote (J. I. Goodman)) the following conclusions: "Under conditions of these inhalation studies there was some evidence of carcinogenic activity of talc in male F344/N rats based on an increased incidence of benign and malignant pheochromocytomas of the adrenal gland. There was clear evidence of carcinogenic activity of talc in female F344/N rats based on increased incidences of alveolar/bronchiolar adenomas and carcinomas of the lung and benign and malignant pheochromocytomas of the adrenal gland. There was no evidence of carcinogenic activity of talc in male or female B6C3F1 mice exposed to 6 or 18 mg/m^3."

The specific aims of this paper are to focus on the studies involving rats, the species in which there was deemed to be a carcinogenic effect, and (1) to provide an overview of the talc bioassay (NTP, 1993) with an emphasis on the rationale for the author's disagreement with the conclusions reached by the other members of the Board regarding the carcinogenicity of talc and (2) to provide a realistic perspective regarding the significance of the talc bioassay.

SYNOPSIS OF THE CARCINOGENICITY STUDY OF TALC

Groups of 50 male and 49 or 50 female F344/N rats were exposed to aerosols containing 0, 6, or 18 mg/m^3 talc, 6 hr a day 5 days per week, until mortality in any

[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.

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TABLE 1
Summary of the Lifetime and 2-Year Carcinogenicity Studies of Talc[a]

	Male F344/N rats	Female F344/N rats
Exposure levels	0, 6, or 18 mg/m^3	0, 6, or 18 mg/m^3
Body weights	High-dose group slightly lower than controls	High-dose group slightly lower than controls
Survival rates	9/50, 14/50, 16/50	11/50, 13/49, 9/50
Neoplastic effects	Adrenal medulla: benign or malignant pheochromocytoma (26/49, 32/48, 37/47)	Lung: alveolar/bronchiolar adenoma or carcinoma (1/50, 0/48, 13/50) Adrenal medulla: benign or malignant pheochromocytoma (13/48, 14/47, 23/49)
Level of evidence of carcinogenic activity	Some evidence	Clear evidence

[a] National Toxicology Program, 1993.

exposure group reached 80% (113 weeks for males and 122 weeks for females). The survival of male and female rats exposed to talc was similar to that of the controls, and the mean body weights of the animals in the high-dose group were only slightly lower (not more than approximately 10% lower) than those of controls after Week 65 (NTP, 1993). The results were interpreted as indicating some evidence of carcinogenicity based on benign and malignant pheochromocytomas in male and female rats and clear evidence of carcinogenicity based on alveolar/bronchiolar adenomas and carcinomas of the lung in female rats. The study is summarized in Table 1.

Groups of 47 to 49 male and 48 to 50 female B6C3F1 mice were exposed to aerosols containing 0, 6, or 18 mg/m^3 talc, 6 hr a day 5 days per week, for up to 103 or 104 weeks. Final mean body weights and survival of the mice exposed to talc were similar to those of the controls (NTP, 1993). The results were interpreted as indicating no evidence of carcinogenic activity in mice.

INTERIM EVALUATIONS IN RATS

In a parallel series of ancillary studies, additional groups of animals were exposed to talc, as described above, and examined for interim pathology evaluations or pulmonary function tests after 6, 11, 18, and 24 months and lung biochemistry and cytology studies after 24 months. These are outlined below.

PARAMETERS ASSESSED IN THE ANCILLARY STUDIES

- Lung talc burden
- Pulmonary function
- Lung biochemistry; bronchoalveolar lavage fluid was analyzed to determine:
  - Cell injury—lactate dehydrogenase.
  - Chronic inflammation—polymorphonuclear leukocytes, pulmonary macrophages, alkaline phosphatase, and protein.
  - Lysosomal activation—β-glucuronidase and acid phosphatase.
  - Response to oxidant injury—increased glutathione reductase.
- Lung collagen metabolism, protein synthesis, and proteinase activity.

LUNG TALC BURDEN, LUNG WEIGHTS, AND RESPIRATORY FUNCTION

The lung talc burden at the 6-, 11-, 18-, and 24-month interim sacrifice times was similar in male and

TABLE 2
Lung Talc Burden (Normalized to Exposure Concentration) of Rats[a]

	Male		Female	
	6 mg/m^3	18 mg/m^3	6 mg/m^3	18 mg/m^3
6-Month interim	0.439 ± 0.040[b]	0.602 ± 0.013*	0.406 ± 0.032	0.464 ± 0.007*
12-Month interim	0.731 ± 0.098	1.165 ± 0.113*	0.785 ± 0.043	0.787 ± 0.187
18-Month interim	1.22 ± 0.12	1.53 ± 0.05	1.28 ± 0.06	1.35 ± 0.04
24-Month interim	1.74 ± 0.21	1.34 ± 0.19	1.52 ± 0.15	1.63 ± 0.13

[a] Table F3, National Toxicology Program, 1993.
[b] Units are presented as mg talc/g control lung/mg/m^3.
* Significantly different ($P \leq 0.05$) from the 6 mg/m^3 group by Dunn's or Shirley's test.

TABLE 3
Total Lung Capacity of Rats[a]

	0 mg/m³	6 mg/m³	18 mg/m³
Male			
6-Month interim	19.86 ± 0.54[b]	19.48 ± 0.46	19.25 ± 0.39
11-Month interim	20.06 ± 0.32	18.44 ± 0.39*	17.67 ± 0.45*
18-Month interim	20.30 ± 0.45	18.87 ± 0.41**	16.34 ± 0.52*
24-Month interim	20.50 ± 0.83	20.20 ± 0.28	16.47 ± 1.53
Female			
6-Month interim	14.20 ± 0.25	14.56 ± 0.27	13.80 ± 0.27
11-Month interim	13.29 ± 0.21	12.91 ± 0.17	12.06 ± 0.26*
18-Month interim	13.94 ± 0.26	12.68 ± 0.28*	11.43 ± 0.31*
24-Month interim	14.85 ± 0.31	13.73 ± 0.34**	11.50 ± 1.07*

[a] Table F10, National Toxicology Program, 1993.
[b] Units are presented as ml; ratio is (dosed group mean/control group mean) × 100.
* $P \leq 0.01$.
** Significantly different ($P \leq 0.05$) from the control by Dunn's and Shirley's test.

female rats exposed to 6 or 18 mg/m³ talc, respectively (Table 2).

The absolute and relative lung weights of male rats exposed to 18 mg/m³ were significantly greater than those of controls at the 6-, 11-, and 18-month interim evaluations and at the end of the lifetime study, while those of female rats exposed to 18 mg/m³ were significantly greater at the 11-, 18-, and 24-month interim evaluations and at the end of the lifetime study. In both males and females, there was a concentration-related impairment of respiratory function which increased in severity with increasing duration of exposure (NTP, 1993). While decreases in total lung capacity were noted in males and females, statistically significant reductions at the 24-month interim were only seen in females and this occurred at both the 6 and 18 mg/m³ doses (Table 3).

BRONCHOALVEOLAR LAVAGE AND LUNG BIOCHEMISTRY

Following the completion of the pulmonary function tests at the 24-month interim evaluation a thorough assessment of biochemical and cellular parameters that could provide insight regarding lung toxicity was made. Bronchoalveolar lavage was performed on the remaining rats in these groups and the lavage fluid was evaluated for enzymes and protein (Table 4) and cell content (Table 5). In addition, lung collagen metabolism and protein synthesis were assessed (Table 6) and proteinase activity in both lavage fluid and lung homogenate supernatant fluid was evaluated (Table 7, males; and Table 8, females). While dose-related increases in these parameters (indicative of toxicity) were noted in both males and females, there was a larger number of parameters that showed statistically significant increases in the females and, in general, the magnitude of the increases was higher in the females.

ASSESSMENT OF THE NTP TALC REPORT

The NTP Talc Report noted that both *in vitro* and *in vivo* studies indicate that talc is not genotoxic and it included a thorough evaluation of lung toxicity (NTP, 1993). However, these data were presented as empirical observations rather than discussed in a manner that would relate them to the risk assessment implications of the bioassay. In other words, relevant data were collected but not used. In addition, the evaluation of the pheochromocytomas was inadequate because it failed to place sufficient emphasis on the spontaneous incidence of this tumor in rats. These deficiencies caused the author to vote against the conclusions presented in the Talc Report when it was reviewed by the NTP Board of Scientific Counselors. The rationale for this decision in the context of a more realistic perspective on the Report is presented below.

The carcinogen bioassay is a qualitative test; it is not a risk assessment. The use of biological information is required in order to place results of the bioassay into proper perspective and to take a rational approach toward risk assessment (Goodman, 1990, 1994). This notion was endorsed in a recent review of the NTP by the NTP Board of Scientific Counselors where it was stated that "Mechanistic studies should pervade the activities of the NTP. . . . With this emphasis the NTP should be able to strengthen its interpretation of test results in the context of human health. Such interpretation should become a part of reports issued by the NTP" (NTP, 1992; Goodman, 1994). It is appropriate to consider the NTP Talc Report in this context.

Talc-induced lung tumors were not detected in male rats, female mice, or male mice. In rats, the principal toxic lesions associated with inhalation exposure to talc included chronic granulomatous inflammation, alveolar epithelial hyperplasia, squamous metaplasia and squamous cysts, and interstitial fibrosis of the lung. These lesions were accompanied by impaired pulmonary function. While the talc burden in the lungs of males and females was similar (Table 2), the degree of chronic toxicity and inflammation was substantially higher in the females (Tables 3–8; NTP, 1993). In mice, inhalation exposure to talc produced some chronic inflammation. In contrast to rats, alveolar epithelial hyperplasia, squamous metaplasia, and interstitial fibrosis were not observed. Overall, markedly less talc-induced lung toxicity was produced in mice than in rats (NTP, 1993). These observations need to be juxtaposed with the findings regarding lung tumors. It is apparent that an increase in lung tumors was seen only in the test animals that clearly exhibited the highest degree of chronic lung toxicity, the female rats exposed to the high dose (18 mg/m³) of talc. The amount of toxicity leads the author to

TABLE 4
Bronchoalveolar Lavage Fluid Enzymes of Rats at the 24-Month Interim Evaluation[a]

	0 mg/m^3	6 mg/m^3	18 mg/m^3
Male			
β-Glucuronidase[b]	1.09 ± 0.40	18.86 ± 3.20*	89.24 ± 14.24**
Lactate dehydrogenase	1634 ± 545	3193 ± 606	8262 ± 380*
Alkaline phosphatase	364.7 ± 147	572.8 ± 86.8	1604.7 ± 143*
Glutathione reductase	103.03 ± 16.43	99.35 ± 19.79	110.99 ± 0.55*
Total protein[c]	1.78 ± 0.40	3.12 ± 0.64	5.79 ± 0.55*
Female			
β-Glucuronidase[b]	3.33 ± 0.97	41.05 ± 4.39**	154.16 ± 17.21**
Lactate dehydrogenase	1655 ± 266	3906 ± 444*	14E3 ± 1E3**
Alkaline phosphatase	427.8 ± 30.9	853.6 ± 79.7**	2594.7 ± 221***
Glutathione reductase	100.6 ± 1.7	135.2 ± 22.4	460.0 ± 44.8*
Total protein[c]	1.20 ± 0.22	4.30 ± 0.36**	12.96 ± 0.28**

[a] Table F4, National Toxicology Program, 1993.
[b] Units presented as mIU/g control lung.
[c] Units presented as mg/g control lung.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's and Shirley's test.
** $P \leq 0.01$.

conclude that the MTD was exceeded in the female rats exposed to the 18 mg/m^3 dose.

The overall incidences of pheochromocytomas (benign, malignant, or complex) of the adrenal medulla that occurred in the 18 mg/m^3 exposed rats were significantly greater than those of the controls (males, $P = 0.006$; females, $P = 0.024$) (NTP, 1993). However, the spontaneous incidence of these tumors was 53% in males and 27% in females. In the author's view, the criterion for statistical significance should be $P < 0.01$. This is consistent with the "guideline" indicating that a compound should be considered carcinogenic if the highest dose produces an increase in a common tumor that is significant at the 1% ($P < 0.01$) level or an increase in a rare tumor that is significant at the 5% ($P < 0.05$) level; otherwise, the compound is regarded as a noncarcinogen (Haseman, 1983; Haseman *et al.*, 1986). A rare neoplasm was defined as a neoplasm occurring with a frequency of less than 1%. Therefore, there is no statistically significant increase in pheochromocytomas in talc-treated female rats and talc should not be deemed carcinogenic at this site in these animals.

There has been a pronounced increase in the spontaneous occurrence of pheochromocytomas in male F344 rats, in studies conducted by the NTP, over the past 10 years (Rao *et al.*, 1990; NTP, 1993). In addition, the high rate of pheochromocytomas in the control female rats in the talc bioassay (13/48, 27%) is in sharp contrast to reports indicating that the spontaneous incidence in control female F344 rats, in NTP studies, has been less than 10% (Rao *et al.*, 1990), e.g., in the range of 5.2–7.1% between 1980 and 1983 (Haseman and Rao, 1992). Unfortunately, the NTP failed to make a comparison of the spontaneous pheochromocytomas observed in the talc

TABLE 5
Bronchoalveolar Lavage Fluid Cell Populations of Rats at the 24-Month Interim Evaluation[a]

	0 mg/m^3	6 mg/m^3	18 mg/m^3
Male			
Polymorphonuclear cells[b]	0.333 ± 0.167	24.417 ± 2.557*	32.50 ± 3.000*
Lymphocytes	0.000 ± 0.000	0.500 ± 0.258	0.500 ± 0.500
Macrophages	93.67 ± 3.3.72	70.25 ± 2.53*	62.75 ± 1.75*
Epithelial cells	6.00 ± 3.61	4.83 ± 1.41	4.25 ± 1.75
Female			
Polymorphonuclear cells	0.625 ± 0.315	25.778 ± 2.673**	37.000 ± 1.528**
Lymphocytes	0.000 ± 0.000	0.722 ± 0.188*	1.333 ± 0.667*
Macrophages	91.38 ± 1.75	71.22 ± 2.95**	57.33 ± 4.67**
Epithelial cells	8.00 ± 2.01	2.28 ± 0.50*	4.33 ± 2.60

[a] Table F5, National Toxicology Program, 1993.
[b] Units presented as percentage of total cells.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's and Shirley's test.
** $P \leq 0.01$.

TABLE 6
Lung Collagen Metabolism and Protein Synthesis in Rats at the 24-Month Interim Evaluation[a]

	0 mg/m³	6 mg/m³	18 mg/m³
Male			
Lavage fluid collagenous peptides[b]	39.79 ± 5.07	46.99 ± 6.51	79.21 ± 13.73
Total lung collagen[c]	13.87 ± 0.60	15.98 ± 0.39*	18.88 ± 3.35*
Collagen production[d]	1.58 ± 0.17	1.60 ± 0.17	1.63 ± 0.22
Collagen degradation[e]	31.67 ± 1.72	27.74 ± 1.42	9.18 ± 2.38*
Noncollagenous protein synthesis[f]	142.1 ± 14.5	199.8 ± 22.1*	312.2 ± 10.6**
Female			
Lavage fluid collagenous peptides	78.27 ± 11.64	115.36 ± 8.61*	174.71 ± 13.56**
Total lung collagen	14.32 ± 0.66	19.95 ± 1.58*	36.47 ± 3.39**
Collagen production	0.982 ± 0.185	1.804 ± 0.144*	2.264 ± 0.347**
Collagen degradation	14.41 ± 2.44	21.59 ± 4.99	9.38 ± 1.63
Noncollagenous protein synthesis	173.9 ± 34.5	325.8 ± 90.9	554.3 ± 107*

[a] Table F7, National Toxicology Program, 1993.
[b] Units are presented as μg/g control lung.
[c] Units are presented as mg/g control lung.
[d] Units are presented as percentage new protein.
[e] Units are presented as percentage new collagen.
[f] Units are presented as dpm × 10^3/g control lung.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's and Shirley's test.
** $P \leq 0.01$.

bioassay with the historical spontaneous incidence of pheochromocytomas in rats. This would have been particularly appropriate (as the author indicated during the Board's review of the NTP Talc Report, June 23–24, 1992).

The marked increase in the spontaneous incidence of pheochromocytomas in female rats coupled with the lack of a statistically significant increase of this tumor in the treated animals (when the appropriate P value is employed) and the trend toward an increasing spontaneous incidence in males indicate that the data presented in the NTP Talc Report do not warrant a conclusion that talc causes pheochromocytomas in rats.

CONCLUSIONS

The appropriate conclusion to be drawn from the NTP Talc Report is that the MTD was exceeded in the female rats exposed to the high dose and talc is not expected to cause lung tumors under conditions of exposure that fail to result in marked chronic lung toxicity. Clear evidence of carcinogenic activity of talc was

TABLE 7
Proteinase Activity in Lavage Fluid and Lung Homogenate Supernatant Fluid of Male Rats at the 24-Month Interim Evaluation[a]

	0 mg/m³	6 mg/m³	18 mg/m³
Lavage fluid			
Acid proteinase	0.994 ± 0.329[c]	1.866 ± 0.174	4.307 ± 0.218*
Cathepsin D	0.147 ± 0.147	0.599 ± 0.150	2.420 ± 0.147**
Cathepsin B	0.924 ± 0.415	1.267 ± 0.094	1.887 ± 0.365
Homogenate supernatant fluid			
Acid proteinase	10.92 ± 0.64	17.51 ± 0.90*	25.13 ± 1.50**
Cathepsin D	8.53 ± 0.91	14.04 ± 0.62*	21.03 ± 1.56**
Cathepsin B	2.39 ± 0.41	3.48 ± 0.37	4.10 ± 0.06*
Neutral proteinase	0.715 ± 0.168	2.417 ± 0.304*	4.505[c]
PMN elastase cathepsin G	0.490 ± 0.218	1.936 ± 0.242*	4.457 ± 0.377**
Macrophage elastase collagenase	0.225 ± 0.099	0.482 ± 0.077	0.000[c]

[a] Table F8, National Toxicology Program, 1993.
[b] Units are presented as mg/hr/mg control lung.
[c] $n = 1$; no statistic calculated.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's and Shirley's test.
** $P \leq 0.01$.

TABLE 8
Proteinase Activity in Lavage Fluid and Lung Homogenate Supernatant Fluid of Female Rats at the 24-Month Interim Evaluation[a]

	0 mg/m^3	6 mg/m^3	18 mg/m^3
Lavage fluid			
Acid proteinase	1.52 ± 0.12[b]	3.46 ± 0.33*	6.05 ± 0.73**
Cathepsin D	0.015 ± 0.015	1.310 ± 0.292*	4.043 ± 0.578**
Cathepsin B	1.61 ± 0.26	2.15 ± 0.22	2.01 ± 0.17
Homogenate supernatant fluid			
Acid proteinase	14.04 ± 0.95	29.43 ± 1.18**	38.61 ± 1.81**
Cathepsin D	10.05 ± 0.68	22.97 ± 1.07**	30.25 ± 1.60**
Cathepsin B	3.99 ± 0.58	6.46 ± 0.60*	8.37 ± 0.42**
Neutral proteinase	0.648 ± 0.087	5.040 ± 0.418**	12.293 ± 1.598**
PMN elastase cathepsin G	0.785 ± 0.142	4.351 ± 0.261**	10.313 ± 2.694**
Macrophage elastase collagenase	0.054 ± 0.037	0.068 ± 0.175*	2.012 ± 1.126*

[a] Table F8, National Toxicology Program, 1993.
[b] Units are presented as mg/hr/mg control lung.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's and Shirley's test.
** $P \leq 0.01$.

seen only in female rats exposed to the high dose of talc and only under circumstances in which there was evidence of marked chronic lung toxicity. These remarks are not intended to connote that the talc bioassay is invalid. The good news is that under the conditions of this study mice did not develop tumors, male rats did not develop lung tumors, and female rats exposed to the 6 mg/m^3 dose did not develop lung tumors. Furthermore, it is doubtful that the pheochromocytomas observed in the talc-treated rats were treatment related. Thus, at the high dose, talc exhibited equivocal evidence of carcinogenic activity toward the adrenal medulla of male rats.

There is a considerable debate regarding the proper criteria for a MTD (Kociba, 1987). However, in a practical sense the chief concern with the MTD is how the resulting data are used in the risk assessment process (McConnell, 1989). Any high dose, no matter how high, that permits animals to live long enough to develop tumors is not necessarily an appropriate high dose. The recent review of the NTP addressed this issue and concluded that "the implicit assumptions underlying extrapolation from the MTD . . . do not appear to be valid. Therefore, both the criteria for selection of the high dose used and the default criteria that are employed for extrapolation from high dose to low dose must be reevaluated in a critical manner" (NTP, 1992; Goodman, 1994). A consideration of the data presented in the NTP Talc Report leads the author to conclude that it would not be appropriate to use the lung tumor end point in female rats as the basis for a risk assessment involving a linear extrapolation to estimate a theoretical risk to humans that might be exposed to relatively low doses of talc.

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SECTION III A

16 · The Aetiology of Ovarian Cancer

M.S. Baylis, W.J. Henderson, C.G. Pierrepoint and K. Griffiths

Introduction

The estimated number of new notifications of ovarian cancer in 1983 in the United States will be 18 200 with 11 500 women dying from the disease in the same period [1]. New registrations of ovarian cancer in England and Wales in 1974 numbered 4178 and 5 years later, 3784 women had died of the disease. Such data illustrate the poor prognosis associated with this condition, with less than 25% of women expecting to survive 5 years (Fig. 16.1). The risk of developing ovarian cancer is similar to that for carcinoma of the cervix, 1 in 80, yet as many women die from ovarian cancer as from the other female genital tract malignancies combined. A factor which gives rise for concern is the increasing incidence of the disease. From 1931 to 1970 in England and Wales the mortality rates per million women rose by 15% each decennium [2]. During the period 1971–1980, however, the rate of increase had plateaued to 6.5% (Fig. 16.2).

The diversity of the structure and functions of the ovary results in a great variety of tumours of complex histological origin [3,4].

Tumours of epithelial origin constitute 58% of all ovarian neoplasms and 80–90% of all ovarian carcinomas [5,6]. There is an age specificity for the various histological sub-groups of ovarian malignancy. Sex cord tumours are very rare before the third decade but increase in frequency to the fifth, when their incidence remains constant. Germ cell tumours occur in the 10–50 year old age group, while epithelial tumours rarely appear before the age of 10. They then increase exponentially until 40 years of age and have their highest incidence in women in their early sixties. Varying nomenclature and different histological classifications have made comparative histopathological and epidemiological studies virtually impossible. The fact that more than 70 different types of neoplasms have been described

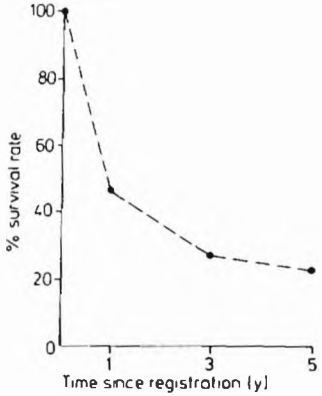

Fig. 16.1. The percentage survival rate over a 5-year period of women registering with ovarian cancer in England and Wales

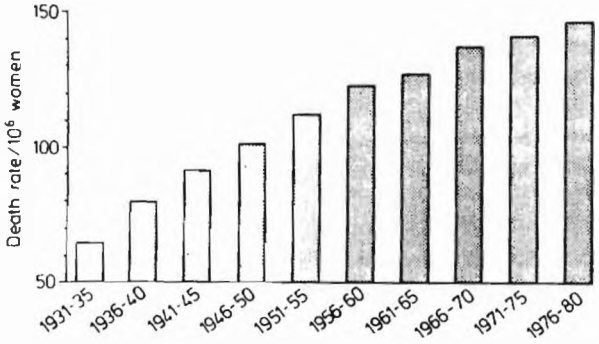

Fig. 16.2. The death rate of women from ovarian cancer from 1931 to 1980 in England and Wales.

highlights the difficulties. The World Health Organisation classification of ovarian tumours [7] should, in future, permit more meaningful comparisons to be undertaken.

Possible Causal Factors

Environmental

Analysis of international mortality rates shows that Northern Europe has four times the incidence rate of Asia [8]. Studies of Japanese migrants living in the

United States [9] show that these Asiatic people exhibit rates of ovarian cancer incidence intermediate between those of their country of origin and their country of adoption. A number of reports have appeared in the literature indicating that certain families show a susceptibility to ovarian malignancy [10] with serous cystadenocarcinomas the most common histological type observed. Prophylactic bilateral oophorectomy has been advocated for these women as soon as they have completed their families, although such an aggressive approach does not guarantee immunity to the development of pelvic cancer, which may exhibit a histological picture similar to that of the ovary and therefore supports the concept of 'generalised instability' of coelomic epithelium. It has been reported that 5%–14% of women with the Peutz-Jeghers syndrome develop ovarian tumours [11,12,13]. Similarly, the basal cell naevus syndrome is associated with the development of ovarian fibromas, although other cell types also occur [14]. The high incidence of ovarian malignancy in the industrialised countries of Northern Europe and North America points strongly to environmental factors playing a significant role in the initiation of the malignant process. A number of studies have shown an association between smoking and the early, spontaneous onset of the menopause [15,16]. There was also a relationship with the level of smoking [17]. Studies of the rodent ovary demonstrated the presence of a non-specific microsomal mono-oxygenase, which metabolises polycyclic aromatic hydrocarbons, present in cigarette smoke and industrial pollutant, to active carcinogenic compounds. Benzo(*a*)pyrene, also a constituent of cigarette smoke, was found to be toxic to the primordial follicles of rodents and to initial ovarian tumours in mice [18]. Mattison and Thorgeirsson found evidence of a similar microsomal mono-oxygenase system in the human ovary and proposed an association between polycyclic aromatic hydrocarbons and the occurrence of an early menopause in smokers and also a possible carcinogenic role for the induction of ovarian cancer [19]. It is said that the menopause in women occurs when the oocytes are depleted, which normally occurs by the process of atresia [20]. Early ovarian failure may therefore occur if the rate of oocyte loss is accelerated. There is no evidence at present, however, to suggest those women who smoke are more susceptible to ovarian malignancy than non-smokers.

Infectious Diseases

Infectious diseases have been considered as a possible aetiological factor in the causation of ovarian cancer. A higher incidence of rubella infection between the ages of 12 and 18 years in ovarian cancer patients than in controls has been reported [21], whilst there was a lower frequency of mumps [22] and of pneumonia and influenza [23].

Hormonal

It is considered that endogenous hormones play a role in the aetiology of ovarian cancer as, in all international reviews of cancer rates, a strong relationship is known to exist between the incidence of ovarian and breast cancer. Women who develop primary carcinoma of the breast have twice the expected risk of developing a primary ovarian cancer and, vice versa, women with primary ovarian cancer

run 3 to 4 times the risk of developing a primary breast malignancy. One hypothesis to explain ovarian epithelial cancer relates growth regulating hormone excess involving the gonadotrophins which act indirectly on the epithelial cells, causing them to replicate following ovulation and cover the denuded surface of the ovary. The epithelium may be incorporated into the stroma of the ovary during the formation of the corpus luteum [24], resulting in inclusion cysts which, because of the multipotentiality of the surface epithelium, may give rise to malignant tumours exhibiting Müllerian cell types [25]. This hypothesis also suggests that the interruption of the normal cyclical control of the ovary by gonadotrophin would be protective and reduce the number of inclusion cysts [26]. This would also account for the rarity of ovarian epithelial neoplasia prior to puberty. A number of case control studies support this concept, showing that pregnancy and lactation have a protective role [23,27,28,29]. Furthermore, in women under 50, incomplete pregnancies were found to be protective, as was the use of oral contraceptives. Beral inversely correlated death from ovarian cancer with the number of children born [30]. It would seem that nulliparous Caucasian women of northern European descent, who live in an industrialised country and who also have a family history of cancer, are at greatest risk of developing epithelial neoplasia of the ovary.

The incidence of breast, endometrial and ovarian cancer was noted by Stadel [31] to be inversely correlated with the dietary intake of iodine, a deficiency which may lead to primary hypothyroidism. Primary hyperthyroidism is associated with elevated prolactin levels [32] and also a 'potentiation' of the effects of gonadotrophins [33], a situation which might lead to excessive oestrogen synthesis and secretion and a possible overall increased risk of ovarian, endometrial and breast malignancy. Biskind and Biskind [34] demonstrated that ovarian tumours of granulosa cell origin could be induced in the rat, probably as a result of excessive gonadotrophin release brought about by castration of the animal and transplantation of part or whole of one ovary into the spleen. As the blood leaving the spleen drains directly to the liver the steroids produced by the transplanted ovary are immediately metabolised and rendered ineffective in the normal feedback of hypothalamus and pituitary. Studies from this Institute [35] confirmed the elevated levels of follicle-stimulating hormone (FSH) and luteinising hormone (LH) following this transplantation procedure. This is an interesting model but is of limited value as granulosa cell tumours are rare in women.

Excessive gonadotrophin stimulation in the rat may also be achieved by immunising with testosterone 3-(*O*-carboxymethyl imino-bovine serum albumin)-T-3-BSA) [36]. This leads to a grossly elevated, total serum testosterone concentration, with a decrease in free circulating, biologically-active testosterone which is bound by the anti-testosterone antibodies. Exogenous hormones have also been implicated in the aetiology of ovarian cancer [37] in a follow-up survey of 908 women who had received conjugated equine oestrogen postmenopausally. It was also stated that there was a greater risk of developing ovarian cancer in those women who had, in addition, taken stilboestrol.

Talc and Asbestos

It is of interest that those women employed in the rubber, electrical and textile industries are at greater risk of developing ovarian malignancy than those

involved in other manufacturing industries [38,39]. The association between asbestos exposure and lung cancer in man was first suggested by Lynch and Smith in 1935 [40]. Further studies confirmed that certain types of asbestos, in addition to their fibrogenic properties, also possessed carcinogenic potential [41]. In 1960, 32 cases of pleural mesothelioma in persons exposed to crocidolite in South Africa were described [42]. Eleven cases of peritoneal tumours associated with asbestos exposure were also reported by Keal [43] and a further nine by Entiknap and Smither [44]. Moreover, Graham and Graham [45] reported the presence of birefringent crystals in human ovarian cancers and related them to asbestos but they were unable to characterise them. Certain ovarian tumours show histological similarity to pleural and peritoneal mesotheliomas. This may be explained by the fact that the ovarian surface epithelium is of mesothelial origin, which confers upon it its unique ability to produce ovarian tumours of multiple cell types including combinations of serous, mucinous, endometrioid, mesonephroid and mesothelial cell types [46].

An extraction replication technique was developed in the Tenovus Institute to investigate foreign particulate material present in biological tissues [47]. This technique was used to investigate and identify various types of contaminating particles in sections of tissue from, for example, the lung and ovaries [48]. It failed to demonstrate the presence of asbestos in human ovaries, as suggested by Graham and Graham, but did establish the presence of talc particles in both normal and neoplastic tissue [49]. Unequivocal identification of individual particles was achieved by using an electron microscope microanalyser (EMMA). There is good evidence that talc may reach the ovary by traversing the female genital tract. Egli and Newton [50] instilled carbon black particles suspended in a 30% dextran solution into the posterior fornix of vaginas of three women undergoing hysterectomy. At laparotomy, in two of the three patients, carbon particules could be found at the fimbrial ostia of the fallopian tube within 40 min of the vaginal instillation. Further confirmation of the migration of particulate material from the vagina to the peritoneal cavity, with uptake into the ovary, was provided by studies using technetium-99 labelled human albumin spheres instilled into the vagina for the purpose of hysterosalpingography by radionuclide scintigraphy [51,52]. Work from this laboratory [53] has shown that when a suspension of talc is introduced either into the posterior aspect of the uterus or even into the vagina of rats, the material can be located in the ovaries within 4 days of the instillation.

Talc is widely used in the pharmaceutical and cosmetic industries and has been used in association with certain types of contraceptive. Many women dust their perinea with talc and some apply it to their sanitary wear [54]. Furthermore, talc particles have been shown to embed in both cultured human lung fibroblasts and in rat ovarian epithelial cells, probably by the process of pinocytosis. Oxygen incineration studies of human ovarian tissue indicated that the amount of talc present could be of the order of 2×10^5 particles per mm^3 [55]. Reports that commercial talcs are contaminated with particles of asbestos [10] have not been substantiated in this Institute, where nearly all forms of natural talc produced from various countries around the world have been analysed. It is well established that talc can induce granulomata in the peritoneal cavity [56] and contamination of the ovary may result in disturbances in the ovarian stroma leading to altered steroid biosynthesis.

In this laboratory, a suspension of asbestos-free Italian 00000 talc in phosphate-

buffered saline was injected below the rat ovarian bursa [57]. Within 6 weeks of injection many animals demonstrated cystic changes which, by 1 year, had developed into ovarian bursal cysts, 5 cm or more in diameter (Fig.16.3). Histo-

Fig. 16.3. Bilateral ovarian bursal cysts from a rat 12 months after the sub-bursal injection of a suspension of talc.

Fig. 16.4. Rat ovarian surface germinal epithelium subsequent to the sub-bursal injection of a suspension of talc. Note papillary formation and the multilayering of the surface epithelium. Stained with H & E. (× 200)

logical examination of these talc-treated ovaries showed multilayering of the surface germinal epithelium (Fig. 16.4) with papillary outgrowths, a pathology not inconsistent with preneoplasia and which resembled the histological findings in the ovaries of guinea pigs treated with intraperitoneal asbestos [45]. The possibility that contaminating talc particles may play a role in initiating ovarian dysfunction, which could lead to neoplasia, requires careful consideration. The possible adverse affects of presence of talc in the ovary are being intensively investigated in this Institute. Several different animal species are being used, including the chicken, which is known to have a high incidence of ovarian adenocarcinoma when kept in artificial light [2]. The possibility that talc may act as a co-carcinogen is being examined. Its adsorptive properties are well known and, once located in the ovary, may retain circulating carcinogens for longer periods than normal. Equivalent studies are being carried out in vitro since it has been shown that talc will penetrate epithelial cells in culture and, although transformation of the cells has not been shown, the addition of a chemical such as dimethylbenzanthracene, which does not usually induce ovarian cancer, may well induce such changes.

It certainly cannot be said at present that talc causes ovarian cancer. Its presence in such tumours was unexpected and unwarranted. It is because the cause of ovarian carcinoma is unknown and that talc has a chemical similarity with asbestos that these investigations were instigated.

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BRIEF SUMMARY (FOR FULL PRESCRIBING INFORMATION AND PATIENT INFORMATION, SEE PACKAGE INSERT)

PREMARIN® Brand of conjugated estrogens tablets, USP
PREMARIN® Brand of conjugated estrogens Vaginal Cream, in a nonliquefying base

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.
Close clinical surveillance of all women taking estrogens is important. Adequate diagnostic measures, including endometrial sampling when indicated, should be undertaken to rule out malignancy in all cases of undiagnosed persistent or recurring abnormal vaginal bleeding. There is currently no evidence that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.
Estrogen therapy during pregnancy is associated with an increased risk of congenital defects in the reproductive organs of the male and female fetus, an increased risk of vaginal adenosis, squamous cell dysplasia of the uterine cervix, and vaginal cancer in the female later in life. The 1985 DES Task Force concluded that women who used DES during their pregnancies may subsequently experience an increased risk of breast cancer. However, a causal relationship is still unproven, and the observed level of risk is similar to that for a number of other breast cancer risk factors.

There is no indication for estrogen therapy during pregnancy. Estrogens are ineffective for the prevention or treatment of threatened or habitual abortion.

DESCRIPTION: PREMARIN (conjugated estrogens, USP) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters. Tablets are available in 0.3 mg, 0.625 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens. Cream is available as 0.625 mg conjugated estrogens per gram.

INDICATIONS AND USAGE: Moderate to severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause and they should not be used to treat these conditions.) Prevention and management of osteoporosis (abnormally low bone mass). Atrophic vaginitis. Hypoestrogenism due to hypogonadism, castration or primary ovarian failure.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected pregnancy (see Boxed Warning). 2. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 3. Known or suspected estrogen-dependent neoplasia. 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. Estrogen replacement therapy has not been reported to increase the risk of thrombophlebitis and/or thromboembolic disease. However, there is insufficient information regarding women who have had previous thromboembolic disease.

PREMARIN Tablets and Vaginal Cream should not be used in patients hypersensitive to their ingredients.

WARNINGS: Some studies suggest a possible increased incidence of breast cancer in women taking higher doses of estrogen for prolonged time periods. The majority of studies have not shown an association with usual estrogen replacement doses. Endometrial cancer risk among estrogen users was about 4-fold or greater than in nonusers and appears dependent on treatment duration and estrogen dose. In patients on combined estrogen-progestin therapy, this risk appears to be decreased. (See PRECAUTIONS below.)

Estrogen therapy during pregnancy is associated with an increased risk of fetal congenital reproductive tract disorders.

A 2.5-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens has been reported.

Large doses of estrogen such as those used to treat prostate and breast cancer have been shown to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis in men. This cannot necessarily be extrapolated to women. However, to avoid the theoretical cardiovascular risk caused by high estrogen doses, the doses for estrogen replacement therapy should not exceed the recommended dose.

Blood pressure should be monitored with estrogen use, especially if high doses are used.

Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: The addition of a progestin for 7 or more days of a cycle of estrogen administration reportedly lowers the incidence of endometrial hyperplasia. Studies of endometrium suggest that 10 to 13 days of progestin are needed to provide maximal endometrial maturation and elimination of hyperplastic changes. Additional risks, such as adverse effects on carbohydrate and lipid metabolism, may be associated with the inclusion of progestin in estrogen replacement regimens. The choice of progestin and dosage may be important in minimizing these adverse effects.

Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding and mastodynia. Preexisting uterine leiomyomata may increase in size during estrogen use. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, or metabolic bone diseases associated with hypercalcemia.

The following drug/laboratory test interactions have been reported, some only with estrogen-progestin combinations (oral contraceptives):

1. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability; decreased fibrinolysis.

2. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by T_4 levels determined by column or by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG. Free T_4 concentration is unaltered.

3. Impaired glucose tolerance.

4. Reduced response to metyrapone test.

5. Reduced serum folate concentration.

MUTAGENESIS AND CARCINOGENESIS: Long-term, continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver.

PREGNANCY CATEGORY X: Estrogens should not be used during pregnancy. See **CONTRAINDICATIONS** and Boxed Warning.

NURSING MOTHERS: As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy: changes in vaginal bleeding pattern and abnormal withdrawal bleeding or flow, breakthrough bleeding, spotting, increase in size of uterine fibromyomata, vaginal candidiasis, change in amount of cervical secretion, tenderness or enlargement of breasts, nausea, vomiting, abdominal cramps, bloating, cholestatic jaundice, chloasma or melasma that may persist when drug is discontinued, erythema multiforme, erythema nodosum, hemorrhagic eruption, loss of scalp hair, hirsutism, steepening of corneal curvature, intolerance to contact lenses, headache, migraine, dizziness, mental depression, chorea, increase or decrease in weight, reduced carbohydrate tolerance, aggravation of porphyria, edema, changes in libido.

ACUTE OVERDOSAGE: May cause nausea and vomiting.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of conjugated estrogens tablets, USP

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2. *Given cyclically:* Hypoestrogenism; Osteoporosis.

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Molecular and Biologic Factors in the Pathogenesis of Ovarian Cancer

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The development and progression of epithelial ovarian cancer can be correlated with various biologic and molecular factors. Tumor growth has been associated with aberrant and dysfunctional expression and mutation of various genes. These genetic defects include oncogene overexpression, amplification or mutation, aberrant tumor suppressor gene expression or mutation, and the inappropriate expression of cytokines and growth factors and/or the cellular receptors for these molecules. Dysregulation of host immune responses may also play a permissive role in the pathogenesis of the disease. Since ovarian cancer has been associated with the frequency of ovulation, the repeated proliferation of epithelial cells may increase the chance of a genetic accident that could contribute to the activation of an oncogene or inactivation of a suppressor gene. These events, combined with the inherent ability of ovarian epithelial cells to respond to and produce various cytokines and growth factors, could promote oncogenesis. (J Reprod Med 39:241–248, 1994)

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Introduction

Ovarian cancer, which results in a higher mortality rate than any other gynecologic malignancy, is the fourth leading cause of cancer death in women in the United States.[65] A major factor in this high mortality rate is that at the time of diagnosis most patients already have advanced disease. Early detection of ovarian cancer is hampered by a lack of appropriate tumor markers, and clinically, most ovarian cancer patients fail to develop significant symptoms until they reach advanced disease. Ovarian cancer has a high frequency of metastasis yet generally remains localized within the peritoneal cavity.[11]

Tumor development has been associated with the aberrant, dysfunctional expression and/or mutation of various genes. This can include oncogene overexpression, amplification or mutation, aberrant tumor suppressor (antioncogene) expression or mutation, and the inappropriate expression of cytokines and growth factors and/or the cellular receptors for these molecules. Also, subversion of host antitumor immune responses may play a role in the pathogenesis of cancer.[18,19]

Most ovarian cancers appear to arise from the ovarian epithelium, a single layer of cells found on the surface of the ovary. While several factors have been implicated in the genesis and growth of ovarian cancer, several studies have shown that the frequency of ovulation is associated with an increased risk of the development of ovarian cancer.[45,53,59,65] Because ovulation is associated with disruption of the surface epithelium of the ovary, the ovarian epithelium must proliferate to heal this ovulation-associated wound. Therefore, ovulation must be associated with the production of growth factors that enhance the growth and/or differentiation of ovarian epithelial cells. Certainly, various cytokines and growth factors, including transforming growth factors a (TGF-α) and IL-6, have been seen in ovarian follicular fluid.[20,53,56] Perhaps the repeated proliferation of ovarian epithelial cells associated with ovulation contributes to the development of ovarian cancer by increasing the chance of a genetic accident (error in DNA replication) that could contribute to activation of an oncogene or inactivation of a tumor suppressor gene. Such an event or events, combined with the inherent ability of ovarian epithelial cells to respond to and produce cytokines and growth factors, could explain, in part, the high frequency of ovarian cancer and its association with ovulation.

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Oncogenes

The aberrant expression of various oncogenes in ovarian cancer has been described. They include HER-2/*neu*, *ras*, *myc*, *fms*, *jun* and *myb*.[4,5,9,12,40,63,75] The many oncogenes that have been characterized can be divided into a few major groups based on their biologic role and cellular location.[4,15] For instance, several oncogenes, including HER-2/*neu* and *fms*, appear to encode for transmembrane receptor molecules involved in ligand binding. Other oncogenes, including *ras*, represent inner membrane proteins involved in signal transduction. Finally, some oncogenes, including *myc*, *myb* and *jun*, represent nuclear transcriptional regulatory proteins. Therefore, protooncogenes represent various cellular proteins with normal physiologic roles in various cellular processes involved in the induction of cellular growth and differentiation. These activities are subverted by mutation or overexpression, resulting, in part, in generation of the transformed phenotype.

Recently, the role of HER-2/*neu* in ovarian cancer has received much attention.[8,9,10] HER-2/*neu* is an oncogene product that was seen to be overexpressed in breast cancer; overexpression of HER-2/*neu* in breast cancer was seen to be associated with a poor prognosis.[66] Normal ovarian epithelium expresses low–moderate levels of HER-2/*neu*.[10] HER-2/*neu* is overexpressed in about 30% of ovarian malignancies and appears to be indicative of a poor prognosis and survival.[8,10,66] Therefore, HER-2/*neu* has the potential to be clinically useful as a marker for ovarian cancer.

Lichtenstein and co-workers have shown that HER-2/*neu* overexpressing ovarian tumor cell lines were more resistant to tumor necrosis factor (TNF) or lymphokine-activated killer cell–mediated lysis than cells that expressed lower levels of HER-2/*neu*, suggesting that overexpression of this oncogene might impart a biologic advantage to tumor cells by enhancing their resistance to cytotoxicity.[43]

The HER-2/*neu* oncogene[60] fits into both the category of oncogene and that of growth factor receptor since the HER-2/*neu* gene both is a protooncogene and appears to code for an epidermal growth factor (EGF) receptor–like molecule.[59] Recently, a ligand (growth factor?) that binds to this putative growth factor receptor has been described and partially characterized.[58] Another oncogene that has been seen to be overexpressed in ovarian cancer is *fms*, which also encodes for a growth factor receptor, in this case for the receptor for M-CSF.[35] Ovarian cancer cells also have been seen to produce M-CSF.[35,61]

Since the HER-2/*neu* gene product appears to be a growth factor receptor, antibodies directed to the extracellular portion of this molecule might be able to down-regulate its growth-promoting activity. Bast and co-workers have found that anti-HER-2/*neu* monoclonal antibodies to cells that overexpress HER-2/*neu* can inhibit their growth.[9,61]

Antioncogenes

Another genetic lesion that has been implicated in the genesis and development of ovarian cancer involves the p53 tumor suppressor gene: the p53 gene has been seen to be overexpressed (and mutated) in 30–50% of ovarian cancers.[48,50,67] The p53 gene, on chromosome 17p, is a tumor suppressor gene, and mutations or loss of p53 have been seen in many human cancers. The product of the p53 gene is a nuclear phosphoprotein that can be expressed by normal cells and plays a role in the regulation of cellular growth and development but that is overexpressed when mutated.[11,41,48] The loss of normal p53 function, due to mutation and overexpression or to deletion of the normal p53 gene, is often associated with a malignant phenotype. Mutation of p53 results in the dominant transformed phenotype since the expression of a mutant form of this tumor suppressor gene leads to the dysfunction of normal p53 by preventing the association of p53 to form a functional DNA-binding, regulatory complex.

In recent studies we have seen that p53 tumor suppressor gene expression can be induced in an ovarian cancer cell line by TNF-α together with the induction of cell death by apoptosis.[74] Therefore, a mechanism by which TNF induces tumor cell death may involve the up-regulation of tumor cell p53 gene expression.

Another tumor suppressor gene that has been suggested to play a role in the development of ovarian cancer is the retinoblastoma (RB) locus: a significantly high frequency of allelic deletion of RB has been seen in ovarian cancer tissue.[42]

Growth Factors and Cytokines

Growth factors and cytokines have been seen to play important roles in the development and growth of cancer,[1,22] including ovarian cancer.[45,46,49,51] In fact, it has been proposed that epithelial ovarian cancer may be a cytokine-propelled disease.[45,53]

Among the several growth factors that have been

examined in ovarian cancer are transforming growth factor–β (TGF-β) and EGF and related factors, including TGF-α.[7,9,53] TGF-β is a 24-kd glycoprotein that can inhibit the growth of many epithelial cells.[69] EGF is a 6-kd glycoprotein, with a structure similar to that of TGF-α, that can stimulate the proliferation of normal ovarian epithelial cells; both EGF and TGF-α bind to the same receptor.[7,9] The response of ovarian cancer cell lines to EGF and TGF-α appears variable.[53] In fact, Bast and coworkers have seen that malignant epithelial ovarian cells are less responsive to EGF than are their nonmalignant counterparts.[7] TGF-β, which can act as an autocrine growth-inhibitory factor for normal ovarian epithelial cells, may act in a similar fashion for some ovarian cancer cells.[7] Therefore, loss of the ability to produce active TGF-β or to respond to this factor may have occurred in some ovarian cancers.

Various cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), macrophage/monocyte colony–stimulating factor (M-CSF) and interleukin 6 (IL-6), may also play important roles in ovarian cancer pathogenesis.[7,14,21,34,45,49] Cytokines, including IL-1 and IL-6, have been seen to enhance the proliferation of some ovarian cancer cell lines,[7,23] and various cytokines have been seen to be produced by ovarian cancer cells, including M-CSF, GM-CSF, IL-1, TNF-α and IL-6.[14,21,34]

Many ovarian cancer cells express both M-CSF and *fms*, the M-CSF receptor.[7,21,34,35,61] M-CSF is a homodimeric, 90-kd glycoprotein. Levels of *fms* transcripts were seen to correlate strongly with ovarian tumors of high histologic grade and advanced clinical stage and were associated with a poor outcome.[34] Also, elevated plasma levels of M-CSF were seen in 70–80% of patients with ovarian cancer.[7,34,35] It has been postulated that M-CSF–stimulated macrophages might produce other cytokines, such as IL-1 and IL-6, that can further stimulate tumor cell growth.[34] Therefore, M-CSF could potentially act as both an autocrine-panacrine tumor stimulatory factor and a factor that can modify the tumor cell environment, resulting in enhanced tumor cell growth.

IL-6

IL-6 is a growth-and-differentiation–inducing factor that is potentially relevant to the development and/or progression of ovarian cancer. IL-6 has been shown to act as an autocrine-paracrine growth factor for several types of human tumors, including multiple myeloma cells[36,38] and renal cancer cells.[51] In our own recent work we found IL-6 to act as an autocrine growth factor for acquired immunodeficiency syndrome–associated Kaposi's sarcoma.[52] Also, IL-6 has been shown to act as a programmed cell death–preventing factor in B-cell tumors.[64] Our studies indicate that many epithelial ovarian cancer cells produce IL-6.[70]

IL-6 is a pleiotropic cytokine with a wide range of biologic effects.[30,37] The gene for IL-6 has been cloned and sequenced and the structure of the IL-6 gene described. IL-6 is a 26-kd glycoprotein consisting of 212 amino acids, based on a sequence deduced from cloned IL-6 cDNA, with some sequence homology with human G-CSF as well as several recently described cytokines, including oncostatin M, ciliary neurotrophic growth factor and leukemia inhibitory factor. The gene for IL-6 in humans is on chromosome 7 and consists of 5 exons and 4 introns. The IL-6 biologic activities that have been described are induction of differentiation of activated B cells, support of plasmacytoma and myeloma growth, induction of acute phase reactants and stimulation of hepatocytes, nerve growth factor–like activity, induction of cytotoxic T cells and support of hematopoietic differentiation.[30] Clearly, IL-6 is a pleiotropic factor, with both growth- and differentiation-inducing properties. IL-6 can be produced by several types of cells, including T lymphocytes, monocyte/macrophages, fibroblasts, certain tumor cells, epithelial cells and endometrial cells.

The receptor for IL-6 (IL-6R) has been cloned.[74] It is composed of two polypeptides: an 80-kd IL-6–binding protein, which is associated on IL-6 binding with a dimer composed of a second, 130-kd, signal-transducing molecule. We have detected IL-6R (80 kd) expression in ovarian cancer cells.[71] Recently the gene for NF-IL6, a nuclear transcription factor that binds to a cytokine-responsive (IL-1) element in the IL-6 regulatory region, was cloned.[2] The NF-IL6 protein has some homology with the *fos* and *myc* oncogene products and interacts with the NF-IL6–binding motif within the IL-6 gene regulatory region, inducing IL-6 gene expression. Normally, NF-IL6 is not expressed, but its expression is induced by exposure of IL-6–producing cells to various inducers of IL-6 gene expression, including LPS and cytokines.

We have seen that ovarian cancer cells of epithelial origin (which constitute 90% of ovarian cancers) produce and secrete IL-6.[70] Several epithelial ovarian cancer cell lines, including SKOV-3, OVCAR-3

and OAOV 3, produce substantial amounts of biologically active IL-6.[70] Ovarian cancer cell lines of nonepithelial origin (PA-1 and 222) do not secrete IL-6, although cytoplasmic IL-6 was detected in PA-1 as well as in all the other epithelial ovarian cancer cell lines tested.[70] Primary ovarian tumor cell cultures also produce substantial levels of IL-6,[70] with primary isolates of ovarian tumor cells displaying intracellular IL-6 by immunoperoxidase staining. The IL-6 produced by ovarian cancer cells was examined by radioimmunoprecipitation and found to correspond in molecular weight to monocyte/lymphoid-derived IL-6.[70] Also, IL-6 mRNA expression indicated that most epithelial ovarian tumor cell lines display detectable levels of IL-6 gene expression.[70] Several cytokines (interferon-γ, IFN-γ, IL-1 and TNF) were seen to up-regulate IL-6 production by ovarian tumor cell lines.[70] Primary cultures of normal human ovarian epithelium also were seen to produce IL-6.[41] These results indicate that ovarian cancer cells—both established ovarian cancer cell lines, primary tumor isolates and normal ovarian epithelial cells—produce biologically active IL-6 that is indistinguishable from the IL-6 produced by peripheral blood mononuclear cells.

Ovarian cancer cells also express the IL-6 receptor. In a recent study we detected expression of the 80-kd subunit of the IL-6 receptor in most ovarian cancer cell lines tested by Northern blot analysis.[71] Naturally, if IL-6 is an autocrine positive or negative growth factor, ovarian cancer cells not only should be producing detectable quantities of secreted IL-6 and IL-6 mRNA but also should express detectable quantities of IL-6 receptor. Therefore, the observation that ovarian cancer cells express the IL-6 receptor allows the possibility that IL-6 might play a role as a growth/differentiation/viability factor for these cells.

Elevated levels of IL-6 were detected in ascitic fluid[70] and serum[13] from women with ovarian cancer. Ascitic fluid isolated from patients with ovarian cancer had very high levels of IL-6 (3.2–42 ng/mL), while those from controls without cancer were much lower (1.8–2.6 ng/mL).[70] Elevated serum IL-6 levels were seen to correlate with the presence of more extensive disease: elevated levels (> 0.2 U/mL) of IL-6 were seen in 16/21 (76%) of ovarian cancer patients with macroscopic disease but in only 13% of patients with microscopic disease or in 17% of healthy control donors.[13] The mean serum IL-6 concentration in the group of ovarian cancer patients with macroscopic disease (n = 21) was 0.26 ± 0.04 (SEM); serum levels of IL-6 in healthy adult donors were 0.12 ± 0.03 U/mL IL-6 (maximum < 0.020).[13] Serum IL-6 reached very elevated levels (> 1 U/mL) in some patients.

Recently we examined the *in vivo* biologic effects of IL-6 by correlating levels of IL-6, acute phase proteins (C-reactive protein [CRP], haptoglobin and $\alpha_2$macroglobulin) and IgM/A/G in ovarian cancer patients' ascitic fluid or serum (unpublished observations). Significantly elevated levels of IL-6, CRP, haptoglobin and IgA and decreased levels of IgM were seen in ovarian cancer ascites (n = 36) when compared to peritoneal fluids from a control group (donors with nonmalignant gynecologic conditions, n = 20). Again, in this study elevated serum IL-6 levels (6.5 ± 5.2 vs. 0.2 ± 0.02 U/mL) were seen in ovarian cancer (n = 7) as was increased serum CRP when compared to controls (n = 7). Ascites CRP correlated with IL-6 in both ovarian cancer ($P < .01$) and controls ($P < .001$). These results suggest that the increased levels of IL-6 *in vivo* in patients with ovarian cancer are associated with increased levels of acute phase proteins and IgA. This is particularly interesting since IL-6 can act both as a B-cell stimulating factor, inducing the differentiation of activated B cells to Ig-secreting cells, and as a potent inducer for the production of acute-phase reactants.[30,47,67,68]

Another recent observation suggests that IL-6 can play a role as a growth factor for ovarian cancer cells: activated monocytes produce factors that enhance ovarian tumor cell growth. Working with Robert Bast and colleagues at Duke University, we have seen that culture supernatants from activated human monocytes, which support ovarian tumor growth, contained significant amounts of IL-6.[73] Furthermore, anti–IL-6 serum inhibited part of this monocyte-produced growth-supporting activity. Therefore, IL-6 has the potential to act as a paracrine growth factor for ovarian cancer cells. Also, we have seen that ovarian cancer cells cultured in serum-free conditions, which result in greatly decreased endogenous IL-6 production, require exogenous IL-6 for optimal proliferation (unpublished observation).

While it is clear that IL-6 can act as an autocrine/paracrine growth factor for human tumors, the complete role of IL-6 in the host response to cancer or as a tumor growth–promoting or –suppressing factor is not clear.[48] Certainly, IL-6 has the potential to modulate antitumor immune

responses and may thereby act as an inhibitory factor for tumor cell growth.

Recently we examined the possibility that IL-6 acts as an autocrine growth factor for ovarian cancer cells by inhibiting IL-6 gene expression by exposure to IL-6 antisense oligonucleotides. This was seen to result in greatly decreased cellular proliferation: exposure of ovarian cancer cell lines (CAOV-3, OVCAR-3 and OC-436) to various concentrations of a single-stranded antisense IL-6 oligodeoxynucleotide, specific to a sequence in the second coding exon of the IL-6 gene, resulted in decreased IL-6 production and a >80–85% inhibition of cellular proliferation.[71] However, the addition of exogenous IL-6 failed to restore the proliferation of the antisense-treated cells. Also, antibodies to IL-6 did not consistently inhibit cell growth, nor did rIL-6 enhance growth at low cell concentrations. These results suggest that exogenous IL-6 does not directly induce the proliferation of ovarian cancer cells, although endogenous IL-6 production is needed for optimal cell growth. Since the majority of epithelial ovarian cancers produce IL-6, the direct specific inhibition of IL-6 gene expression may be of potential therapeutic value.

Preliminary studies indicate that various tumor cells can be protected from natural killer (NK) cell-mediated killing by IL-6 (unpublished observations).[33] Cells (CAOV-3 ovarian cancer cells) treated with dexamethasone, which results in greatly decreased IL-6 production,[62] showed greatly increased sensitivity to NK cell-mediated lysis. Addition of exogenous recombinant IL-6 to dexamethasone-treated cells restored resistance to NK lysis. These preliminary results suggest that IL-6 (endogenously produced or exogenous) can protect tumor cells from NK-mediated killing

IL-10

Epithelial malignancies of the ovary remain confined to the peritoneal cavity even in advanced stages of the disease. It has been suggested that the growth of ovarian cancer intraperitoneally could be related to the local deficiency of antitumor immune effector mechanisms, allowing tumor growth within the peritoneal cavity.[12] The presence of immunosuppressive factors in the ascites from ovarian epithelial tumor-bearing patients has been described previously.[3,32] In recent studies we observed that ascitic fluid from patients with ovarian cancer contains significantly elevated levels of IL-10,[28] a cytokine with various immunosuppressive activities.

IL-10 was originally identified as cytokine synthesis inhibitory factor because of its activity as an inhibitor of cytokine production.[25] The gene for IL-10 has been cloned.[54] This 35- to 40-kd cytokine is produced by the "type 2" CD4-positive helper T cell clones (TH2) and inhibits cytokine production of the "type 1" subset (TH1) in murine systems. TH1 and TH2 are two subpopulations of T helper cells, initially defined in mice, that control the nature of an immune response by secreting characteristic and mutually antagonistic sets of cytokines:[55] TH1 clones specifically produce IL-2 and IFN-γ, whereas TH2 clones produce IL-4, -5, -6 and -10. IL-10 inhibits the production of IL-2 and IFN-γ by TH1 cells.[27] While T cells corresponding strictly to TH1 and TH2 subsets have not been found in humans, increasing evidence points to the existence of TH1- and TH2-type responses in humans; a similar dichotomy between TH1- and TH2-type responses has recently been reported for the human.[23,60] Human IL-10 also inhibits the synthesis of IFN-γ and other cytokines by human peripheral blood mononuclear cells.[76] Furthermore, IL-10 is exceedingly potent at suppressing the release of such cytokines as IL-1, IL-6, IL-8 and TNF-α by activated monocytes.[16,24,26] IL-10 further down-regulates class II major histocompatibility complex molecule expression on monocytes, resulting in a strong reduction in antigen-presenting capacity of these cells.[24] Together, these observations support the concept that IL-10 plays an important role not only in the regulation of T cell responses but also as an antiinflammatory mediator. IL-10 is produced by T cells, B cells and monocytes.[76] Among T cells, CD4-positive cells appear to be the main producers of IL-10, whereas CD8-positive cells appear to be poor producers of this cytokine.

Very recently we measured levels of various cytokines, in collaboration with John Abrams, DNAX, Inc., in ascites obtained from women with ovarian cancer and found that levels of various cytokines, measured by enzyme-linked immunosorbent assay, were significantly elevated when compared to levels in peritoneal fluid from women who did not have ovarian cancer. In particular, levels of IL-10 and, as expected, IL-6 were particularly elevated in ovarian cancer ascites.[28] Our preliminary results indicate that ovarian cancer cells do not produce IL-10; ovarian cancer cell lines and primary tumor cells cultured *in vitro* did not produce

detectable levels of IL-10, while several of these lines did produce IL-6. Elevated levels of IL-10 were seen in nearly all ascites samples from women with ovarian cancer. Because peritoneal immunosuppression is characteristically seen in this disease, we are currently examining the role of IL-10 as a potential peritoneal immunosuppressive factor in ovarian cancer.

Peritoneal Cytokines in Ovarian Cancer

Since the development of ovarian cancer may involve cytokine dysregulation, we recently determined, in collaboration with John Abrams, the levels of various cytokines in ascites collected from women with ovarian cancer. As mentioned above, high levels of ascites IL-6 and -10 are seen in the great majority of ovarian cancer cases. Of those cases, 50–60% were seen to also contain significant ascites levels of IL-2, TNF-α, IFN-γ, G-CSF and GM-CSF.[72] Levels of various cytokines, including IL-6, IL-10, TNF-α, G-CSF and GM-CSF, were significantly elevated as compared to levels of these same cytokines seen in peritoneal fluids from normal women. A similar pattern was seen in sera from women with ovarian cancer, with IL-6 and IL-10 the serum cytokines detected most frequently. A significant correlation was seen between IL-6 (and IL-10) and acute phase proteins and immunoglobulins as well as the immunosuppressive activity of ascitic fluids and tumor histologic stage. Studies to determine whether a specific cytokine/tumor marker/acute phase reactant pattern could be useful in monitoring the progress of patients with ovarian cancer are under way.

Certainly it is possible that certain cytokine patterns, in particular high levels of IL-10, could result in a peritoneal environment characterized by immune unresponsiveness and promotion of tumor growth.

Conclusion

The dysregulation of various biologic and molecular factors may play important roles in the development and progression of ovarian cancer. These include oncogene overexpression, mutation or loss of antioncogenes, growth factor and cytokine stimulation, and modification of the host environment and host antitumor immune response. Understanding the specific mechanisms involved in ovarian cancer development and growth will allow opportunities for the design of effective antitumor treatments.

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Insulinlike Growth Factors

Their Regulation of Glucose and Amino Acid Transport in Placental Trophoblasts Isolated from First-Trimester Chorionic Villi

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The transport of glucose and amino acids from the maternal to fetal circulation through the placenta is critical to the delivery of fuel for normal fetal growth and development. Little information indicates that transplacental glucose or amino acid transport is influenced by hormones or polypeptide growth factors. We developed a continuous cell line of cytotrophoblastlike cells derived from first-trimester human chorionic villi as a model system to study the regulation of glucose and amino acid transport by insulinlike growth factors (IGFs). Using immunocytochemical and biochemical criteria, the cells were shown to manifest a trophoblastlike phenotype. The cells were maintained in serum-supplemented medium until confluent, at which time they were shifted to serum-free medium for one day. Experiments were initiated by transferring the cells to glucose-free assay buffer and incubating them with IGF-I, IGF-II or insulin. Glucose uptake was measured by the transport of 2-deoxy-D-[1,2-^{3}H]glucose (2[^{3}H]DG) in the presence or absence of cytochalasin B, which has been shown to competitively inhibit glucose uptake. IGF-I, IGF-II and insulin each enhanced 2[^{3}H]DG transport in a dose-dependent fashion. Amino acid transport was measured by incubation of the cells with IGF-I for 60 minutes, followed by a 5-minute challenge with α-[methyl-^{3}H]aminoisobutyric acid. IGF-I caused a dose-dependent increase in uptake of the amino acid analog. Radioreceptor assays using [^{125}I]insulinlike growth factor I ([^{125}I]IGF-I) demonstrated that the trophoblast-derived cells contained high-affinity, saturable receptors for IGF-I that also bound IGF-II. Insulin bound to this binding site with very low affinity. These data indicate that in contrast to previous reports that glucose and amino acid transport in the placenta are unregulated processes, IGFs may exert local modulation of acute metabolic actions in trophoblast-derived cells via IGF-I receptors. (J Reprod Med 39:249–256, 1994)

Keywords: growth substances, chorionic villi, trophoblast.

Introduction

The human placenta must meet the metabolic demands of the developing fetus through the delivery of important fuels, such as glucose, amino acids and fatty acids. Glucose is the major carbohydrate source for oxidative metabolism in the fetus and is transferred across the placenta via an energy-independent, carrier-mediated process that appears to be unregulated and determined solely by maternal blood glucose levels.[1–3] Derangements in maternal carbohydrate utilization (i.e., diabetes mellitus) that result in maternal hyperglycemia are known to have profound effects on fetal development, well-being, or both, including structural malformations and fetal macrosomia.[4] According to the Pedersen hypothesis, fetal macrosomia in the diabetic pregnancy derives from the delivery of excess glucose to the fetus by the placenta and a compensatory increase in insulin release by the fetal pancreas.[5] Insulin serves as a growth factor to drive cellular hyperplasia and hypertrophy, resulting in an increased risk of diabetic fetopathy.

Controversy exists, however, as to whether

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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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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 (Σ^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 (Σ^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 (Σ^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 (Σ^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[a]				
Gravid	176	376	1.0[c]	
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[c]	
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[c]	
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]				
0	185	330	1.0[c]	
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)[a]				
15–19	26	65	1.0	
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	(0.6–2.7)
≥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[a]				
None	44	107	1.0[c]	
≤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			

[a] All relative risks adjusted for age and social class. [b] Reference category. [c] Data missing for 1 control. [d] Relative risks adjusted for parity. [e] Data missing for 2 cases and 1 control. [f] Women with livebirths only. [g] 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	(0.4–7.8)
> 120	1	4	0.7	(0.1–20.4)
	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	128	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[c]	
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)[d]				
<45	10	34	1.0[c]	
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. [d]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 c[illegible]ssociated with ever having recieved hormone replacemen[illegible]apy (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%[a] 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	422	249	1.0*	
Yes	34	44	1.5	(0.9–2.6)
Women reporting hysterectomy				
Use of hormone replacement therapy				
No	5	62	1.0*	
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*	
Yes	29	34	1.2	(0.7–2.3)

†Post-menopausal women only. ‡Relative risks adjusted for age and social class. *Reference category.

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

Factor	RR	(95% CI)	χ² test for trend (1 d.f.)
Gravidity†‡			
0	1.0*		
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)†			
< 1	1.0*		
1–40	1.3	(0.8–2.0)	
41–120	1.1	(0.5–2.5)	
> 120	1.9	(1.2–3.2)	7.8 $P < 0.05$
Oral contraceptive use			
Never used	1.0*		
≤ 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	0.2	(0.05–0.6)‖	
Age at natural menopause§			
≤ 45 years	1.0		
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¶	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. ‡Reference category. §Sexually active women only. ¶Relative risks not adjusted for sterilization: see text for details. ‖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*				
Never	76	178	1.0†	
Rarely	6	16	0.9	(0.3–2.4)
Monthly	7	24	0.7	(0.3–1.8)
Weekly	57	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. †Reference category. *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; Fesher *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.

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SHORT COMMUNICATION

Pulmonary giant cell carcinoma: the relation to smoking

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The relation of smoking to the occurrence of the most common types of lung cancer has been examined and found positive in epidemiological studies. Types that have been causally ascribed to smoking in such studies have included squamous cell, small and large cell, and adenocarcinomas (US Dept of Health & Human Services, 1982; International Agency for Research on Cancer, 1986). However, rarer types, such as giant and alveolar cell carcinomas, have not been subject to separate study, not only because of their rarity, but because some pathologic classification systems for lung cancer include these types with other histologies (Yesner & Carter, 1982). While all histological types of lung cancer studied thus far have been related to tobacco use and no type has yet been found to be unrelated, there have been questions raised about the relationship of smoking to these rarer types.

Giant cell carcinoma of the lung was first described by Nash and Stout in 1958. Some investigators classify it as a separate entity (Shin et al., 1986). On the other hand, some have included it as a sub-type of adenocarcinoma of the lung, while both the World Health Organization and the Armed Forces Institute of Pathology include it with large cell undifferentiated carcinoma (Razzuk et al., 1970). These several schemes lead to non-uniform criteria in the literature for diagnosis of pulmonary giant cell carcinoma. A survey such as ours cannot resolve this difficulty and, therefore, we must accept the cases reported as genuine for our purpose.

This survey is an attempt to provide some information bearing on the aetiological relationship of giant cell carcinoma of the lung to smoking. We searched the literature, as indexed by Medline through 1986, for case reports of pulmonary giant cell carcinoma which included smoking histories.

We found reports of 119 cases of pulmonary giant cell carcinoma with smoking histories of the patients. Only eight of these cases were female, five smokers and three non-smokers, which are too few to analyse separately. Therefore, we confined our analysis to males. The 111 male cases with smoking data were found in 23 independent studies (Bendel & Ishak, 1961; Broderick et al., 1975; Dailey & Marcuse, 1969; DeAngelis et al., 1961; Flanagan & Roeckel, 1964; Friedberg, 1965; Gajaraj et al., 1971; Guillan & Zelman, 1966; Hathaway et al., 1969; Hellstrom & Fisher, 1963; Horie & Ohta, 1981; Kallenberg & Jaque, 1979; Kennedy, 1969; Lerner, 1967; Naib, 1961; Matsuo et al., 1986; Nash & Stout, 1958; Pfeffer & Stoven, 1978; Pfitzer & Knoblich, 1975; Shin et al., 1986; Thomas, 1962; Wang et al., 1976). (Although not reported as such in the publication, one woman in the study of Shin et al. (1986) was a smoker, as were 8 of the 13 men; Shin, personal communication.) We also found 99 other cases, 81 male and 18 female, in other studies which did not contain smoking information (12 references not cited). Therefore, the male: female sex ratio for pulmonary giant cell carcinoma is 7.4:1.

Of the 111 men with giant cell carcinoma of the lung, only eight (7.2%) had been non-smokers. This 0.93 proportion of smokers among men with giant cell carcinoma of the lung has a 95% confidence interval of 0.86–0.96 (Rothman & Boice, 1979). This confidence interval does not come near to overlapping any of the estimates of the prevalence of smoking among US males and, therefore, is highly statistically significant (Table I). Most reports did not include information on the years or amount smoked, so we could not examine the dose reponse.

Smoking prevalence data, based on a US national health survey (US Dept of Health & Human Services, 1983), show that 52%, 42% and 38% of all males were current smokers in 1965, 1976 and 1980 respectively, while 20%, 30% and 31% were former smokers. Therefore, 72%, 72% and 69% of US males had a positive smoking history in those years. The prevalence of a history of smoking in males is then about 71% over the period 1965–1980. Using that rate, one can calculate a relative risk of 5.3 for pulmonary giant cell carcinoma due to ever having smoked. Many of our 23 studies did not distinguish between current and former smokers. Therefore, the risk estimate of 5.3 is too low if the smoking status in some of these studies included only current smokers. To obtain an upper limit to this estimated risk, we can compare our tabulation to the 44% mean prevalence of current smoking in the US data from those three years. Such calculation yields a risk ratio of 16. This range of risk (5.3–16) is equal to or higher than the relative risks published for other histological types of lung cancer (US Dept of Health & Human Services, 1982; International Agency for Research on Cancer, 1986). Our use of US smoking prevalence for the years 1965–1980 as a population comparison is justified by the fact that the reports we used were from 1958 to 1986, essentially the same period, and that 83% of our reported cases were from the US.

There is a possibility that the authors of the case histories may have mentioned a positive history of smoking more readily than a negative one. This bias cannot be completely excluded. However, in order to have the 95% confidence limits (Rothman & Boice, 1979) of our observed proportion overlap the expected 71% prevalence of a positive history of smoking, at least 20 non-smokers must have been unreported. This would mean that the non-smokers would have to have been at least 70% under-reported for our result to become non-significant, which is an unreasonably high proportion.

In summary, we abstracted smoking data from 23 published clinical reports of giant cell carcinoma of the lung in 111 men, of whom 93% were or had been smokers. This proportion is significantly higher than the 71% prevelance of a history of smoking among US men in the corresponding

Table I Prevalence of smokers among males

	Smokers	*Odds ratio*	*P value*
Giant cell lung cancer cases	93% (98/111)		
vs			
US population 1965–80			
Current smokers	44%	5.3	<0.001
Ever smokers	72%	16	<0.001

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REVIEW ARTICLE

MEDICAL PROGRESS

CANCER OF THE OVARY

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EPITHELIAL cancer of the ovary is the fifth most common malignant condition among women in the United States, with an annual incidence of 22,000 new cases.[1] This disease predominantly affects postmenopausal women in their sixth decade, accounting for approximately 13,300 deaths each year and for over half of all deaths from genital cancer. The highly lethal nature of this tumor is related to the absence of symptoms in the majority of women with early stages of the disease. Seventy percent of women present with advanced disease in which the tumor has spread to the peritoneal surfaces of the upper abdomen. Extensive intraabdominal disease is difficult to eradicate completely by surgery, and many patients have only a partial response to postoperative chemotherapy. This review will discuss selected issues concerning the risk factors for and diagnosis of ovarian cancer, followed by a detailed discussion of treatment options for patients with newly diagnosed disease and those with persistent disease.

Risk Factors for Ovarian Cancer

The average lifetime risk for the development of ovarian cancer in women in the United States is 1 in 70. Women with uninterrupted ovulation appear to be at higher risk for malignant transformation of the ovarian surface epithelium. For instance, a higher risk of ovarian cancer is associated with nulliparity or a first birth after the age of 35.[2] Conversely, a lower risk is associated with childbirth, especially at age 25 or younger, or with the use of oral contraceptives.[2] However, a family history of ovarian cancer, especially if two or more first-degree relatives have been affected, is the most important risk factor.[3,4] At least two well-described familial ovarian cancer syndromes exist, known as the hereditary breast–ovarian cancer syndrome and the Lynch syndrome II.[3] The hereditary breast–ovarian cancer syndrome is characterized by a familial predisposition to breast and ovarian cancer, with an autosomal dominant pattern of inheritance derived from either maternal or paternal genes. The Lynch syndrome II is characterized by a similar mode of inheritance and a familial predisposition to ovarian cancer, endometrial cancer, and colon cancer without polyposis. Familial ovarian cancer accounts for less than 5 percent of all ovarian cancers and tends to occur in women at a younger age than its sporadic counterpart. Although the responsible gene (or genes) has not yet been identified, linkage analysis suggests that a candidate gene referred to as *BRCA-1* is located on the long arm of chromosome 17 at site 17q12-q23.[5]

For women with a strong family history of ovarian cancer, management typically includes frequent screening and consideration of prophylactic oophorectomy after age 35 and when childbearing is complete.[4] It is not yet known whether currently available screening methods will permit the efficient detection of disease at an early stage or improve survival. A screening method that uses the CA-125 serum tumor marker (see below), followed by confirmatory pelvic ultrasonography for women with elevated levels, has a specificity of 99.9 percent in postmenopausal women, although the sensitivity of this combined approach is still only approximately 50 percent.[6,7] Further investigation of such screening strategies will be required before firm recommendations can be made regarding their general use.

Clinical Presentation

The most common symptoms of ovarian cancer are related to extension of the disease outside the pelvis. Many women present with abdominal fullness and early satiety due to ascites and omental tumor implants (Fig. 1). Occasionally, a woman will undergo an extensive gastrointestinal evaluation for presumed hiatal hernia or gallbladder disease before the true cause of these nonspecific symptoms is identified. Abdominal examination may reveal ascites, upper abdominal fullness from omental involvement, umbilical hernia due to increased abdominal pressure, or umbilical lymph-node involvement (Sister Mary Joseph's node). A minority of patients (30 percent) with disease still confined to the pelvis present with occasional pelvic pain due to intermittent ovarian torsion, but most patients with limited disease are asymptomatic and are identified by routine pelvic examination. Pelvic examination may reveal an ovarian mass in a premenopausal woman or a palpable ovary in a postmenopausal woman. Although ovarian cancer is a disease that initially spreads throughout the abdominal cavity, examination may sometimes reveal a pleural effusion or involvement of the axillary or inguinal lymph nodes.[8,9] In addition, patients with abdominal disease frequently have asymptomatic involvement of the pelvic and retroperitoneal nodes, usually documented at the time of surgery.[10] Patients with ovarian cancer may occasionally present with various types of paraneoplastic conditions, such as humorally mediated hypercalcemia (clear-cell histologic features),[12,13] cerebellar degeneration (associated with antibodies to

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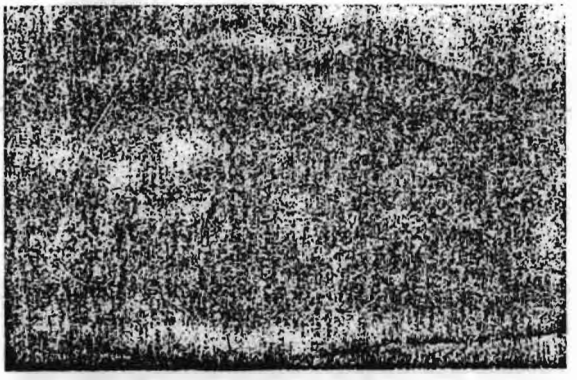

Figure 1. Intraoperative Appearance of Stage III Ovarian Cancer Involving the Upper Abdomen.

The exploratory laparotomy is performed through a vertical, midline incision to permit adequate visualization of the upper abdomen. Several serosal implants are shown in a patient with stage III disease. (Photograph courtesy of Dr. Jonathan Niloff, Department of Gynecologic Oncology, Beth Israel Hospital, Boston.)

Purkinje's cells),[14,15] the sudden appearance of seborrheic keratosis (sign of Leser–Trélat),[16] or chronic intravascular coagulation leading to arterial and venous thrombi (Trousseau's syndrome). In rare cases, patients have presented with brain metastasis as the first sign of disease.[17]

Once a pelvic mass has been detected, confirmatory studies include a pelvic ultrasound examination and a CA-125 determination. Pelvic ultrasonography, especially when performed transvaginally,[8,18] is a more sensitive technique than computed tomography (CT) for imaging the ovary, and it provides information that is predictive of the presence of cancer. Specifically, benign cysts usually appear as single, simple structures without internal echoes, whereas an ovarian tumor may appear as a multicystic complex mass. The CA-125 antigen is a serum tumor marker that is elevated in 80 percent of patients with advanced ovarian cancer.[19,20] Consequently, this tumor marker often provides confirmatory evidence of ovarian cancer in a woman with a pelvic mass and suspicious findings on ultrasound evaluation. In one study of 158 patients with pelvic masses, a CA-125 level above 65 U per milliliter was highly suggestive of cancer, with a positive predictive value of 98 percent in postmenopausal women.[21] However, the CA-125 antigen is not a specific marker for ovarian cancer. Elevated levels of the antigen may also be associated with several other tumors, such as breast, lung, and gastrointestinal cancers; with nonmalignant conditions, such as benign cysts and endometriosis; and with menstruation and pregnancy. In addition, the results of this test are normal in up to 50 percent of patients with early-stage ovarian cancer.[19,20] Thus, an elevated CA-125 level does not guarantee the presence of ovarian cancer, and a normal CA-125 level does not preclude the need for surgery if the pelvic examination and ultrasound study suggest cancer.

Staging and Surgical Treatment

An exploratory laparotomy is often necessary to diagnose ovarian cancer, since ovarian masses may also be caused by benign cysts; by other primary ovarian tumors, such as germ-cell or stromal-cell neoplasms; or by metastasis to the ovary from a primary gastrointestinal or breast cancer.[22,23] The pathological distinction between epithelial ovarian cancer and these other possibilities is usually straightforward; the common histologic features are shown in Table 1.

Once epithelial ovarian cancer has been confirmed, a total abdominal hysterectomy, bilateral salpingo-oophorectomy, and omentectomy are usually performed, along with a careful examination of all serosal surfaces, biopsies of grossly involved areas, and collection of ascites or peritoneal washings for cytologic studies. If the disease appears to be limited to the ovary, the retroperitoneal nodes are also examined, since evidence of lymphatic involvement alters the postoperative treatment approach (see below). Occurrence of intraoperative rupture of the primary ovarian mass is also noted. Finally, an attempt is made to debulk all gross disease, and the amount of residual peritoneal or serosal implants remaining at the completion of surgery is noted. The staging system defined by the International Federation of Gynecologic Oncologists (see Table 2) assumes that an adequate staging operation has been performed.

Adverse prognostic factors in ovarian cancer include an advanced stage of disease, a high tumor grade, the presence of residual disease after initial surgery, older age, clear-cell or mucinous histologic features, overexpression of the epidermal-growth factor

Table 1. Common Histologic Features and Clinical Correlates of Epithelial Ovarian Cancer.

Histologic Feature	Clinical Correlates
Papillary serous	The most common type of ovarian cancer
Endometrioid	Occasionally associated with primary endometrial cancer or endometriosis; bilateral presentation in up to 40 percent of cases
Mucinous	Often associated with normal or only minimally elevated CA-125 levels
Clear cell	Stage for stage, the worst prognosis, because of relative insensitivity to chemotherapy, associated with endometriosis and humorally mediated hypercalcemia

Table 2. FIGO Staging System for Epithelial Cancer of the Ovary.*

Stage I: tumor limited to the ovaries
IA One ovary, no ascites, intact capsule
IB Both ovaries, no ascites, intact capsule
IC Ruptured capsule, capsular involvement, positive peritoneal washings, or malignant ascites
Stage II: ovarian tumor with pelvic extension
IIA Pelvic extension to uterus or tubes
IIB Pelvic extension to other pelvic organs (bladder, rectum, or vagina)
IIC Pelvic extension, plus findings indicated for IC
Stage III: tumor outside the pelvis or with positive nodes
IIIA Microscopic seeding outside the true pelvis
IIIB Gross deposits ≤2 cm
IIIC Gross deposits >2 cm or positive nodes
Stage IV: distant organ involvement, including liver parenchyma or pleural space

*FIGO denotes International Federation of Gynecology Obstetrics.

receptor, and amplification of the *c-neu* proto-oncogene.[24,25] Patients with stage III disease and residual peritoneal or serosal implants under 0.5 cm in diameter have a median survival of 40 months, and those with residual implants of 0.5 to 2 cm have a median survival of 18 months. Patients with bulky residual tumors that are over 2 cm in diameter have a median survival in the range of 6 to 12 months, and overall long-term survival is less than 10 percent.[30,31] The strong correlation between the amount of residual disease and survival in patients with stage III disease has been used as the main justification for debulking, although it is possible that this correlation simply represents a selection phenomenon whereby patients with more indolent or chemosensitive disease are identified on the basis of their ability to undergo successful debulking, which is partly a function of the volume and location of the disease.[32] Not surprisingly, the ability to debulk tumors in patients with stage IV disease is not necessarily associated with prolonged survival,[33] although selected patients may still benefit from the relief of imminent bowel obstruction before chemotherapy is started.

Postoperative Treatment

Combination chemotherapy that includes a platinum analogue — either cisplatin or carboplatin — is the standard postoperative therapy for patients with residual disease or those at high risk for recurrent disease after initial surgery. Although alkylating agents such as melphalan induce responses in about 30 percent of patients with advanced disease,[34] platinum-based chemotherapy has resulted in a substantially improved response rate of approximately 60 percent and prolongation of median survival by about six months.[35,36]

Limited Disease (Stages I and II)

Patients with stage IA or IB tumors (Table 2) that are well or moderately well differentiated have a five-year disease-free survival of 91 to 98 percent, which is not improved by adjuvant treatment.[37] In selected patients with stage IA tumors that are well or moderately well differentiated who wish to remain fertile, a unilateral salpingo-oophorectomy may be performed, without evidence of compromised survival.[38] Patients with either stage IC (e.g., capsular involvement or positive washings), poorly differentiated stage I, or any stage II disease have a higher risk of relapse, and these patients are often considered for postoperative adjuvant therapy.[37,39] In a trial by the Ovarian Cancer Study Group, such higher-risk patients were randomly assigned to receive either 12 months of oral melphalan or one dose of intraperitoneal chromic phosphate ^{32}P, resulting in equivalent 5-year disease-free survival rates in the 80 percent range.[37] However, prolonged melphalan treatment is associated with an increased risk of leukemia, and the distribution of intraperitoneal ^{32}P may not be uniform in patients who have undergone surgery and in whom intraabdominal adhesions subsequently develop. In a current study by the Gynecologic Oncology Group, such patients are randomly assigned to receive either one dose of intraperitoneal ^{32}P or three cycles of intravenous cyclophosphamide and cisplatin. Patients with stage IC, poorly differentiated stage I, or any stage II ovarian cancer who are not enrolled in clinical studies are often treated with adjuvant platinum-based chemotherapy as described below.

Advanced Disease (Stages III and IV)

The majority of women with ovarian cancer present with stage III or IV disease. The treatment of advanced disease with platinum-based chemotherapy has resulted in an improved response rate and longer median survival, but the overall survival rate is still only about 20 percent. Since this is a heterogeneous group of patients, overall survival is a function of both the stage and the amount of residual disease after initial debulking. Patients with stage III disease who have undergone optional debulking (<1 to 2 cm of residual tumor) have a four-year survival rate of approximately 30 percent[40]; those with stage IV disease or stage III disease and suboptimal debulking (>2 cm of residual tumor) have less than a 10 percent chance of long-term survival.[41,42]

Although many types of platinum-based combination chemotherapy have been investigated for the treatment of advanced ovarian cancer, the regimen of cisplatin plus cyclophosphamide is just as effective as more complicated and toxic regimens, such as those containing altretamine or doxorubicin hydrochloride.[36,42,43] However, the toxic effects of cisplatin, including renal insufficiency, peripheral neuropathy, ototoxicity, and nausea, have prompted the investigation of less toxic platinum analogues such as carboplatin. In two recent randomized trials comparing cisplatin plus cyclophosphamide with carboplatin plus cyclophosphamide to treat advanced ovarian cancer, the carboplatin regimen was better tolerated, with less nausea, less renal toxicity, and less neurotoxicity.[44,45]

The primary toxic effect of carboplatin is bone marrow suppression, with cumulative thrombocytopenia that occasionally interferes with the chemotherapy dosage. Nevertheless, the two regimens have been shown to be equally effective,[46,48] and combination therapy consisting of carboplatin plus cyclophosphamide for six cycles (which can be given on an outpatient basis) is now a widely accepted regimen for the treatment of advanced-stage ovarian cancer. Continuing chemotherapy beyond six cycles does not extend survival.[47,48]

The majority of patients with advanced-stage ovarian cancer have a complete clinical response (as defined by a normal physical examination, CT scan, and CA-125 level) to surgical debulking and postoperative chemotherapy (Fig. 2). However, only 50 percent of these patients have a complete pathological response (as defined by negative biopsy specimens obtained during a second-look laparotomy).[49,50] The high false negative rate of clinical evaluation relates to the fact that persistent disease is usually manifested by either small-volume gross deposits or microscopic disease, both being below the level of detection by CT scanning or CA-125 testing. Thus, the second-look laparotomy is the most sensitive way of detecting persistent disease after platinum chemotherapy, although there are currently no standard second-line salvage regimens that are curative for this

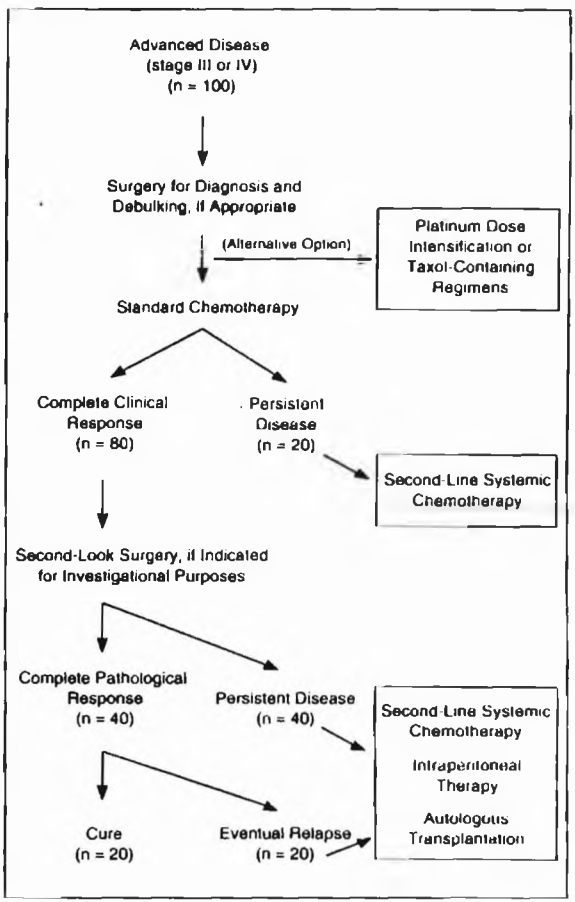

Figure 2. Representative Outcome in 100 Patients with Advanced-Stage Ovarian Cancer.

Exploratory laparotomy with debulking is appropriate for patients with stage III disease. Debulking should be used selectively in stage IV disease for specific indications such as imminent bowel obstruction. The most common regimen is standard-dose cyclophosphamide with carboplatin or cisplatin. Taxol plus cisplatin is a more active regimen for selected patients with bulky residual disease, although the effect of this treatment on overall survival is not yet known. Several investigational regimens containing taxol, high-dose carboplatin, or both are also available for patients with appropriate stage III or IV disease. A complete clinical response is defined as a normal physical examination, CT scan, and CA-125 level. Second-look surgery is performed if the finding of residual disease would make the patient eligible for innovative second-line treatment programs. The relapse rate for patients with a complete pathological response is about 50 percent. Persistent clinical disease is usually manifested by an elevated CA-125 level or an abnormal physical examination or CT scan. Symptomatic residual disease is usually treated with second-line chemotherapy regimens such as taxol. Patients are occasionally considered for secondary debulking, after which they may be eligible for investigational intraperitoneal therapy, if appropriate. Persistent, minimal disease at second-look surgery may be treated with second-line chemotherapy regimens, intraperitoneal chemotherapy, or autologous transplantation. A rapid recurrence (within six months) is often treated with agents that are potentially not cross-resistant to platinum, such as taxol. A late recurrence (after six months) may respond to additional standard doses of platinum-based chemotherapy. Like patients with persistent disease, those with recurrent disease are occasionally considered for secondary debulking, followed by investigational intraperitoneal chemotherapy. Appropriate patients who have an excellent response to second-line chemotherapy may also be considered for autologous transplantation.

patient group. In addition, patients who do have a complete pathological response as determined by second-look surgery still have a 50 percent chance of relapse.[11,12] Thus, even though laparotomy is more sensitive than clinical evaluation, the detection of active disease has little effect on survival, and the surgical procedure has a high false negative rate. On the basis of these considerations, second-look laparotomy is no longer performed routinely, unless the detection of disease would make the patient eligible for innovative treatment approaches such as those outlined below.

Methods to Obtain a Complete Pathological Response in Advanced Ovarian Cancer

The inability to obtain a complete pathological response with standard doses of carboplatin and cyclophosphamide is the chief obstacle to cure in advanced ovarian cancer (Fig. 2). Strategies to increase the rate of complete pathological responses include the use of new drugs with unique mechanisms of action, such as taxol, and dose intensification to improve the activity of platinum analogues. Taxol is a diterpene plant product isolated from the bark of the Pacific yew tree, *Taxus brevifolia*. It exerts cytotoxic effects through a unique mechanism of action involving microtubule polymerization, rendering the mitotic spindle dysfunctional and resulting in an arrest between the second gap (G_2) phase and the mitotic (M) phase of the cell cycle. As discussed below, this drug was originally noted to be effective in platinum-refractory ovarian cancer, suggesting that taxol and cisplatin do not share common mechanisms of drug resistance. In a recent trial by the Gynecologic Oncology Group, the two drugs were combined to treat patients with newly diagnosed, advanced disease.[13] A total of 394 patients with residual tumors over 1 cm in diameter were randomly assigned to receive either cyclophosphamide plus cisplatin or taxol plus cisplatin every three weeks for a total of six cycles. Although the patients receiving taxol had a greater degree of neutropenia, the incidence of documented episodes of sepsis was similar for the two groups, and the taxol–cisplatin regimen was generally well tolerated. The overall response rate of the taxol–cisplatin group was significantly higher than that of the cyclophosphamide–cisplatin group (79 percent vs. 63 percent, $P<0.01$), and the median periods of survival without progression of disease were 17.9 and 13.8 months, respectively. The rates of complete pathological responses for the taxol–cisplatin group and the cyclophosphamide–cisplatin group were not significantly different (26 percent vs. 19 percent, respectively; $P = 0.07$), suggesting that the increased response rate with taxol may not necessarily result in improved long-term survival. A longer period of follow-up will be required to address this issue. Nevertheless, this study has shown that taxol combined with cisplatin is a highly active regimen that represents a reasonable alternative to standard chemotherapy for selected patients with newly diagnosed, advanced disease.

Dose intensification is another strategy for improving the response rate in ovarian cancer. The theory underlying a higher platinum dose is that many tumors exhibit a steep dose–response curve to this drug in vitro, meaning that a relatively small increase in the dose may result in a large increase in the number of tumor cells killed. Support for this concept comes from a retrospective study by Levin and Hryniuk demonstrating that a higher platinum dose is associated with a superior outcome in patients with ovarian cancer.[52] A recent randomized study compared two doses of cisplatin — 50 versus 100 mg per square meter of body-surface area — administered with cyclophosphamide every three weeks for a total of six cycles.[53] The median survival was significantly improved in the higher-dose group, with the effect being most prominent in patients with residual tumors under 2 cm in diameter after initial surgery (median survival, three years). Since this study used doses of cisplatin that are still within the standard range, the results do not address the value of using even higher chemotherapy doses to treat advanced ovarian cancer. Regimens of systemic high-dose cisplatin or carboplatin may induce additional responses in 20 to 30 percent of patients with residual disease after standard doses of platinum chemotherapy (usually in those with a previous response to standard-dose platinum), providing further support for the concept of a steep dose–response curve.[54,55]

Among newly diagnosed patients, however, convincing improvements in the rate of complete pathological responses or survival have not yet been observed with systemic dose intensification of platinum analogues.[54,56,59] As Jodrell et al. suggest, this lack of improvement may reflect a plateau in the dose–response curve, so that higher platinum doses may not necessarily yield improved response rates.[60] In addition, some patients are unable to tolerate sequential cycles of high-dose cisplatin because of nonhematologic toxicity, such as nephrotoxicity and neuropathy, which may be severe at doses as high as 200 mg per square meter of body-surface area per cycle. Likewise, many patients are unable to tolerate sequential cycles of high-dose carboplatin because of cumulative bone marrow toxicity, usually in the form of persistent thrombocytopenia, which is often unresponsive to treatment with hematopoietic growth factor.[61-63] Recently, several groups have begun to treat newly diagnosed, advanced-stage ovarian cancer with high-dose carboplatin followed by an infusion of autologous peripheral-blood progenitor cells in an attempt to increase the dose without incurring excessive bone marrow toxicity.[64,65] The effect of this strategy on the rate of complete pathological responses among patients with newly diagnosed, advanced disease will require further investigation.

Treatment Options for Persistent or Recurrent Disease

Patients who have persistent or recurrent disease after initial chemotherapy are generally not curable.

Persistent disease is often heralded by a CA-125 level that does not return to normal after three cycles of platinum treatment[69] or that remains elevated after completion of chemotherapy.[70-72] Similarly, a new elevation in the CA-125 level is often the first sign of relapse and may occur before a clinically apparent tumor (as detected by physical examination or CT scanning), with a median lead time of three months.[71] However, since second-line treatment approaches are potentially toxic and generally palliative in nature, the decision to treat patients with persistent or recurrent disease is often based on either the presence of symptoms, such as abdominal discomfort resulting from a documented mass or ascites, or the eligibility of patients for innovative treatment approaches (see below).

Patients with disease that persists after initial chemotherapy or recurs within six months after treatment are unlikely to benefit from additional standard-dose platinum.[73] In contrast, patients with disease that recurs more than six months after their initial treatment may have a good chance of benefiting from second-line cisplatin or carboplatin (27 to 59 percent response rate),[73] although such responses are generally not associated with long-term survival. A description of treatment options for the management of persistent or recurrent disease is presented below and summarized in Figure 2.

Secondary Debulking Surgery

Patients with persistent or recurrent disease are sometimes treated with another debulking procedure in an attempt to improve their survival. Proponents of this procedure have reported an improved median survival rate for patients in whom secondary debulking resulted in minimal residual disease, although most patients treated in this way have been carefully selected and are nevertheless destined to die of their disease.[42,74-76] As with initial debulking, it is difficult to know whether the secondary debulking itself has a therapeutic effect or even a durable palliative effect, or whether the patients in whom the procedure is successful are those who have more indolent disease. Since secondary debulking is not curative and may be associated with considerable morbidity in patients who may otherwise be asymptomatic at the time, its routine use cannot be recommended. However, there are patients who may benefit from secondary debulking, including those with imminent bowel obstruction due to a single dominant pelvic mass and those who have a relapse many years after the initial diagnosis, in whom an exploratory laparotomy to verify the diagnosis and debulk the tumor may be followed by several more years of disease-free survival. Finally, secondary debulking of gross disease may be appropriate for patients who are eligible for treatment programs involving intraperitoneal chemotherapy, since it is known that this form of treatment induces responses only if there is minimal disease, as discussed below. In a noninvestigational setting, however, secondary debulking should have a limited role in the palliative care of patients with ovarian cancer, and its use should be individualized.

Intraperitoneal Chemotherapy

Intraperitoneal chemotherapy consists of administering drugs such as cisplatin and etoposide through an indwelling catheter inserted into the peritoneal cavity. Although this procedure has been used for several years to treat patients with residual disease after initial systemic platinum therapy, its impact on survival in these patients is still unknown owing to the lack of randomized trials in this area until recently. Appropriate drugs for intraperitoneal therapy are those that have a favorable ratio of peritoneal to plasma drug concentration, those that are nonirritating when instilled, and those in which a small increment in the dose concentration may result in an additional cytotoxic response (i.e., drugs with a steep dose–response curve). Cisplatin and taxol display such characteristics, and dose schedules have been established that provide a pharmacologic advantage with acceptable toxicity.[77,78] Since intraperitoneal chemotherapy is a form of topical treatment, drug diffusion into tissue is limited to the first several millimeters of tumor at best, suggesting that bulky tumor masses cannot be expected to respond (although responses can still be induced with systemically absorbed drug). Several phase I and phase II studies using platinum-based intraperitoneal regimens have reported an acceptable level of toxicity and a complete surgical response rate as high as 20 to 30 percent in patients with residual small-volume disease who have previously had a response to systemic platinum.[77,79-81] Nevertheless, the importance of these responses, in terms of both palliation and cure, remains uncertain, and most patients eventually die of progressive disease. Additional studies have shown that the patients who are most likely to benefit from intraperitoneal treatment are those with minimal tumors (<1 to 2 cm) who do not have diffuse carcinomatosis, evidence of progressive disease during therapy with systemic platinum, extensive intraabdominal adhesions that would prevent homogeneous drug distribution, or disease in inaccessible sites such as the lymph nodes or liver parenchyma.[82,83] These restrictions suggest that intraperitoneal therapy may be appropriate for only a small subgroup of patients with persistent or rapidly recurring disease.

It is possible that a dose-intensification strategy such as intraperitoneal therapy will be most effective early in the treatment of the disease, when a substantial fraction of tumor cells are still chemosensitive. There are at least two randomized trials under way that should determine the value, if any, of intraperitoneal chemotherapy as a means of dose intensification in this setting. The Gynecologic Oncology Group is conducting a randomized trial of intraperitoneal versus intravenous cisplatin (with a standard dose of intravenous cyclophosphamide in both arms) in patients with newly diagnosed stage III disease that has been optimally debulked. The European

Organization for the Research and Treatment of Cancer is conducting a randomized trial comparing intraperitoneal platinum-based therapy with no further treatment as a means of improving the complete pathological response in patients with advanced-stage disease.

Radiotherapy

There is no convincing evidence that whole-abdomen radiotherapy can improve survival in patients who have persistent residual disease after platinum chemotherapy.[30,84-87] Patients with macroscopic residual disease, especially if poorly differentiated, clearly do not benefit from whole-abdomen radiotherapy,[87] and the toxic effects of this treatment include small-bowel obstruction, radiation enteritis, and myelosuppression. Methods to overcome mechanisms of resistance to radiotherapy after failure of platinum therapy or to decrease toxicity through hyperfractionation techniques are currently under investigation.[88-90] Although radiotherapy cannot be routinely recommended as a salvage treatment in ovarian cancer, it occasionally provides palliation for patients with recurrent pelvic disease who are not candidates for systemic chemotherapy.

Systemic, Non–Cross-Resistant Chemotherapy

Systemic treatment with agents that are not cross-resistant to platinum represents the mainstay of treatment for patients with platinum-refractory disease, as defined above. Although several drugs exhibit activity in this patient population, response rates are low (10 to 15 percent), and most responses are partial and short-lived.

Potentially useful drugs in treating platinum-refractory disease include altretamine,[91,92] melphalan, ifosfamide,[93] tamoxifen,[94] and taxol.[95-97] As stated above, the most active agent in this category is taxol, which induces response rates of approximately 25 percent in patients with platinum-refractory disease. In contrast to most other chemotherapy agents, taxol may require at least three or four cycles of administration before a response is evident. The drug is generally administered intravenously over a period of 24 hours on an inpatient basis, with dexamethasone, cimetidine, and diphenhydramine prophylaxis to prevent the hypersensitivity reactions frequently observed in the past, thought to be due to either taxol itself or its Cremophor vehicle.[96] The main toxic effect of taxol is neutropenia, often without suppression of red-cell or platelet counts, as well as cardiac-conduction disturbances and peripheral neuropathy. The dose of taxol can be escalated through the use of hematopoietic growth factor,[96] although it is unclear whether higher doses of taxol result in improved response rates for patients with platinum-refractory disease. Taxol, which is now available commercially for the treatment of platinum-refractory ovarian cancer, has recently been shown to produce a substantial improvement in the overall response rate when used in conjunction with cisplatin to treat patients with newly diagnosed disease who have residual tumors over 1 cm in diameter.[51]

Autologous Bone Marrow Transplantation

Techniques to administer very-high-dose, myeloablative chemotherapy followed by bone marrow rescue are now well established in many treatment centers. This strategy is potentially curative for patients with tumors such as lymphoma, in whom both chemosensitivity and a minimal volume of disease before transplantation are prerequisites for obtaining an optimal response.[100-103] Most ablative regimens used in ovarian-cancer transplantation programs include potentially active agents such as an alkylating agent (i.e., melphalan, cyclophosphamide, or ifosfamide), a platinum analogue such as carboplatin, and other drugs such as etoposide.

The benefits of transplantation in ovarian cancer are difficult to assess owing to both the relatively small numbers of patients who have undergone this procedure and selection bias.[102,104] Transplantation has generally been reserved for patients with persistent residual disease after standard chemotherapy, and response rates as high as 75 percent have been reported.[104] Some researchers have even reported small numbers of long-term survivors, although many of these patients received transplants for persistent microscopic disease, a group that may have had an indolent clinical course regardless of the treatment.[86,102] However, much of the early transplantation experience involved patients who had received extensive prior chemotherapy, resulting in a level of drug resistance that could not be surmounted by dose escalation alone. These patients tended to have either no response or a short-lived partial response to transplantation, at the expense of considerable toxicity (due to a relatively small marrow reserve). Therefore, the most appropriate patients for transplantation are those who have had a clear response to initial platinum-based therapy but nevertheless have persistent, nonbulky disease; who are otherwise young and healthy; and who understand the risks of autologous transplantation (including a 5 to 10 percent chance of treatment-related death).

Summary and Future Directions

Treatment of ovarian cancer is both frustrating and encouraging. In view of its propensity to remain in the abdominal cavity and its responsiveness to chemotherapy, ovarian cancer would appear to be a curable disease. In reality, however, by the time most patients are given a diagnosis, the tumor has progressed to a stage in its natural history at which it is partially resistant to drug therapy. It is possible that the recognition of familial cancer syndromes and the use of improved methods of early detection will eventually permit diagnosis at an earlier, more limited stage of the disease, when drug resistance has not yet developed and the majority of patients can be cured by surgery and standard-dose adjuvant chemotherapy.

For patients with advanced disease, the inability to achieve and maintain a complete pathological response with platinum-based therapy is the chief obstacle to cure. Ongoing efforts to improve the rate of complete pathological response include intensification of the platinum dose and the use of new drugs with novel mechanisms of action, such as taxol. Treatment of persistent residual or rapidly recurrent disease continues to be a major problem, but there is a promising role for non–cross-resistant drugs such as taxol, a possible role for intraperitoneal therapy, and an evolving role for autologous bone marrow transplantation. Several other innovative approaches that may eventually benefit patients with advanced disease include reversal of platinum resistance with buthionine sulfoximine,[108] intraperitoneal administration of autologous lymphokine-activated killer cells,[109] or intraperitoneal administration of tumor-directed antibodies conjugated to toxins or radioisotopes.[110,111]

Thus, although ovarian cancer can be a particularly difficult disease to treat, we now have several promising drugs and strategies that may eventually result in improved survival for patients with this disease. However, it is likely that cure will result only from a combination of treatment strategies tailored to the needs of individual patients. For example, it is possible that in the future patients with advanced-stage disease may best be treated by surgery, followed by postoperative chemotherapy with taxol and high-dose carboplatin, with immediate consolidation of an excellent response by autologous bone marrow transplantation or by intraperitoneal administration of antibody–toxin conjugates. The impact of these new treatment approaches on survival will be determined only through the continued enrollment of patients in well-designed clinical trials.

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A Brief Original Contribution

LACTASE PERSISTENCE AND MILK CONSUMPTION AS DETERMINANTS OF OVARIAN CANCER RISK

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Cramer, D. W. (Brigham and Women's Hospital, Boston, MA 02115). Lactase persistence and milk consumption as determinants of ovarian cancer risk. *Am J Epidemiol* 1989;130:904–10.

Using published data, largely from the 1970s, the author compared ovarian cancer incidence, per capita milk consumption, and population estimates of lactase persistence (the ability to digest lactose after infancy) in 27 countries. Significant positive correlations were noted between ovarian cancer incidence, per capita milk consumption, and lactase persistence. Lactase persistence showed a stronger association than milk consumption or animal fat consumption in multiple regression models. The author speculates that toxicity from the lactose component of milk and, more specifically, galactose, the digestion of which is facilitated by lactase persistence, may provide a biologic basis for the correlation.

galactosidase; lactose; milk; ovarian neoplasms

A correlation between ovarian cancer mortality and per capita consumption of milk has been described and has been attributed to the fat content of dairy products (1). A nutrient which distinguishes milk products is lactose, a disaccharide composed of galactose and glucose. The ability to digest lactose after childhood, called lactase persistence, varies widely geographically and racially. Its correlation with ovarian cancer might suggest the importance of lactose or its component sugars in ovarian cancer etiology. This brief report describes correlations between ovarian cancer incidence, milk consumption, and lactase persistence and speculates on their possibly biologic basis.

MATERIALS AND METHODS

Estimates, by country, for the following variables were sought: ovarian cancer incidence, percentage of adults who are lactase-persistent, and per capita consumption of milk (estimated in fluid weight) and animal fat (from nondairy sources). Data on the incidence of ovarian cancer came from *Cancer Incidence in Five Continents* (2) or from National Cancer Institute data (3) for the period 1970–1980 and are stated in terms of cumulative incidence, estimated by summing age-specific incidences from birth to age 75 years. Information on population frequencies of lactase persistence came from reports based upon jejunal biopsies in hospitalized patients, serial blood galactose or glucose determinations after an oral lactose load, or hydrogen breath determinations after an oral lactose load (4–26). Information on consumption of various foodstuffs was obtained from the food balance sheets published by the United Nations (27). Food balance sheets show, for primary

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food commodities, the amount potentially available for human consumption by totalling quantities produced and imported; subtracting exports, feed to livestock, and other nonfood uses; and then dividing by the total population. Statistical correlations on the above data were performed using standard linear or multiple regression techniques (28).

Results

Table 1 shows estimates for the cumulative incidence of ovarian cancer, per capita supply of milk (in grams per day), and proportion of the population who have lactase persistence. The cumulative (lifetime) risk for ovarian cancer varies from 0.4 percent in Japan and Senegal to 2.2 percent in Sweden. The per capita supply of milk varies from 13 g/day in China to 857 g/day in New Zealand. The estimated proportion of the population who have lactase persistence varies from 0 percent in Singapore and the Netherlands Antilles to 99 percent in Sweden. A significant correlation (R^2 = 0.29) was noted between the cumulative

Table 1

Cumulative incidence of ovarian cancer, per capita supply of milk, and percentage of population with lactase persistence in 32 countries

Country (abbreviation)	Cumulative incidence* (%)	Per capita milk supply (g/day)†	Percentage with lactase persistence	Reference for column 3
Australia (AUS)	1.21	331	82	4
Brazil (BRA)	0.94	183	31	5
Canada (CAN)	1.58	468	NA‡	
Colombia (COL)	1.22	168	46	6
Cuba (CUB)	0.58	422	NA	
Czechoslovakia (CZE)	1.02	426	88	7
Denmark (DEN)	2.03	432	97	8
Finland (FIN)	1.44	711	83	9
France (FRA)	0.75	347	58	10
German Democratic Republic (GDR)	1.63	389	78	7
Federal Republic of Germany (FRG)	1.81	329	88	7
Hong Kong (HK)	0.68	95	NA	
Hungary (HUN)	0.84	316	63	11
India (IND)	0.85	105	36	12
Israel (ISR)	1.66	315	34	13
Italy (ITA)	1.47	300	49	14
Jamaica (JAM)	0.91	78	41	15
Japan (JAP)	0.43	135	10	16
Netherlands Antilles (NEA)	0.55	421	0	17
New Zealand (Maori) (NZ)	1.37	857	36	18
Norway (NOR)	1.87	693	NA	
Poland (POL)	1.00	437	62	19
Romania (ROM)	0.79	404	44	11
Senegal (SEN)	0.38	97	65	20
China (Shanghai) (SHA)	0.47	13	8	21
Singapore (ethnic Chinese) (SIN)	0.69	113	0	22
Spain (SPA)	0.63	329	72	23
Sweden (SWE)	2.12	502	99	9
Switzerland (SWI)	1.91	461	84	24
United Kingdom (Scotland) (UK)	1.50	455	95	25
United States (Iowa) (USA)	1.64	462	81	26
Yugoslavia (YUG)	1.40	298	NA	

* Based upon age-specific incidence rates in the mid-1970s (2, 3). For countries with more than one registry site, the registry closest to the site for the survey of lactase persistence was selected.

† From United Nations food balance sheets (1979–1981) averages (27).

‡ NA, not available.

incidence of ovarian cancer and the per capita supply of milk ($p = 0.004$) (table 2). Linear regression indicates that for each 100-g increase in the daily per capita supply of fluid milk, there is a net increase of 0.14 percent in the cumulative incidence of ovarian cancer (figure 1). A stronger correlation ($R^2 = 0.44$) was noted between the cumulative incidence of ovarian cancer and lactase persistence ($p = 0.0002$) (table 2). Linear regression indicates that for each 10-percent increase in the population frequency of lactase persistence, there is a net increase of 0.1 percent in the cumulative risk of ovarian cancer (figure 2).

Some countries which appear to be outliers in the model between milk consumption and ovarian cancer are not outliers in the lactase persistence and ovarian cancer model (e.g., New Zealand) and vice versa (e.g., Senegal). This suggests that ovarian cancer occurrence might be better explained by considering both lactase persistence and milk consumption in a multiple regression model. For this two-parameter model, the overall R^2 was 0.50, some improvement over the linear models, with lactase persistence showing a stronger association (table 2). Lactase persistence also exerted a greater effect on ovarian cancer incidence than per capita nondairy animal fats in a regression model including these two variables (table 2).

Discussion

The ability to digest lactose from the jejunum after infancy, called lactase persistence, arose as a selective advantage sometime around 4,000 B.C. in certain populations practicing dairying (29). It is transmitted as an autosomal dominant trait and remains a characteristic of people with dairying ancestry (30). However, the majority of the world's population, along with most mammals, do not possess this characteristic and are described as having primary adult lactase deficiency. Surprisingly little has been written about the possible relation between lactase persistence and chronic disease patterns (31, 32). This report has confirmed, using incidence, a previous report correlating mortality rates for ovarian cancer with per capita milk consumption (1) and has described a stronger correlation between lactase persistence and ovarian cancer incidence. Lactase persistence does not appear to be merely a surrogate variable for milk consumption or fat consumption, since it showed a stronger association than either of these variables in multiple regression models; and a model containing both lactase persistence and milk consumption better explained the geographic variation of ovarian cancer than simple linear models involving each variable separately.

Table 2

*Correlations and parameters for regression models of ovarian cancer, milk supply, and lactase persistence, with the incidence of ovarian cancer as the dependent variable**

R^2 for model (p)	Intercept parameter	Milk supply parameter (p)	Lactase persistence parameter (p)	Animal fat parameter† (p)
0.29 (0.004)	0.673	0.0014 (0.004)		
0.44 (0.0002)	0.524		0.011 (0.0002)	
0.36 (0.004)	0.516	0.0008 (0.20)		0.008 (0.10)
0.19 (0.0003)	0.389		0.008 (0.009)	0.006 (0.13)
0.50 (0.0002)	0.391	0.0008 (0.09)	0.009 (0.004)	

* See table 1 for source of incidence and nutrient information.

† Animal fat from nondairy sources was calculated by subtracting animal fat from milk products from total animal fat using data provided in reference 27.

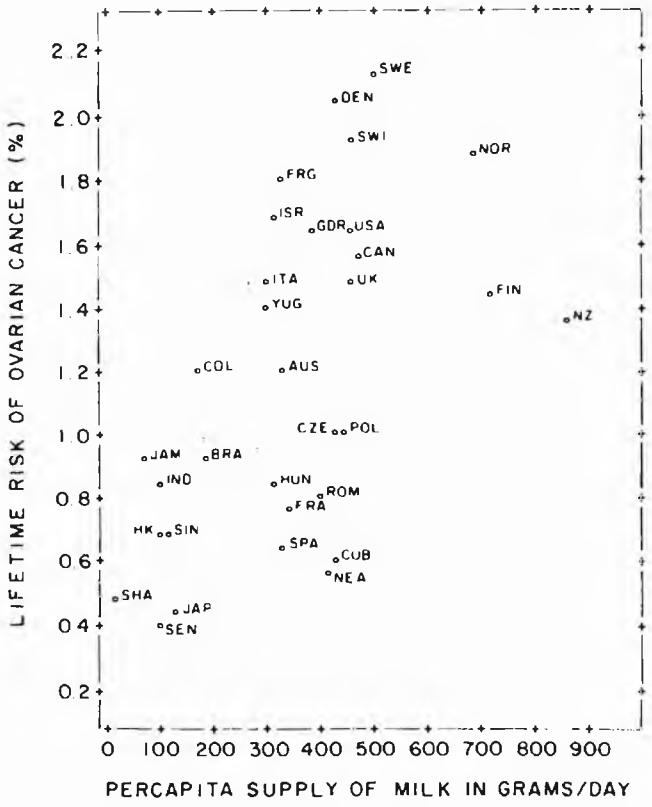

FIGURE 1. Correlation between ovarian cancer incidence and per capita milk consumption in 32 countries. See table 1 for country abbreviations.

Readers will be aware of the limitations of ecologic studies such as this one, including inaccuracies of the data and potential confounding factors. Data on the per capita supply of foodstuffs are limited by the accuracy of the gross data provided by the countries. Estimates of lactase persistence vary because of differences in techniques used and the specific population sampled (33). Jejunal biopsy is a precise technique but is limited to a select group of hospitalized patients. A preferred test is the hydrogen breath test after oral lactose, which is based upon the observation that undigested lactose is fermented by colonic bacteria, releasing hydrogen which is absorbed in the blood and exhaled. Probably most reliable are the data on ovarian cancer occurrence, because they come from established registries using standardized age and disease categories. Other factors associated with both lactase persistence and ovarian cancer could account for the correlations observed, and it must be conceded that not all potential confounding factors have been considered. Despite these limitations, the author believes that these data provide descriptive support for a model of ovarian cancer as a consequence of galactose toxicity.

A population with high levels of both lactose consumption and lactase persistence is a population that will be exposed to galactose – the carbohydrate that is combined with glucose in the disaccharide lactose and whose digestion is facilitated by lactase persistence. There is evidence that

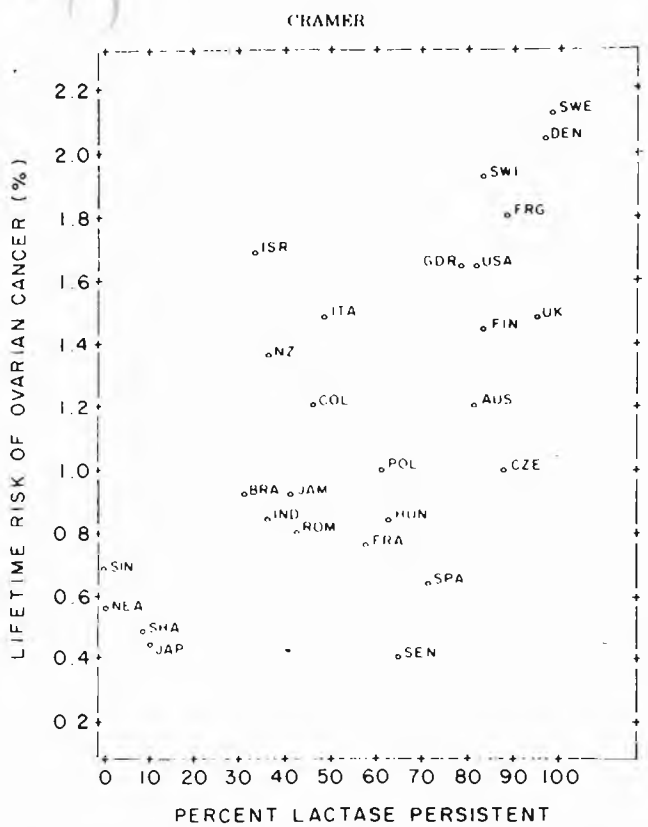

FIGURE 2. Correlation between ovarian cancer incidence and lactase persistence in 27 countries. See table 1 for country abbreviations.

galactose may be toxic to ovarian germ cells from animal studies showing that female rodents fed high-galactose diets produced offspring with reduced oocyte numbers (34) and that nonpregnant females developed ovulatory dysfunction (35). Galactosemic women who are unable to metabolize galactose develop premature menopause (36), and this investigator has recently reported that carriers for galactosemia also have an earlier menopause (37).

Experimental evidence supports ovarian failure (hypogonadism) as a predisposing factor to ovarian cancer. Rodents receiving ovarian irradiation or oocyte toxins such as polycyclic hydrocarbons or having congenital deficiency of oocytes developed ovarian tumors, primarily of stromal types (38). That gonadotropin stimulation was a necessary component of the model (hypergonadotropic hypogonadism) was inferred from the ability of pituitary ablation to block tumor development. But the relevance of the rodent models has been doubted, because rodents develop stromal tumors of the ovary and humans most commonly develop epithelial tumors of the ovary. However, humans have much greater stromal epithelial admixture in their ovaries (inclusion cysts), leading to the possibility that, under the same stimulus, humans may develop different tumor types than rodents. This model is discussed in more detail elsewhere (39).

In conclusion, the author believes that these descriptive data on lactase persis-

tence and milk consumption in relation to ovarian cancer are pertinent to its etiology and that additional studies regarding the consumption, absorption, and metabolism of galactose in relation to ovarian cancer are warranted.

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RELATION OF BODY FATNESS AND ITS DISTRIBUTION TO CARDIOVASCULAR RISK FACTORS IN YOUNG BLACKS AND WHITES

THE ROLE OF INSULIN

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Folsom, A. R. (Div. of Epidemiology, U. of Minnesota School of Public Health, Minneapolis, MN 55455), G. L. Burke, C. Ballew, D. R. Jacobs, Jr., W. L. Haskell, R. P. Donahue, K. Liu, and J. E. Hilner. Relation of body fatness and its distribution to cardiovascular risk factors in young blacks and whites: the role of insulin. *Am J Epidemiol* 1989;130:911–24.

Persons whose body fat is distributed predominantly in the abdomen compared with the hips are at increased risk of several chronic diseases. This study examined the cross-sectional relation of percent body fat, computed from skinfold thickness, and fat distribution, measured by the waist-to-hip girth ratio, to physiologic cardiovascular risk factors in a biracial sample (blacks and whites) of young adults aged 18–30 years. The subjects were persons who were examined at baseline (1984–1986) in the Coronary Artery Risk Development in Young Adults Study in four US metropolitan areas. The two hypotheses tested were that 1) after adjusting for percent body fat, waist-to-hip girth ratio is associated with several physiologic risk factors, and 2) fasting concentrations of serum insulin partly explain such associations. Percent body fat was significantly associated with all measured blood lipids, lipoproteins, apolipoproteins, uric acid, and blood pressure. Waist-to-hip girth ratio was significantly, although more weakly, associated in multivariate models with blood concentrations of triglycerides, high density lipoprotein (HDL) cholesterol, HDL_2 cholesterol, apolipoproteins A-I and B, low density lipoprotein cholesterol (in women only), uric acid, and systolic blood pressure, but was not associated in either sex with total cholesterol, HDL_3 cholesterol, or diastolic blood pressure. Fasting serum insulin concentrations were significantly associated with percent body fat (Pearson $r = 0.45–0.53$), waist-to-hip girth ratio (Pearson $r = 0.16–0.27$), and most of the physiologic risk factors. Inclusion of fasting insulin in multivariate models reduced, but rarely eliminated, associations between waist-to-hip girth ratio and the physiologic risk factors. These findings suggest that obese young adults, especially those with abdominal fat preponderance, carry a physiologic profile that places them at higher risk of cardiovascular disease, and that fasting insulin concentrations are only partly explanatory.

adipose tissue; apolipoproteins; blood pressure; insulin; lipids; lipoproteins; obesity

The relations of blood lipids, glucose, uric acid, and blood pressure to body fatness are well established. Several investigators have recently hypothesized that these relations

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Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein.

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GALACTOSE CONSUMPTION AND METABOLISM IN RELATION TO THE RISK OF OVARIAN CANCER

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Summary In a case-control study, consumption of dairy foods by 235 white women with epithelial ovarian cancer and by 239 control women, and activity of red blood cell galactose-1-phosphate uridyl transferase (transferase) in a subset of 145 cases and 127 controls were determined. Yogurt was consumed at least monthly by 49% of cases and 36% of controls. The mean transferase activity of cases was significantly lower than that of controls. When a ratio of lactose consumption to transferase (L/T) was calculated, cases had a mean L/T of 1·17 compared with 0·98 for controls; there was a highly significant trend for increasing ovarian cancer risk with increasing L/T ratio. Lactose consumption may be a dietary risk factor and transferase a genetic risk factor for ovarian cancer.

Introduction

FORTY years ago, Gardner[1] proposed that ovarian cancer was caused by hypergonadotropic hypogonadism—ie, high secretions of gonadotropins due to ovarian failure or lack of feedback control on the pituitary. In 1983 we[2] restated that theory and began a case-control study to examine novel exposures that might be associated with ovarian cancer through this mechanism. Because dietary galactose consumption and a key enzyme involved in its metabolism, galactose-1-phosphate uridyltransferase, have also been linked with hypergonadotropic hypogonadism, we have conducted a case-control study to investigate the relation between ovarian cancer and galactose consumption and metabolism.

Subjects and Methods

Subjects

The study was restricted to English-speaking white residents of Massachusetts within an 80 km radius of Boston who were aged between 18 and 76. Cases were women with ovarian cancer diagnosed at ten participating hospitals in the greater Boston area between July, 1983, and September, 1987, and identified through tumour boards, pathology registers, or the Massachusetts State Cancer Registry. During the study period, 394 potential cases were identified; 42 women (11%) could not be interviewed because the physician denied contact, 43 (11%) refused, and 37 (9%) had died or had moved before contact. For each of the 272 interviewed cases, slides of surgical specimens were reviewed. 10 cases were excluded as tumours metastatic to the ovaries, and the remaining cases were classified according to histological criteria of the World Health Organisation classification of ovarian tumours.[3] The present analysis is restricted to the 235 women with epithelial ovarian cancer including tumours of borderline malignancy.

Control women were identified through the Massachusetts Town Books—annual publications that list residents by name, age, and address. For each interviewed case a list of 5 potential controls was prepared by random selection from those women with listed phone numbers who matched cases by area of residence, race, and age within two years. These potential controls were contacted until an eligible control was identified who agreed to participate. Women who said that they had had bilateral oophorectomy were excluded. Of 526 potential control names on our lists, 129 (25%) could not be contacted because they had moved, died, or were otherwise unreachable, 54 (10%) were ineligible because of oophorectomy or incorrect age or race match, and 102 (19%) declined to participate. Thus, 239 controls were included in this study.

Dietary History

The women were interviewed to assess reproductive, medical, and family history. Dietary history was obtained with a self-administered semiquantitative 116-item food frequency questionnaire developed and validated for the Nurses' Health Study.[4,5] Cases and controls were asked to focus on their dietary patterns of the previous five years. Cases were reminded not to show changes in food preferences since the diagnosis of their cancer. Questionnaires were checked for completeness and open-ended questions were coded by a nutritionist; questionnaires were then read by an optical scanner. Nutrient summary scores were prepared with computer algorithms based on nutrient values for standard food portions.[6] Galactose consumption was measured indirectly: it is equivalent to lactose consumption since lactose is virtually the only source of dietary galactose. 236 ml of milk contains about 11 g of lactose and 5·5 g of galactose. We estimated lactose consumption from the frequency of use of eleven dairy products including whole milk, skimmed milk, ice cream, various cheeses, and yogurt but it was not feasible to estimate lactose intake from fillers in food products or in medications.

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TABLE I—CLINICAL AND DEMOGRAPHIC CHARACTERISTICS

	All subjects		Subgroup with quantitative transferase measurements	
	No of cases (%) (n = 235)	No of controls (%) (n = 239)	No of cases (%) (n = 145)	No of controls (%) (n = 127)
Age (yr)				
< 35	31 (13.2)	34 (14.2)	18 (12.4)	17 (13.4)
35–49	65 (27.7)	67 (28.0)	37 (25.5)	32 (25.2)
50–64	93 (39.6)	88 (36.8)	67 (46.2)	58 (45.7)
≥ 65	46 (19.6)	50 (20.9)	23 (15.9)	20 (15.7)
Parity				
0	79 (33.6)	43 (18.0)	48 (33.1)	21 (16.5)
1	31 (13.2)	27 (11.3)	17 (11.7)	16 (12.6)
2	40 (17.0)	63 (26.4)	24 (16.6)	33 (26.0)
3	49 (20.9)	43 (18.0)	32 (22.1)	29 (22.8)
≥ 4	36 (15.3)	63 (26.4)	24 (16.6)	28 (22.1)
Religion				
Catholic	118 (50.2)	132 (55.2)	78 (53.8)	69 (54.3)
Jewish	35 (14.9)	21 (8.8)	22 (15.2)	17 (13.4)
Protestant	66 (28.1)	76 (31.8)	40 (27.6)	37 (29.1)
Other	16 (6.8)	10 (4.2)	5 (3.5)	4 (3.2)
Education (yr)				
≤ 12	93 (39.6)	115 (48.1)	57 (39.3)	64 (50.4)
> 12	142 (60.4)	124 (51.9)	88 (60.7)	63 (49.6)
Marital status				
Never married	40 (17.0)	26 (10.9)	23 (15.9)	12 (9.5)
Ever married	195 (83.0)	213 (89.1)	122 (84.1)	115 (90.6)
Oral contraceptives				
Never used	168 (71.5)	157 (65.7)	102 (70.3)	85 (66.9)
Ever used	67 (28.5)	82 (34.3)	43 (29.7)	42 (33.1)

Transferase Activity

213 (91%) cases and 170 (71%) controls provided a blood specimen for analysis of galactose-1-phosphate uridyltransferase (transferase). Initially finger-prick specimens were obtained to measure transferase by the Beutler test, a qualitative technique developed for newborn screening for galactosaemia: in this test a fluorescent reaction is delayed in subjects with reduced enzyme activity.[8] These analyses were done at the newborn screening laboratory of the Massachusetts Department of Public Health. Later in the study venepuncture specimens were obtained for more precise quantitative measurements of transferase activity with a carbon-14 (^{14}C) labelling method.[9] Transferase activity is recorded in micromoles of uridine diphosphate galactose formed from ^{14}C-labelled galactose-1-phosphate precursor per hour per gram of haemoglobin (μmol/h per g Hb). These analyses were done at the Children's Hospital of Los Angeles on heparinised blood shipped by air express at room temperature within 1 or 2 days of collection. Samples were analysed within an average of 1·6 days of shipment in cases and 1·5 days in controls. Blood collection was delayed for at least 3 months in any patient who had had a transfusion. Finger-prick specimens only were available from 68 cases and 43 controls, venepuncture specimens only were available from 57 cases and 79 controls, and both were available from 88 cases and 48 controls. Analyses of transferase activity focused on the 145 cases and 127 controls who gave venepuncture samples.

Statistical Analyses

Differences between cases and controls in the distribution of quantitative variables such as lactose consumption or transferase activity were measured by *t* tests or by Wilcoxon rank sum tests depending on the normality of the distribution.[9] Relative risks for any particular amount of lactose consumption or transferase activity were determined with multivariate logistic regression for matched-pair data to control for potential confounding factors.[9] A matched-pair technique was used because cases and controls were matched only loosely for demographic factors and because blood results were not always available for both case and control members of a pair. In these analyses, risks were adjusted for potential confounding factors including age (< 50, ≥ 50), parity (0, 1–2, > 2), religion (Jewish, non-Jewish), education (≤ 12 years, > 12), and oral contraceptive use (never or ever). Also, total calorie intake was included in the models to try to adjust for any general over-reporting or under-reporting of quantities consumed.

TABLE II—ADJUSTED RELATIVE RISKS FOR OVARIAN CANCER ASSOCIATED WITH CONSUMPTION OF VARIOUS TYPES OF DAIRY PRODUCTS

Dairy product	No of cases (%) (n = 235)	No of controls (%) (n = 239)	RR (95% CI)
Cream cheese (28 g)			
Never or < monthly	118 (50)	131 (55)	1·0
≥ Monthly	117 (50)	108 (45)	1·2 (0·8–1·7)
Cottage cheese (113 g)			
Never or < monthly	85 (36)	106 (44)	1·0
≥ Monthly	150 (64)	133 (56)	1·4 (1·0–2·1)*
Ice cream (113 g)			
Never or < monthly	54 (23)	49 (21)	1·0
≥ Monthly	181 (77)	190 (79)	0·8 (0·5–1·1)
Ice milk (113 g)			
Never or < monthly	176 (75)	184 (77)	1·0
≥ Monthly	59 (25)	55 (23)	1·1 (0·7–1·6)
Skimmed milk (246 ml)			
Never or < monthly	112 (48)	121 (51)	1·0
≥ Monthly	123 (52)	118 (49)	1·0 (0·7–1·5)
Whole milk (246 ml)			
Never or < monthly	116 (49)	121 (51)	1·0
≥ Monthly	119 (51)	118 (49)	1·1 (0·7–1·6)
Yogurt (226 g)			
Never or < monthly	121 (51)	153 (64)	1·0
≥ Monthly	114 (49)	86 (36)	1·7 (1·1–2·4)†

RR = relative risk; all risks are relative to never eating the product or eating it on a less than monthly basis and are adjusted for age (< 50, ≥ 50), parity (0, 1–2, > 2), education (≤ 12, > 12), oral contraceptive use (ever, never), total calorie intake, and religion.
*p = 0·08; †p = 0·01.

Results

Table I shows clinical and demographic characteristics of all cases and controls and of those in whom quantitative transferase was measured. Age, a key matching characteristic, did not differ between cases and controls. Cases were more likely to be Jewish, college educated, never married, nulliparous, and never users of oral contraceptives than controls. Characteristics of the subgroup of cases and controls in whom quantitative transferase was measured, reflected general differences between cases and controls: thus, there was no major bias in the selection of the group from whom specimens were taken by venepuncture.

Table II shows risk for ovarian cancer associated with regular consumption of various types of dairy products relative to never use or use less than once a month of each product. Risks for regular use of these products were generally more than 1; yogurt and cottage cheese were the only products whose regular use especially distinguished cases from controls. Eating yogurt at least once a month was associated with a relative risk for ovarian cancer of 1·7 ($p = 0·01$); for cottage cheese this risk was 1·4 ($p = 0·08$).

Total lactose consumption by all cases and controls was skewed right and not normally distributed. The median and mean lactose consumption for cases were 11·8 and 14·8 g/day, respectively, and for controls were 11·0 and 13·3 g/day. These differences were not significant. In the upper range of lactose consumption, 51 (22%) cases consumed ≥ 22 g of lactose a day compared with 37 (16%) controls (adjusted relative risk for ovarian cancer 1·6; $p = 0·08$, 95% confidence interval [CI] 1·0–2·7).

According to the Beutler test, 51 (33%) of 156 cases had a 50% reduction of transferase activity compared with 18

TABLE III—LACTOSE CONSUMPTION IN ALL CASES AND CONTROLS AND TRANSFERASE IN THE SUBSET OF SUBJECTS WITH VENEPUNCTURE BLOOD SAMPLES BY VARIOUS CHARACTERISTICS

	Lactose consumption				Transferase activity			
	Cases (n = 235)		Controls (n = 298)		Cases (n = 145)		Controls (n = 127)	
	No.	Median*	No.	Median*	No.	Mean†	No.	Mean†
Age (yrs)								
< 50	98	11·8	101	12·9	55	21·6	49	23·2§
≥ 50	137	12·1	197	10·5‡§	90	21·9	78	22·6
Parity								
0	24	11·2	43	11·9	18	22·2	21	22·0
1–2	71	10·8	90	10·2	41	22·1	49	22·8
≥ 3	85	11·4	106	11·4	56	21·2	57	21·1§
Religion								
Jewish	35	11·7	21	7·4	23	21·8	17	21·8
Non-Jewish	200	11·8	276	11·0	123	21·8	110	23·0§
Education (yrs)								
≤ 12	91	11·0	115	10·1	57	22·1	64	22·8
> 12	132	12·4	174	12·8	88	21·6	63	22·9§
Marital status								
Never married	41	12·4	36	11·1	21	22·9	12	22·4
Ever married	195	11·8	259	10·8	122	21·6	115	22·9§
Oral contraceptives								
Never used	168	11·5	157	9·8‡	102	21·8	85	22·6
Ever used	67	9·6	82	11·2‡	43	21·9	42	23·3

*g/day
†μmol uridine diphosphate galactose h per g Hb
‡p < 0·05 for difference between this and its complementary category among controls only
§p ≤ 0·05 between cases and controls for this category

(20%) of 91 controls (p = 0·03). This observation, suggesting that there was lower transferase activity in ovarian cancer cases, prompted further study with the quantitative technique for analysis of transferase activity in 145 cases and 127 controls. Transferase activity was normally distributed in cases and controls: mean transferase activity for cases (21·8 μmol/h per g Hb) was lower than that (22·8 μmol/h per g Hb) for controls (p = 0·03). At the lower end of the transferase distribution, there were 58 (40%) cases compared with 35 (28%) controls with transferase measurements below 21 μmol/h per g Hb (adjusted relative risk 1·7; p = 0·04, 95% CI 1·0–2·9). Further adjustment for interval between specimen shipment and analysis or for year of analysis did not alter this association.

TABLE IV—RELATIVE RISKS FOR OVARIAN CANCER BY LACTOSE CONSUMPTION AND TRANSFERASE ACTIVITY

Combination		No. of subjects (%)		
Lactose consumption (g/day)	Transferase activity (μmol/h per g Hb)	Cases (n = 145)	Controls (n = 127)	RR* (95% CI)
≤ 11	≥ 22·9	29 (20)	36 (28)	1·0†
≤ 11	< 22·9	30 (21)	35 (28)	1·1 (0·5–2·2)
> 11	≥ 22·9	31 (22)	29 (23)	1·3 (0·6–2·7)§
> 11	< 22·9	55 (38)	27 (21)	2·2 (1·1–4·5)‡

*All risks are relative to reference category.
†Reference category.
‡p < 0·05

Table III shows median lactose consumption for all cases and controls and mean transferase activity for the subset of subjects with venepuncture specimens by various characteristics. More lactose was consumed by controls under 50 years old than by those older than 50 (p = 0·04). Possibly related to this age difference was the observation that more lactose was consumed by those controls who had used oral contraceptives than by those who had not (p = 0·007). Other non-significant differences included greater lactose consumption by non-Jewish compared with Jewish controls, more educated compared with less educated controls, and never married compared with ever married controls. Case subjects older than 50 consumed more lactose than older controls (p = 0·05); likewise, case subjects who had never used oral contraceptives consumed more lactose than controls who had never used them (p = 0·01). There were no significant differences in transferase activity by demographic characteristics within the control group. Cases had lower transferase activity than

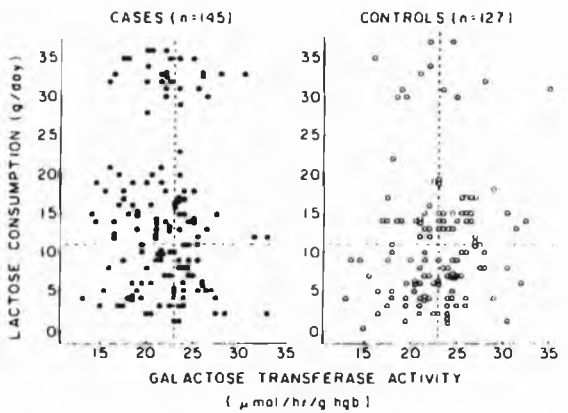

Fig 1—Correlation between lactose consumption and transferase activity in ovarian cancer cases and controls.

TABLE V—RELATIVE RISKS FOR OVARIAN CANCER RANGED WITH L/T CATEGORIES

L/T category	No of cases (%) (n = 145)	No of controls (%) (n = 127)	RR (95% CI)*
< 1·0	52 (36)	67 (53)	1·0†
1·0–1·24	31 (22)	29 (23)	1·3 (0·7–2·5)
1·25–1·49	25 (17)	16 (12)	2·1 (1·0–4·4)
≥ 1·5	37 (26)	15 (12)	2·9 (1·4–6·1)‡

*All risks are relative to reference category.
†Reference category.
‡p < 0·001 (Mantel extension test for trend; χ² = 14, p < 0·001).

controls: this was most striking among subjects younger than 50, among those with 3 or more children, among non-Jewish subjects, and among ever married subjects.

There was no correlation between transferase activity and lactose consumption. In fig 1 the quadrants defined by the control medians for transferase activity and lactose consumption contain dissimilar ratios of cases to controls with an excess of cases in the quadrant defined by higher lactose consumption and lower transferase activity. Women with lactose consumption of > 11 g/day and transferase activity of < 22·9 μmol/h per g Hb had a risk for ovarian cancer of 2·2 (p = 0·03, 95% CI 1·1–4·5) relative to women with transferase activity of ≥ 22·9 μmol/h per g Hb and lactose consumption of ≤ 11 g/day (table IV). This finding suggested that lactose consumption and transferase activity, combined as a single variable, might be a good predictor of risk for ovarian cancer. Fig 2 shows the distribution of cases and controls by L/T, a variable we define as the natural log of lactose consumption by an individual (× 10) divided by their transferase activity. When the log of lactose intake was used, the distribution in cases and controls became normal.

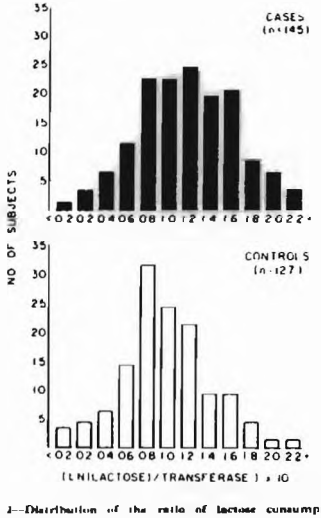

Fig 1—Distribution of the ratio of lactose consumption to transferase activity in ovarian cancer cases and controls.

TABLE VI—MEAN TRANSFERASE ACTIVITY AND MEAN L/T RATIO IN NORMAL AND THEIR OVARIAN CANCER HISTOLOGICAL GRADE OR TYPE OF TUMOUR

Tumour	Mean (SEM) galactose transferase (μmol h per g Hb)	Mean (SEM) L/T ratio
Histological type		
Serous (n = 73)	21·3 (0·4)	1·2 (0·05)
Mucinous (n = 25)	21·6 (0·7)	1·2 (0·09)
Endometrioid (n = 12)	22·9 (0·7)	1·1 (0·08)
Other (n = 15)	22·0 (0·9)	1·3 (0·14)
*Histological grade**		
0 (n = 41)	22·1 (0·6)	1·2 (0·08)
1 (n = 7)	20·6 (0·7)	1·1 (0·19)
2 (n = 47)	22·4 (0·5)	1·1 (0·07)
3 (n = 34)	21·2 (0·6)	1·2 (0·08)

*Grade unspecified in 16 cases with undifferentiated or mixed epithelial tumours.

The mean (SEM) L/T ratio for cases was 1·17 (0·01) and for controls was 0·98 (0·04), indicating that women with ovarian cancer tended to have consumed more lactose relative to their transferase activity than control women (p = 0·0005). In table V this finding is expressed in terms of relative risks adjusted for potential confounders. The trend in risk by L/T categories was highly significant (p < 0·001).

Table VI shows transferase activity and L/T ratios in ovarian cancer cases according to histological type or grade of ovarian cancer. Women with tumours of a particular cell type or grade did not have lower transferase activity or higher L/T ratios, although the deviation from control means for transferase and L/T was least for the endometrioid tumours.

Discussion

Lactose, found naturally only in milk, is a disaccharide composed of galactose and glucose in equal amounts. Infants can digest lactose by hydrolysing it by the action of the jejunal enzyme lactase—an ability lost with age in most individuals of non-European descent.[10] Some adults retain lactase activity and can digest lactose; but those without lactase activity can still absorb galactose by eating products in which the lactose has been partly hydrolysed, such as yogurt, or by having gut bacteria that can break down undigested lactose.[11] Once absorbed, galactose may be used in carbohydrate side chains of glycoproteins and lipids or metabolised for energy by conversion to glucose mainly by the Leloir pathway.[12] Absence or severe deficiency of galactose-1-phosphate uridyltransferase causes galactosaemia.[13]

We have found that women with ovarian cancer used dairy products with a higher content of prehydrolysed lactose—ie, yogurt and cottage cheese—more often than control women and had lower concentrations of a key enzyme that converts galactose to glucose. Risk for ovarian cancer was strongly related to the L/T ratio: thus, cases were more likely to have consumed more lactose relative to their ability to metabolise it than controls.

These observations are compatible with the general model of ovarian cancer as a consequence of hypergonadotropic hypogonadism as evidenced by animal and epidemiological studies.[14–16] Ovarian tumours—mainly stromal types—developed in rodents that had received ovarian irradiation[14] or oocyte toxins,[15] or that had

congenital deficiency of oocytes.[16] In mice, removal of the pituitary gland blocks tumour development.[17] There is doubt about the relevance of these animal models to man. However, a stimulus that causes stromal tumours in rodents may also promote a different type of ovarian neoplasm in human beings. Human ovaries have numerous inclusion cysts—islands of ovarian surface mesothelium entrapped within the stroma—and most human epithelial ovarian tumours arise from the differentiation and proliferation of this entrapped epithelium.[18] Epithelial differentiation and proliferation depend on the presence of homologous stroma and its hormonal receptor status.[19] Additionally, ovarian cancers developed in women who had received radiation for cervical cancer or who were among atomic bomb survivors.[20,21] There is also experimental and clinical evidence that there is an association of galactose consumption and galactose metabolism with hypergonadotropic hypogonadism. Female offspring of rodents fed with a 50% galactose diet are born with fewer oocytes[22] and ovulatory disturbances develop in non-pregnant females fed on the same diet;[23] hypergonadotropic hypogonadism also occurs in galactosaemic women or carriers with reduced transferase.[24,25]

We believe that the above observations provide a credible biological basis for the associations between ovarian cancer, increased galactose consumption, and decreased transferase activity. Nevertheless, potential biases must be considered. We do not believe that the lower transferase activity for cases is accounted for by a difference in the reading or handling of specimens: subject status was unknown to laboratory personnel. Adjustment for interval between specimen shipment and analysis or year of specimen analysis did not alter the risk associated with low transferase activity. It is also noteworthy that the lower transferase activity for cases confirmed by the quantitative test was initially suggested by a different qualitative test done by a different laboratory.

Does lower transferase activity precede ovarian cancer, representing a possible genetic marker for increased susceptibility, or does lower transferase activity follow the disease, representing a response to the tumour or to its treatment? If the deficient transferase activity is a consequence of tumour development, the lowest measurements might be expected in cases with higher grade tumours or perhaps in women with a particular cell type. Neither finding was observed. Additionally, because transferase activity is measured within the red blood cell and not in the serum, a tumour marker is less likely to be identified. Our finding of lower red blood cell galactose transferase activity should not be confused with reports of raised serum galactosyl transferase as a possible tumour marker in women with ovarian cancer.[27] Galactosyl transferase, which transfers galactose to terminal oligosaccharide chains during glycoprotein synthesis,[12] uses the product from the galactose transferase reaction: genes that code for the two enzymes are both located on chromosome 9.[28] Thus, the possibility that raised serum galactosyl transferase is due to the lower galactose transferase activity and represents a related genetic marker rather than a tumour marker should be investigated.

We do not believe that cases preferentially recalled dairy product use since its association with ovarian cancer has not been previously reported. Two case-control studies of dietary factors and ovarian cancer did not find an effect of milk consumption on ovarian cancer risk;[29,30] but these studies did not examine other products such as yogurt, nor did they calculate total lactose exposure. Could other factors associated with both ovarian cancer and lactose consumption account for the association? The factors that might influence lactose consumption—ie, age, religion, marital status, education, and oral contraceptive use—may also be associated with risk for ovarian cancer, and therefore we adjusted for these factors in our analyses. Dietary factors may also be confounders. Because diets high in lactose may also be high in animal fat, it may be difficult to state which of these components of the diet is potentially harmful. Using more limited dietary histories, we previously concluded that the principal association was with fat.[31] That galactose is the harmful component rather than fat comes from our observation of the present study that the ability of galactose consumption to predict risk for ovarian cancer is improved when the L/T ratio is considered. Furthermore, the principal dairy products which distinguished cases from controls were yogurt and cottage cheese, foods with little fat but high lactose. Although this observation might be due to intention to lose weight among cases, excess risk was not seen for use of skimmed or ice milk, which are also low fat but lack a high content of prehydrolysed lactose. Additionally, adjustment for weight or height did not alter the increased risk associated with regular use of yogurt and cottage cheese. The production of both yogurt and cottage cheese usually starts with skimmed milk. Lactose is added in the form of nonfat dried milk followed by lactobacilli which start the fermentation process and partly hydrolyse the lactose into its component sugars.[32] In yogurt, up to 40% of the lactose is already hydrolysed so that even lactase deficient individuals can absorb galactose.[33] The specific and significant association between ovarian cancer and yogurt consumption is notable because its consumption in the US has more than quadrupled since 1970.[33]

The issue of gastrointestinal absorption of lactose was not addressed in this study. Generally, individuals who are lactase deficient and cannot digest lactose have gastrointestinal symptoms from dairy products and avoid them, whereas those with lactase persistence are symptom-free and continue to use the products throughout life. Our observation that greater lactose consumption was most striking for older cases may point to a higher prevalence of lactase persistence among women with ovarian cancer. World wide, ovarian cancer risk is strongly correlated with lactase persistence and per caput milk consumption, further epidemiological evidence that lactose rather than fat is the key dietary variable for ovarian cancer.[34]

This study provides a basis for further research on galactose consumption as an environmental factor and galactose-1-phosphate uridyltransferase as a genetic factor in the aetiology of ovarian cancer. Further human epidemiological studies and relevant animal experiments are warranted before any public health recommendations can be made. If our findings are confirmed, however, avoidance of lactose-rich food by adults may be a way of primary prevention of ovarian cancer, particularly in those women with low transferase activity.

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PREDOMINANCE OF BORRELIA BURGDORFERI SPECIFIC B CELLS IN CEREBROSPINAL FLUID IN NEUROBORRELIOSIS

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Summary A nitrocellulose immunospot assay that allows the counting of cells secreting IgG, IgA, or IgM antibodies to *Borrelia burgdorferi* was used to compare B cell response to *B burgdorferi* at the cellular level in cerebrospinal fluid (CSF) and blood from patients with neuroborreliosis with that in patients with aseptic meningoencephalitis (AM) or non-inflammatory neurological diseases. 13 of the 14 patients with untreated neuroborreliosis had CSF cells secreting IgG antibodies to *B burgdorferi* (mean 17 cells per 10^4 CSF cells), whereas 8 of 12 patients examined had cells secreting IgA antibodies (mean 6 cells) and 10 of 12 had cells secreting IgM antibodies (mean 6 cells) per 10^4 CSF cells. IgG antibody producing cells predominated except in 2 patients with mainly or only IgM secreting cells. Cells secreting antibodies to *B burgdorferi* were rarely found in the blood and then at very low numbers, which reflects preferential compartmentalisation of the specific B cell response to the CSF. The cells were not detectable in CSF or blood from the two control groups. Evaluation of humoral immunity at the cellular level is a novel approach to the detection and localisation of immune events in neuroinflammatory disorders.

Introduction

LYME disease may cause meningoradiculitis, meningitis, or neuritis of one or more cranial nerves.[1] Less frequently, there is clinical evidence of neuronal lesions in the central nervous system (CNS).[2] Cerebrospinal fluid (CSF) findings characteristically consist of mononuclear pleocytosis and blood-brain barrier (BBB) damage (both of which may be pronounced), and evidence of central nervous system synthesis of IgG, IgA, and especially IgM.[3,4] A history of tick-bite and of erythema migrans may point to the diagnosis, which is usually verified by demonstration of IgG and IgM antibodies against *Borrelia burgdorferi* in serum, but sometimes in CSF only.[5]

Since levels of specific antibodies present in free form in body fluids such as the CSF may be influenced by a variety of factors, including binding to target antigens and altered antibody catabolism, we have proposed that counting of cells secreting specific antibodies of different isotypes might reflect the B cell response.[6,7]

Patients and Methods

Paired specimens of CSF and peripheral blood were obtained from 14 consecutive patients (9 females) with clinical and CSF findings (table I) that suggested neuroborreliosis. 11 patients had serological evidence in the serum and CSF of *B burgdorferi* infection, and 3 had such evidence in the CSF only. Most patients had pronounced mononuclear pleocytosis in the CSF, and only 1 had a CSF cell count which was borderline. Albumin and IgG were measured in CSF and the corresponding plasma sample by

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Epidemiology, etiology, and fertility drugs in ovarian epithelial carcinoma: Where are we today?

4232 p. 435

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Objective: To review studies that have examined the epidemiology and etiology of the development of epithelial carcinoma of the ovary.

Data Identification: Important published studies related to the topic were identified through a computerized bibliography search.

Conclusion: A review of the literature reveals that the etiology of epithelial ovarian cancer is probably multifactorial and that genetic, environmental, hormonal, and viral factors appear to be directly or indirectly related to the development of the disease. An attempt to implicate specific agents has not produced conclusive results. However, based on large epidemiologic studies, it seems that there is a clear trend of decreasing risk with increasing number of pregnancies, deliveries, use of oral contraceptives, and the duration of breast feeding. An increased risk was found to be associated with ovarian dysfunction leading to infertility and exposure to asbestos and talc. The recent observation that infertile women who used fertility drugs might experience an increased risk for the development of epithelial ovarian cancer should be examined very carefully because of the small number of patients in the study, lack of appropriate information about the type of infertility, drugs used, dosage, and duration of treatment. Because there are no screening tests that are consistently accurate enough to detect ovarian cancer at an early stage, translating the current information into disease prevention requires careful clinical evaluation with a routine follow-up of patients at risk. Fertil Steril 1994;62:433–48

Key Words: Epithelial ovarian cancer, infertility, oral contraceptives, breast feeding, pregnancy, fertility drugs, asbestos

Ovarian cancer is the sixth most common malignancy in women in the United States (1). The mortality for ovarian cancer in Northwestern Europe and the United States is approximately 7.3 to 13 per 100,000 women, making ovarian cancer the most frequent cause of death from gynecologic malignancies and the fifth leading cause of death from cancer in women (2).

The cells of origin of ovarian epithelial cancer in human beings, which comprises some 94% of cases of ovarian cancer in the United States (3), are generally agreed to be from the surface epithelium of the ovary (4–6). In the adult, the ovarian surface epithelium arises after invagination of the coelomic mesothelium cover of the embryonic gonadal ridge and consists of cuboidal to low columnar pseudostratified epithelium (7). This mesothelial layer is set off from the hormonally active stroma by a basement membrane and a thin connective tissue layer, homologous to the tunica albuginea of the testis (8).

The age-specific incidence rate increases from 2/100,000 for women in their 20s to 55/100,000 at the age of 70 (1). From reports by Barber (9) and Coppleson (10), it can be estimated that approximately 1 out of 424 women will experience ovarian cancer before age 40, and, according to the Third National Cancer Survey, it is predicted that 1 of every 70 newborn females will eventually have this disease (1). The reported incidence of ovarian

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cancer during pregnancy varies from 1 in 6,429 (11) to 1 in 25,000 (12). In our experience the incidence was 1 in 47,115 deliveries (13) considering the rarity of such events during gestation.

Ovarian cancer typically remains clinically silent until it is far advanced; morbidity and death are generally sequela of intraperitoneal carcinomatosis. Therefore, epidemiologic studies of ovarian cancer have attempted to identify risk factors that might help define groups of women at unusually high risk as it has become clear that improved prevention and detection of these groups of women are necessary.

Attempts to implicate specific agents in the etiology of human ovarian cancer have not produced conclusive results, and review of the literature reveals that there is currently no evidence to implicate any single etiologic factor and that genetic, environmental, hormonal, and viral factors appear to be directly or indirectly related to the disease.

Recently, in a series of publications by Whittemore et al. (14–16), the authors described findings for invasive epithelial tumors in white women based on a collaborative analysis of data from 12 case-control studies of ovarian cancer conducted in the United States. Accumulating data from 3 (17–19) out of the 12 studies, an increased risk for invasive epithelial ovarian cancer was found in women who used fertility drugs. By contrast, infertile women who did not use fertility drugs experienced no increased risk. This new epidemiologic association between fertility drugs and ovarian cancer raised much discussion, because, if verified, it could have far-reaching implications for current clinical treatment as well as public health.

As a result of the discussion and disagreement with the results of Whittemore et al. (15), it was found appropriate to review the current literature regarding the different epidemiologic risk factors and the suggested mechanisms for the development of ovarian epithelial cancer, as well as to summarize the new epidemiologic results and the comments to these findings, as discussed in the literature.

EPIDEMIOLOGIC FACTORS AND MECHANISMS OF ACTION

The events leading to malignant transformation and progression of ovarian cells are not known. It is likely that multiple events are necessary for transformation. In the present state of knowledge, it appears that the etiology of ovarian cancer is multifactorial. Attempts to implicate specific agents in the etiology of human ovarian cancer have not produced conclusive results. Epidemiologic and etiologic studies have identified a group of risk factors that appears to be directly or indirectly related to human ovarian cancer. These factors can be grouped into three main categories: genetic, environmental, and hormonal.

The Genetic Hypothesis

The occurrence of familial ovarian cancer has been increasingly recognized (20–23), emphasizing the etiologic influence of genetic factors in a proportion of cases. Familial ovarian carcinoma is transmitted as an autosomal dominant trait (20). After analysis of 10 families in which 25 women developed ovarian cancer over three or four generations, Lynch et al. (24, 25) concluded that the disease is heterogeneous, with at least three genotypes predisposing to distinctive hereditary syndromes: [1] site-specific familial ovarian cancer syndrome, where women in these families are at risk of developing ovarian cancer only; [2] ovarian carcinoma in association with carcinoma of the breast (26, 27), where a woman may have 50% risk for ovarian cancer if her mother or sister had breast cancer and/or ovarian cancer; and [3] cancer family syndrome (Lynch syndrome II), characterized by hereditary nonpolyposis colorectal cancer with proximal colonic cancer predominance, endometrial cancer, and ovarian carcinoma (28, 29). Consistent with autosomal dominant inheritance factor, the risk for ovarian cancer among first degree relatives may be as high as 50% in these families.

These syndromes are characterized by significantly early age at onset and an excess of multiple primary cancers. A review paper by Hientz et al. (2) summarizes 140 families who have been reported with familial ovarian cancer. Out of the 140 families, 94 were reported by the Familial Ovarian Cancer Registry (21). In these reported studies, in 131 families, 321 women developed ovarian cancer; the number of observed generations varies from two to four per family. A tendency toward earlier onset, with a mean age of 47.7 years compared with 59 years for epithelial ovarian cancer in the general population (30), was noted. In addition, a predominance of a serous type of tumor with a trend toward more poorly differentiated adenocarcinomas and a high proportion of bilateral disease was described. The hereditary phenomena in these families is con-

sistent with an autosomal dominant gene with variable penetration, transmitted through men or women (24, 25, 30, 31). The Familial Ovarian Cancer Registry, which was established at Roswell Park Cancer Institute in 1981, assessed through December 1990 1,336 cases of ovarian cancer in 587 families (32), which made it clear that familial ovarian cancer is not a rare occurrence.

The exact pathogenesis of these syndromes is not understood. Three theoretical explanations are possible (24): [1] different genetic susceptibility of ovarian tissue to carcinogenic influences, [2] a genetic defect that induces a hormonal imbalance that may be potentially carcinogenic, and [3] a genetic defect causing an altered immunologic response resulting in ovarian cancer.

The most reproducible and specific structural changes in ovarian cancer are short-arm deletions of chromosome 3 (del[3][p21–p13]) and long-arm deletions of chromosome 6 (del[6][q15–p23]) (33). Similar abnormalities of both chromosomes have been seen in several other malignancies, with breakpoints clustering near the locations of some oncogenes (33).

Variable number tandem repeat probes were used to analyze DNA from a bank of over 50 primary ovarian tumors and peripheral blood lymphocyte matched control DNA. Allele loss was seen on the long arm of chromosome 17 in over 80% of malignant ovarian neoplasms (34). The finding of substantial allele loss on 17p has been confirmed by others (35). Mapping of the short arm of chromosome 11 identified allele loss in 45% of patients. It can be speculated that allele loss on chromosome 11 may represent deletion of tumor-suppressor genes (36), which appear to inhibit cellular expression of the transformed phenotype (37, 38), contributing to the development and progression of ovarian cancer. Crickard et al. (39) reported two patients with borderline serous tumors illustrating trisomy of chromosome 2,7 and 10. Trisomy of chromosome 10 was also found in five of six serous borderline tumors in comparison with normal karyotypes seen in 18 benign cystadenomas (40).

Epidemiologic evidence raises the possibility of defective cellular repair after ovulation as a major risk factor in ovarian cancer. The molecular evidence suggests that this repetitive proliferation allows promotion of cells already bearing allele loss, and, by inference, those carrying inactivated tumor-suppressor genes will lead to uncontrolled cell division and malignant transformation.

Support for the genetic hypothesis was given by the observation of decreased serum activity of α-L-fucosidase in the serum of ovarian cancer patients (41). This observation, along with the report that women with ovarian cancer may possess alleles that lead to a deficiency of α-L-fucosidase activity (41), suggests that the enzyme deficiency may be hereditary and associated with an increased risk for the development of ovarian cancer. If the current knowledge of the genetic epidemiology and mechanism of pathogenesis are to be translated into disease prevention, more attention and effort should be invested into the identification of women at genetic risk.

Environmental Factors and Carcinogens

The fact that the highest incidence of ovarian cancer is found in industrialized countries suggests a relationship between this tumor and environmental factors associated with industrial chemicals and diet. This hypothesis has been strengthened by the observation that the incidence of ovarian cancer in Japanese women who were born in Japan and emigrated to the United States increased (1, 42, 43). Epidemiologic, experimental, and clinical data seem to focus on a possible relationship between ovarian cancer and materials of the talc-asbestos group (hydrous magnesium silicates) (44–49). The clear relationship between asbestos exposure and pleural and peritoneal mesotheliomas, the similarity of ovarian cancer to mesotheliomas, and the ability of talc to enter the pelvic cavity are in support of this theory.

Graham and Graham (47) found particles similar to talc in ovaries with ovarian carcinoma. They also were able to induce ovarian neoplasms in Guinea pigs with asbestos, which suggested that ovarian cancer could be related to asbestos exposure. Newhouse et al. (50) found a slightly elevated frequency of ovarian carcinoma in female asbestos workers. Cramer et al. (44) found that 42.8% of ovarian cancer patients regularly used talc powder in the perineal area, either as a dusting powder on the perineum or on sanitary napkins, in contrast to 28.4% of a control, yielding a relative risk (RR) of 1.92 ($P < 0.003$) for developing the disease. In addition to this, women who had regularly engaged in both practices, powdering the perineum and sanitary napkins, had an adjusted RR of 3.28 ($P < 0.001$) compared with women with neither exposure.

It has been suggested that cosmetic talc powder (46) on a dusty contraceptive diaphragm can enter the vagina and comes in contact with the ovaries after passage through the uterus and fallopian tubes (51). That this route of contamination is feasible was shown in 1961 by Egli and Newton (52) using carbon particles and in 1981 by Venter (53) using labeled human albumin microspheres. If exposure to talc on contraceptive devices is associated with increased risk of ovarian cancer, then observations of decreased risk among parous, lactating, or pill-using women may somewhat reflect their nonusage of such methods.

The "pelvic contamination" theory proposed that environmental carcinogens might ascend the genital tract and act upon the surface of the ovary (51). Talc has been proposed as one such substance (44, 46) as it was suggested that the talc (perhaps contaminated with asbestos) alters the responsiveness of the ovarian epithelium, which ultimately proliferates autonomously.

In an experimental model, in mice, ovarian tumor was induced by administering carcinogenic polycyclic aromatic hydrocarbons, like 7,12-dimethylbenz[*a*]anthracene (54), which undergoes hormone-dependent metabolism to reactive intermediates in the rat ovary (55). It was also shown that monooxygenase activity in proliferating human granulosa cells caused 7,12-dimethylbenz[*a*]-anthracene to metabolize under the influence of gonadotropins and raised steroid concentrations (56). Linking the above observations into a theory, it might be suggested that when granulosa cells are in the phase of rapid growth under gonadotropin and estrogen stimulation, microsomal cytochrome P450–dependent hydroxylase systems convert 7,12-dimethylbenz[*a*]anthracene to reactive epoxide intermediates, forming covalent linkages to protein DNA that may induce follicular cell destruction or transformation to a cancer cell (56).

One of the major dietary differences among people in industrialized countries is the high intake of meat and animal fat, which has been reported to be associated with an increase of ovarian cancer in several studies (57, 58). Using published data, largely from the 1970s, Cramer (59) compared ovarian cancer incidence, per capita milk consumption, and population estimates of lactase persistence in 27 countries. (A nutrient that distinguishes milk products is lactose, a disaccharide composed of galactose. The ability to digest lactose after childhood, called lactase persistence, varies widely geographically and racially.) Significant positive correlations were noted between ovarian cancer incidence, per capita milk consumption, and lactase persistence. In 1989, Cramer et al. (60) proposed that increased dietary galactose consumption and low serum levels of galactose-1-phosphate uridyltransferase, which prevents the degradation of galactose to glucose, may be linked to an increase of ovarian cancer.

To explore the possible importance of the animal fat content of milk in milk–ovarian cancer association, a case-control study of 303 ovarian cancer cases and 606 age-matched nonmalignant-disease controls seen between 1982 and 1988 was performed (61). Total frequency of usual milk intake was not associated with increased risk. Usually drinking more than one glass of whole milk daily relative to never drinking whole milk was associated with a RR of 3.1 (95% confidence interval [CI] 1.8 to 5.5). Consumption of reduced-fat milk was associated with reduced RR. Among persons who reported drinking milk regularly, persons reporting drinking only whole milk were at increased risk (RR = 2.6, 95% CI 1.7 to 4.0) relative to persons who drank only skim milk or 2% milk. Therefore, it was suggested that milk is related to ovarian cancer when it is the vehicle for animal fat consumption (61).

Hormones and Related Conditions

Because it was noted that women with ovarian cancer had an increased incidence of nulliparity, lower rate of pregnancies, increased incidence of miscarriage, and high rate of infertility, it was suggested that malignancies of the ovary, especially epithelial carcinoma, are caused by endocrine and physiological disorders associated with ovulation that impairs the ability to conceive. According to this theory, pregnancy, oral contraception, and lactation, all of which suppress ovulation, protect against ovarian cancer. In this section, the association between conditions that suppress ovulation are reviewed along with other conditions associated with infertility.

Parity

Many studies have shown that pregnancy confers a high degree of protection against the development of ovarian cancer compared with nulliparous women (50, 62–68). Most of the authors agree that protection increases with the increasing number of term pregnancies (50, 63, 66–68). A pooled analysis of three European case-control studies (69), provid-

ing a total database of 1,140 cases and 2,724 controls, shows that the risk for developing ovarian cancer decreases with increasing number of births, and the trend in risk was significant ($P < 0.01$). In comparison with nulliparous women, those who reported four or more births had a 40% reduction in risk of ovarian cancer (RR of 0.6, 95% CI 0.4 to 0.8).

In each study, as well as in the overall database, an inverse association between the number of abortions and ovarian cancer was noted. The estimated RR for those who experienced two or more abortions was 0.7 (95% CI 0.6 to 0.9). This study also presents data regarding the protective effects of full-term pregnancy upon the age at which ovarian cancer occurs. An estimated RR of 1.4 (95% CI 1.1 to 1.7) was found for age 35 or more at first birth compared with age 25 or less. The high risk of first delivery at age 35 is comparable in this study to the RR of nulliparous women. The influence of age at the time of delivery and the association of ovarian cancer received support from the study by La Vecchia et al. (70). In their study late age at first birth was associated with increased risk in comparison with those who had a child before the age of 22 years. The RR for those who first gave birth at age 22 to 24, 25 to 27, and 28 or more were 2.7, 3.2, and 4.0, respectively. Comparable results were found in a large prospective study in Norway (71). The risk for ovarian cancer decreased significantly with increasing parity. A parity of one or two was found to be linked with a risk reduction of 10% to 20%. The estimated odd ratio for women with five or more births compared with one was 0.46.

Lactation

There is no consensus in the literature regarding the association between breastfeeding and ovarian cancer, but the majority of studies observed a decreasing risk. Four studies found no association (18, 67, 72, 73). In another study, a 50% reduction risk was found in association with a history of lactation (74), and in others, lactation was found to be a protective factor (15, 17, 19, 75, 76). Based on the recent studies by Whittemore et al. (15, 16), each month of breastfeeding was associated with an overall risk reduction of 0.99 ($P < 0.01$). It was also noted that the risk reduction per month of breastfeeding within 6 months of delivery exceeds that for subsequent breastfeeding.

Birth Control Pill

The hypothesis that the use of an oral contraceptive (OC) reduces the risk of epithelial ovarian cancer has been consistenly supported (50, 64, 66, 76–78). The risk of epithelial ovarian cancer in relation to the use of OCs was evaluated in several case-control study (50, 66, 76).

Detailed analysis by Weiss et al. (76) revealed a significantly decreased risk for ovarian cancer at age 35 to 55, especially if OCs had been used for more than 3 years. A case-control study by Cramer et al. (79) of 144 white women under the age of 60 who had ovarian cancer and 139 white women under 60 who were selected from the general population revealed a decreased risk for ovarian cancer associated with the use of OCs in subjects 40 to 59 years of age at the time of the study. The RR adjusted for parity was 0.11, with 95% confidence limits of 0.04 to 0.33 ($P < 0.00001$).

In a case-control study reported by the Centers for Disease Control (80) regarding cancer and steroid hormones, the major finding was that women who had ever used OCs had a significantly lower risk of ovarian cancer. The decrease in risk was directly related to the duration of OC use. After a minimum of 3 months, the RR declined from 1.0 to 0.6, and it declined further to 0.4 for women who used OCs for more than 5 years.

In a report (75) that evaluated the results of data gathered from a case-control study of 546 women 20 to 54 years of age with ovarian cancer and 4,228 controls, it was found that women who had used OCs had a risk for epithelial ovarian cancer of 0.6 (95% CI, 0.5 to 0.7) as compared with those who had never used them. This protective effect was seen in women who had used OCs for as little as 3 to 6 months, and it continued for 15 years after OCs were stopped. Furthermore, the results indicated that the use of OCs even for a few months reduced the risk of epithelial ovarian cancer by 40% for women 20 to 54 years of age. The effect probably takes 5 to 10 years to become apparent, but it persists long after the use of OCs ends, and protection exists regardless of the drug combination used for contraception. Looking at the association between nulliparous women who usually are located in a high risk group revealed low risk for developing the disease after the use of OCs. Their risk for developing the disease was even lower than that for parous women. A protective effect of OCs among nulliparous women also was found by Rosenberg et al. (77) and Cramer et al. (79) but only in very small samples.

Gwinn et al. (81) estimated the effects of cumulative months of pregnancy, breastfeeding, and OC

use on the risk of developing epithelial ovarian cancer, following the hypothesis that all three conditions cause cessation of ovulation. They used data from the Cancer and Steroid Hormone Study, a multicenter case control study. Estimated RR of epithelial ovarian cancer was 0.6 (95% CI 0.5 to 0.8) for women who had been pregnant, 0.6 (95% CI 0.5 to 0.8) for women who had breast fed, and 0.5 (95% CI 0.5 to 0.7) for women who had used OCs. Logistic regression analysis revealed a strong trend in decreasing risk of epithelial ovarian cancer with increasing cumulative months of pregnancy. In contrast, a marked reduction in risk was associated with having breast fed or used OCs, whereas the decrease in risk from additional months of either of these exposures was less than that for pregnancy.

Possible Mechanisms of Pathogenesis. Several hypotheses to explain the mechanism of this biological action have emerged. This is based on epidemiologic observations that increased parity, use of contraceptive pill, and breastfeeding, the three major causes of anovulation during reproductive life, and to a lesser degree repeated miscarriage, early onset of menopause, and late menarche give some protection (50, 67, 75, 77, 79, 82–85) against the development of ovarian cancer.

Fathalla (86) was the first to suggest that the key step in the pathogenesis of epithelial ovarian cancer is the disruption of the ovarian epithelium, occurring at the time of ovulation, leading to malignant transformation, named the "incessant ovulation" theory. Further support for the ovulation theory came from studies showing that in the absence of ovulation, before puberty, and in patients with gonadal dysgenesis, ovarian neoplasms of surface epithelial origin are extremely rare, whereas germ-cell and mesenchymal tumors occasionally arise (87, 88). Zajicek (89) have further hypothesized that epithelial tumors of the ovary may arise as a result of epithelial inclusions of the surface epithelium of the ovary, which frequently occur after ovulation. Pathologic review of the epithelial inclusion cysts found in the ovary at autopsy suggests that, after follicular rupture, the follicular cavity can become lined with epithelium from the ovarian surface, from the fallopian tubes, or from the endometrium (90).

Cramer and Welch (8) have tried to unite the different etiologic factors into a model of tumorogenesis that attempts to reconcile the existing epidemiologic and pathologic data regarding ovarian cancer. According to this model, the first step toward malignant growth is the formation of an inclusion cyst by entrapment or invagination of surface epithelium in the ovarian stroma, disruptin the connective tissue that separates the surface epithelium from the ovarian cortex and allowing the cells to be in close proximity to steroid producing cells. This most probably occurs as a sequela of ovulation or indirect stimulation through inflammation due to chemical irritants or carcinogens.

The second step is direct or indirect stimulation of the included epithelium by extraglandular or intraglandular estrogen, growth factors, follicular fluid, or gonadotropins resulting in differentiation, proliferation, and sometimes malignant transformation. However, the final stimulus to malignant transformation remains unknown.

Support for the incessant ovulation hypothesis comes from studies looking at the side of ovulation that has been found to occur significantly more often in the right ovary (65%). In a study performed by Potashnik et al. (91), 64% of ovulations were detected in the right ovary ($P < 0.01$). Based on this observation, Cruickshank (92) raised the hypothesis that ovarian cancer should be found more frequently in the right ovary. In a study of women with epithelial ovarian cancer in Scotland, he found significantly more tumors on the right side than on the left (59% vs. 41%), the 95% CI for tumors on t right being 51% to 67%. However, an important observation of this study is that 60% of the patients with left-side tumors presented with disease at an earlier stage, in contrast to 58% of those with right-side tumor, who presented with disease at a later stage.

Going further with the assumption that formation of inclusion cysts is the mechanism through which frequent ovulation leads to increased risk of ovarian cancer, one might expect to find more germinal inclusion cysts in women with ovarian cancer. However, in a study done by Westhoff et al. (93), the authors counted the germinal inclusion cysts in a single slide of sections from ovaries of 148 women who underwent incidental oophorectomy and from contralateral ovaries of 37 women with unilateral ovarian cancer. The mean number of germinal inclusion cysts was 2.7 for cases and 3.6 for controls. Conditional logistic regression analysis showed that germinal inclusion cysts were not associated with ovarian cancer (odds ratio = 0.98, 95% CI 0.92 to 1.04). These findings do not support the hypothesis that increased formation of inclu-

sion cysts per se is a risk factor for the development of ovarian cancer.

A hypergonadotropic state has also been entertained as a possible component in the etiology of epithelial ovarian cancer (94). It is also well established that some of the protective effects against ovarian cancer are associated with suppressed gonadotropin concentration. Among these are pregnancy, use of OCs, and lactation. Evidence that gonadotropin may be involved in ovarian neoplasia is indirect. In general, gonadotropin levels, particularly FSH, increase gradually with age, peak in the immediate postmenopausal years, and decline in the oldest age groups (95, 96). This pattern correlates with the age-specific rates of ovarian cancer (94), although the peak in incidence is some 15 to 20 years after the peak in gonadotropin levels. Support for the gonadotropin theory can be implied from experimental ovarian tumorogenesis. McGowan and Davis (97) have observed that after intrasplenic ovarian transplant, tumors developed because of increased pituitary gonadotropin secretion, resulting from hepatic inactivation of estrogen in the venous blood before it could exert feedback on the pituitary (98, 99). However, these tumors generally originated from the stromal cells of the ovary and do not duplicate the complex epithelial patterns observed in human ovarian tumors (100).

There are several physiological conditions that can cause hypersecretion of gonadotropins early in life and therefore might be expected to increase the risk of ovarian cancer. Among these are premature ovarian failure and postradiation state. Pelvic irradiation sufficient to cause menopause may be linked to ovarian cancer. However, several recent studies have found a nonsignificant excess of women with ovarian cancer who had prior irradiation (18, 65, 101).

Viruses, particularly the mumps virus, may be a cause of premature ovarian failure and hypergonadotropic state. However, a case-control study of ovarian cancer found that patients were less likely to recall a history of mumps parotitis than were the controls, suggesting a protective effect of mumps (73).

It was also observed that during lactation, FSH rises and values remain elevated until the return of ovarian estrogenic function. Therefore, it might be expected that breastfeeding will increase the risk of the disease. Yet, the data show reduced risk associated with breast feeding (16).

In addition to the above information and along with the abovementioned hypothesis, women using noncontraceptive estrogens would also be at lower risk of ovarian cancer, because exogenous estrogen administration is known to decrease gonadotropin concentration (102–104). However, the available data reveal no effect of hormone replacement therapy on ovarian carcinoma risk (2, 24, 28, 29, 105).

It is postulated that gonadotropin receptors on the surface of target cells regulate cellular processes by transducing the binding event into the formation of the intracellular messenger, cyclic adenosine monophosphate (106). This process is accomplished by receptor-coupled activation of adenylate cyclase in the plasma membrane. The data of Graves et al. (107) suggest that ovarian cancer cells maintained a functional nucleotide regulatory protein-adenylate cyclase unit reminiscent of normal cells. As such, failure of gonadotropins to stimulate adenylate cyclase may be due to a lack of gonadotropin receptors or an error in receptor activation of the nucleotide protein. In the Graves et al. (107) study, none of the ovarian tumors classified as epithelial, whether well differentiated or not, exhibited gonadotropin-responsive adenylate cyclase.

The epithelial tumors are morphologically analogous to epithelia of various portions of the mullerian tract, for example, the fallopian tube, endocervix, and endometrium (108). It is not surprising, therefore, that the epithelial tumors are gonadotropin insensitive, because the normal tissue analogues have not been considered targets for these hormones. The absence of a gonadotropin-responsive system in epithelial tumors (107) suggests that the most common type of ovarian cancer is not a target tissue for gonadotropin. The ability or inability to bind gonadotropin does not establish definitively that a tissue is responsive or insensitive to these hormones. Another study (109) trying to evaluate the incidence and localization of specific gonadotropin binding in the various types of ovarian tumors, with both the biochemical binding study and surface-binding autoradiography, demonstrated that none of the 11 epithelial malignant ovarian tumors examined contained the binding sites for gonadotropins. As such, due to the various inconsistencies, the contribution of pituitary gonadotropins to the etiology of cancer of the human ovary remains poorly supported at present (110–112).

Mechanisms of Protection. The proposed biological mechanism of protective effect for pregnancy, contraceptive pill, and lactation involves suppres-

sion of ovulation (16, 83, 86) or alterations in the production of pituitary gonadotropins (94). The hypothesis has been supported by the studies of Casagrande et al. (66), Hildreth et al. (67), and Franceschi et al. (68), who showed that the index of "ovulation years," the total time of exposure to ovulation, also contributed significantly to the risk of ovarian cancer. However, this observation was not confirmed in a study conducted by Gwinn et al. (81), who compared the month by month effect of pregnancy, use of contraceptive pill, and breastfeeding on risk of ovarian cancer. These authors noticed that equal months of exposure do not necessarily result in equal periods of anovulation.

If suppression of ovulation was the single underlying protective mechanism, one would expect a given period of a particular exposure to produce the same reduction in risk, regardless of previous exposure. This was not the result in the Gwinn et al. (81) study as it was suggested that each month of pregnancy was associated with a 2.6% reduction in the estimated relative risk, each month of breastfeeding reduced risk by 2.4%, whereas each month of OC use reduced it by 0.8%. The observation that anovulation due to pregnancy is more protective than anovulation due to the use of OC (15, 81) indicates that aspects of ovarian function and suppression other than ovulation may be more relevant to the risk of ovarian cancer. This notion is also supported by the estimation that a woman who had one pregnancy and breast fed may have skipped approximately 3% of the ovulations that would have occurred if she had never been pregnant. However, a single pregnancy is associated with a reduction in incidence by approximately one half (113).

Another theory involves the speculation that pregnancy may induce changes in the pituitary gland that in some way permanently altered the secretion of gonadotropins that stimulate the ovary. It was shown that pregnancy induces anatomical changes in the pituitary, which seem to correlate with the number of previous pregnancies (114). It was also known that the protective effect increases with increasing parity (2, 115). However, the risk reduction among parous associated with each term pregnancy declined with age, which contradicts the notion that pregnancy induces permanent changes that continue to reduce ovarian cancer risk throughout life (16).

One other possible interpretation would be that the endocrinologic status after pregnancy and the use of contraceptive pill protects against ovarian cancer and the lack of that protection makes infertile women at high risk for ovarian cancer. It was observed that one of the hormonal effects of child bearing is the sustained rise in the concentration of sex hormone-binding globulin after pregnancy (113). Hormonal contraceptive regimens induce a similar effect. This may explain benefits extending beyond the period of hormonal consumption.

Local Hormone Production as a Mechanism of Proliferation. There is some evidence suggesting that hormones can cause differentiation and proliferation of the surface endometrium. Henderson et al. (116) recently stated that "neoplasia is the consequence of excessive hormonal stimulation of a particular target organ, the normal growth of which is under hormonal control." Estrogen increases granulosa cell proliferation, and hence the frequency of mitotic activity may lead to malignant phenotypes (116, 117). It is known that the surface epithelium of the ovary can differentiate into endosalpingeal, endometrial, and endocervical-like epithelium (118). It is likely that this differentiation is mediated through estrogen and/or progesterone sensitivity of the ovarian surface epithelium. Cytoplasmic receptors for estrogen and progesterone have been found in most benign and malignant ovarian tumors (119–121).

Support evidence for this proposed mechanism was found in an animal experimental model. The earliest report, in 1962, concerned eight female dogs treated with stilbestrol (122). Within 19 months, ovarian carcinoma had developed in six dogs. It was also demonstrated that cell cultures from in situ ovarian cancer tissue secreted estrogen and/or progesterone and that this secretion was regulated by cyclic adenosine monophosphate in the majority of cultures and by hCG and FSH in approximately 30% of the cultures (123). In addition, cultured ovarian cancer cells from an omental metastasis continued to produce estrogen, indicating that FSH and hCG have time- and dose-related positive effects on cell division in cells from metastatic ovarian carcinoma (124). However, because epidemiologic data found a decreased risk for epithelial ovarian cancer with the use of estrogen in the premenopausal or postmenopausal period (125), it might be possible to speculate that gonadotropins promote ovarian cancer cell growth in vivo under conditions where ovarian estrogen secretion is drastically decreased or virtually nil. However, the epidemiologic observation that estrogen replacement therapy reduces risk for the development of

epithelial ovarian cancer was not supported by others (16).

Recently, a new family of low-molecular-weight peptide growth factors has been described (126). These growth factors are secreted by normal tissues, and they act to regulate growth in the local environment in which they are produced. According to this hypothesis, constitutive growth factor production would permit autonomous growth of cancer cells.

It was shown that growth of ovarian epithelial cells, in vitro, is greatly enhanced by the presence of hydrocortisone and epidermal growth factor (EGF), suggesting that specific growth factors can stimulate epithelial proliferation. It is hypothesized that such factors may promote repair of postovulatory defects in the ovarian epithelium. This hypothesis received some support from the observation that some virally transformed cells produced growth factors and did not require exogenous growth factors in culture (127–131). The observation that rabbit ovarian follicular fluid stimulates proliferation of cultured rabbit ovarian epithelium supports this hypothesis. Lately, in vitro studies of cancer cells have demonstrated abnormalities of both growth factor production and growth factor receptor number and function (126).

In a study performed by Berchuck et al. (132), the response of four epithelial ovarian cancer cell lines, previously established from ascites of patients with ovarian cancer, were tested for proliferation after stimulation by EGF, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and transforming growth factor-beta (TGF-β). All four epithelial ovarian cancer cell lines were found to differ in their response to the mitogenes tested. Two of the four cell lines increased proliferation in response to EGF. One cell line responded to FGF, and none of the cell lines responded to PDGF. These results are consistent with a previous report (133). The action of each of these peptides is mediated by a distinct membrane receptor, which in turn activates a postreceptor signaling mechanism and leads eventually to a mitogenic response (127).

In another observation, EGF and TGF-α induced in vitro growth of a wide variety of tumor types, including human ovarian carcinoma (132, 134). Bauknecht et al. (135) detected EGF receptors on 45% of 141 ovarian tumor specimens, and this has been reported to correlate favorably with response to chemotherapy and overall survival in ovarian cancer patients (136). This correlation suggests that EGF, TGF-α, and/or their receptors may contribute to the pathogenesis of the disease. However, the infrequency with which autocrine growth is seen in nonmalignant cells suggests that additional mechanisms exist to prevent uncontrolled cell proliferation.

Infertility

It is postulated that a common factor, probably of gonadal nature, is responsible for both ovarian carcinogenesis and decreased fertility. Ovarian cancer risk among nulliparous (but not parous) women was positively associated with a history of unsuccessful attempts to conceive (137). The finding that married nulliparous women have a higher risk of ovarian cancer than do single nulliparous women (19, 63) suggests that infertility may be a causative factor rather than childbearing as a protective factor.

Several authors have identified infertility as an independent risk factor on top of the nulliparity effect (19, 63, 74, 137). It was speculated by Whittemore et al. (137) that it is not the number of years of uninterrupted ovulation that increases the risk of developing cancer but the inability to conceive, due to endocrine disorders that exposed these women to a doubled risk of developing ovarian cancer compared with women who were fertile. This group of investigators noted that among all women, risk increased with increasing years of unprotected intercourse (P value for trend = 0.02). Risk among women having 10 years or more of unprotected intercourse was 1.8 relative to that among women having <2 years (P = 0.01) (137).

In another observation among nulligravid women, those with 10 years of unprotected intercourse had a risk of over six times that of nulligravid women who reported <3 months of unprotected intercourse (138). In a study performed by Harlow et al. (74) regarding patients with borderline tumors, the results suggested that married nulliparous women who reported a history of infertility were at a substantially greater risk, over six times more, of being diagnosed with a borderline tumor than married nulliparous women with no reported infertility problem. However, a noncausal explanation for this association might be that an ovarian tumor during its early preclinical development interferes with fertility in some manner.

The above findings are supported by a study of Lais et al. (139) in which a silent ovarian carcinoma

was found in 6 out of 571 patients undergoing microsurgery for infertility. The prevalence of ovarian cancer in these patients was 1% (95% confidence limits of 0.4 to 2.3%), compared with only 1 of 5,806 patients undergoing nonspecific abdominal surgery. Five of 6 patients were nulligravid, and only 1 patient had a previous pregnancy. This patient spontaneously aborted one preterm infant and had one tubal ectopic pregnancy.

Mechanism of Pathogenesis

Virus Infection. Several mechanisms of action were suggested. It was postulated that with the ability of the rubella virus to persist for years after the initial infection, one could suggest a theory that a change in ovarian function or morphology is produced that, although it does not interfere with ovulation, might alter cell surfaces, making impregnantation difficult or impossible (64).

Interference with Normal Process of Ovulation. It was also hypothesized that luteinized unruptured follicle syndrome, which represents another abnormal concomitant of ovulation, may be involved in ovarian carcinogenesis. The luteinized unruptured follicle syndrome has been associated with endocrine abnormalities that may play a role in the neoplastic process. Luteal phase peritoneal concentrations of progesterone and E_2 are lower in luteinized unruptured follicle cycles than in cycles characterized by an ovarian stigma or by subsequent conception (140). The unruptured follicles contain high concentrations of progesterone, estrogen, androgens, and LH (140). They can form cysts that remain in the ovarian stroma (141), and they may be related to the inclusion cycts found at autopsy.

Infertility Drugs. The question whether there is a possible causal relationship between ovulation induction and the development of ovarian cancer is an intriguing one. Several cases were reported in which an association between treatment for infertility and epithelial ovarian cancer of low-grade malignancy or epithelial ovarian carcinoma have been described.

The first report came from Bamford and Steele in 1982 (142). They described a case in which a patient with primary infertility was treated with CC and hMG for 11 cycles, in which 2 years were allowed to pass between the first 8 cycles and the last 3. During the last treatment cycle, a mass was palpable that was found to be ovarian endometroid tumor with encroachment of the serosal coat and a well-differentiated endometrial adenocarcinoma. In the same year Atlas and Menczer (143) reported a ca of borderline serous papillary cystadenocarcinoma after treatment of one cycle with CC. Ben-Hur et al. in 1986 (144) reported two cases in which papillary serous cystadenocarcinoma were diagnosed after treatment with CC and bromocriptine in one patient and CC only in the second one.

Carter and Joyce (145) in 1987 reported a case of papillary serous adenocarcinoma after treatment with CC, hMG, and hCG for an IVF program. In 1989 Kulkarni and McGarry (146) reported a case of serous papillary cystadenocarcinoma in a patient 8 years after several treatment cycles with CC and cyclofenil, which at that time ended with pregnancy and normal delivery. Dietl in 1991 (147) reported a case of papillary serous adenocarcinoma in patients with endometriosis who received treatment for several months with CC, hMG, hCG, and two types of GnRH agonist (GnRH-a). Goldberg and Runowicz (148) in 1992 reported three cases of borderline serous adenocarcinoma of the ovary associated with infertility and ovulation induction. In two patients treatment for two and three cycles with hMG for an IVF program was administered. In the third patient two treatment cycles with hMG were administered for ovulation induction.

The last report in the literature came from N man et al. (149) about two patients who developed a borderline malignancy of the ovary after treatment with hMG, GnRH-a, and hCG in the context of an IVF program. Even though such individual reports do not prove a direct connection between stimulation and the induction of ovarian neoplasms, it is nevertheless possible that the stimulation or other associated factors substantially stimulated an existent tumor, as in most cases the tumor was found shortly after ovulation induction treatment and it developed with remarkable rapidity.

Most recently, a series of articles published by Whittemore et al. (14–16) has focused attention on the association between infertility drugs and invasive epithelial ovarian cancer. The authors described findings for invasive ovarian epithelial tumors in white women based on a collaborative analysis of data from 12 case-control studies of ovarian cancer in the United States. The original studies were based on data collected between 1956 and 1986 and included 2,197 women with invasive epithelial ovarian cancer, 327 women with ovarian tumors of low malignant potential, and 8,893 controls. The study involved personal interviews with

study subjects. In general, most of the study findings were consistent with the current consensus; the risk of ovarian epithelial cancer decreased with increasing parity, the use of OCs, and breastfeeding. The risk increased with increasing duration of unprotected intercourse regardless of parity and gravidity.

However, the most striking newest data regard the use of fertility drugs. Of the 12 case-control studies, 3 studies appeared that contain data on past use of fertility drugs (17–19). The global population of these three studies was 2,278 women, of whom 711 women with invasive ovarian cancer and 1,211 controls were included in the analysis. Thirty-one women were found to have some history of previous use of fertility drugs. The specific drugs involved are not known nor are the dosage, duration of therapy, or type of infertility treatment.

Regarding the use of fertility drugs, it was found that the risk for invasive epithelial ovarian cancer among women who were diagnosed as being infertile and who had received fertility drugs was almost three times that of women who had no history of infertility (odds ratio [OR] = 2.8, 95% CI 1.3 to 6.1). Infertile women who had not used fertility drugs had no increased risk (OR = 0.91, 95% CI 0.66 to 1.3). Those infertile women who used fertility drugs and became pregnant did not have significant increased risk for ovarian cancer (OR = 1.4, 95% CI 0.52 to 3.6). However, among infertile women who had never become pregnant, the use of fertility drugs was associated with significantly increased risk of cancer (OR = 27.0, 95% CI 2.3 to 315.6). Fertility drugs had been used by 12 of 34 nulligravid cases compared with one of 23 nulligravid controls. The authors proposed the hypothesis that the increased risk associated with infertility may be due to the use of fertility drugs.

The data and the hypothesis presented by Whittemore et al. (15) and by Harris et al. (150) have prompted a worldwide concern for women's health and therefore raised an extensive discussion among epidemiologists, endocrinologists, oncologists, and gynecologists. It is not the purpose of this review to argue with the methods or the statistical analysis nor the results of the study of Whittemore et al. (15). However, some points regarding the use of fertility drugs, as raised in the Whittemore et al. (15) paper and those published in the literature, are discussed.

The most extensive research concerning the association of treatment of infertility and carcinoma of the ovary was published by Ron et al. (151). This study, conducted in Israel, gathered information about 2,632 Israeli women who were treated for infertility between 1964 and 1974. Analysis by infertility diagnosis demonstrated no significant excess of total cancer incidence. The standardized incidence ratio was 1.3 (95% CI = 0.8 to 1.8) for infertility due to hormonal deficiency, 0.7 (95% CI = 0.3 to 1.4) for mechanical infertility, 1.6 (95% CI = 0.6–3.6) for infertility of the male partner, and 1.1 (95% CI = 0.5–2.2) for unclassified diagnosis. Drugs that were evaluated in the study were hMG, CC, and hCG. However, because women received many different hormones during their course of treatment, it was difficult to distinguish independent treatment effects. Among women with the diagnosis of hormonal infertility, neither hMG nor CC was significantly associated with a cancer risk greater than the risk of infertile women receiving other hormones or no hormones. The authors summarized their paper by stating that no evidence of association between ovulation induction drugs and cancer of the ovary should be found.

Under the auspices of the International Federation of Fertility Studies, a group of clinical and epidemiologic experts (152) has made several comments regarding the study of Whittemore et al. (15). One of the comments is that the new fertility drugs were registered in the United States quite late in relation to the study period. Clomiphene citrate was registered in 1967, hMG was registered in 1969, and bromocriptine in 1978; before that period, treatment for infertility included pituitary irradiation, pregnant mare serum gonadotropins, conjugated estrogen, and diethyl stilbestrol, none of which is used any more (152).

In the Whittemore et al. (15) study, where infertility treatments were analyzed, the average age of women with cancer was 53 years, where the cancer was diagnosed between 1977 and 1981. If it is assumed that infertility treatment took place between the ages of 30 and 40, those women were then treated between 1954 and 1967 on average. These results are not compatible with use of the new fertility drugs that were registered from 1967 and onward. Caro et al. (153) raised the point that at the time the original studies were carried out, infertility drugs were used almost routinely in women diagnosed with ovulatory infertility, which represents 20% to 40% of female infertility. For example, among 708 infertile women (154, 155), 47% had anovulatory infertility and 90% of those received

drugs. Caro et al. (153) also raised the possibility that women who received treatment for infertility in the past who did not conceive were more liable to remember that they had been treated than those who did conceive (156).

It might be possible that infertility is due to a condition that in itself could be associated with ovarian cancer, and infertility is an expression of a common underlying phenomenon. It cannot be totally ruled out that ovulation induction might activate certain types of pre-existing ovarian cancer.

In summary, a number of potentially important risk factors have been identified. These are a family history of gynecologic cancer and cystic ovaries, nulliparity or low gravidity, difficulty in conceiving and infertility, and lack of exposure to OCs. If the above studies of ovarian cancer are to be translated into disease prevention, in view of the relatively high risk for infertile women to develop ovarian carcinoma, there is a need for careful clinical evaluation with ultrasound or other modern techniques before and during ovulation induction treatment. There is an urgent need for a prospective multicenter trial in which the scientific community would be able to confirm or refute the data regarding fertility drugs. Until then, use of fertility drugs should be handled with care, following pretreatment and post-treatment monitoring of the patients.

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