

*file Talc*

Br. J. exp. Path. (1984) 65, 101-106

## Effects of talc on the rat ovary

T.C. Hamilton<sup>1\*</sup>, H. Fox<sup>2</sup>, C.H. Buckley<sup>2</sup>, W.J. Henderson<sup>1</sup> and K. Griffiths<sup>1</sup>  
<sup>1</sup>Tenovus Institute for Cancer Research, The Welsh National School of Medicine, The Heath, Cardiff CF4 4XX, Wales and <sup>2</sup>Department of Pathology, University of Manchester, Manchester M13 9PT, UK

Received for publication 26 July 1983

**Summary.** Exposure of rat ovaries to talc was accomplished by intrabursal injection. As early as 1 and up to 18 months after treatment, the ovaries and associated tissue were cystic in appearance; these changes were the result of bursal distention. Histologically the ovarian tissue was decreased in amount and spread as a remnant on the inner wall of the bursa. In four of 10 treated animals but in no controls, focal areas of papillary change were noted in the surface epithelium of the ovary. Polarized light and electron microscope microanalysis confirmed the presence of talc in the surface epithelium, ovarian cortex, and connective tissue matrix of the bursa. Although the changes in the ovarian surface may be related to direct effects of talc exposure, it is postulated that these changes might also be related to constant exposure to the high concentrations of steroid hormones which have undoubtedly accumulated in the intrabursal space.

**Keywords:** talc, ovary, mineral dust, steroid hormones

Several factors have been suggested to be of importance in the aetiology of ovarian cancer (Fathalla 1972; Lingeman 1974, 1983; Henderson *et al.* 1979; Longo & Young 1979; Hamilton & Davies 1983). The possibility that an ascending carcinogen is implicated in the aetiology of ovarian epithelial cancer is one that has been widely canvassed (Fathalla 1972; Lingeman 1974; Henderson *et al.* 1979; Longo & Young 1979), and suspicion in this respect has fallen particularly on talc (Anonymous 1977; Henderson *et al.* 1979; Longo & Young 1979; Roe 1979). Until recently the only evidence to support this suspicion has been the finding of talc particles deeply embedded within ovarian carcinomatous tissue (Henderson *et al.* 1971), but Cramer *et al.* (1982) have now reported an epidemiological study which showed that women using dusting powders which contained talc, either on their perineum or on sanitary

towels, had substantially higher risk of developing ovarian carcinoma than did those not using talc-containing preparations. An epidemiological study which describes a non-specified increased cancer risk amongst workers in talc-associated Russian industries (Katsnelson & Mokronosava 1979) also may implicate talc in the malignant process.

Cramer *et al.* (1982) have reviewed the evidence linking talc and ovarian carcinoma and concluded that there is an urgent need for animal studies to help determine the relationship between this ubiquitous environmental agent and ovarian neoplasia. We report here the preliminary results of an in-vivo study of the effects of intrabursal talc injection on the rat ovaries.

### Material and methods

Female Sprague-Dawley rats bred in the

\* Present address: Medicine Branch, Division of Cancer Treatment, National Cancer Institute, Building 10, Room 12N226, 9000 Rockville Pike, Bethesda, Maryland 20205, USA.

animal husbandry unit of the Tenovus Institute were used at 10–15 weeks of age. Animals were fed a standard Laboratory diet (Pilsbury Ltd, Birmingham, UK) and were provided with tap water *ad libitum*. Animals were housed in rooms maintained at 23–24°C in 12 h of artificial light and 12 h darkness. After procedures described below, animals were kept in close proximity to male rats.

The talc preparation used in the procedures to be described was Italian 00000 (100 mg/ml) in phosphate-buffered saline, sterilized by autoclave before use. This preparation was composed of a heterogeneously sized population of platy crystals (size range: 0.3–14  $\mu\text{m}$ ) and contained no asbestos as judged by electron microscope microanalysis (observation by W.J.H.).

The procedure for surgical implantation of talc was as follows. Ovaries were located by blunt forceps used as probes via incisions through the skin and abdominal musculature and were pulled through the opening still attached to the fallopian tube and uterus. The bursa was immobilized with fine forceps and pulled sufficiently away from the ovarian surface to allow a 25 gauge needle, attached to a 1-ml syringe, to pierce the bursa without damage to the underlying ovarian tissue. The talc suspension (100  $\mu\text{l}$ ) was then infused and the needle removed. The ovary was returned to the peritoneum and the muscular wall of the abdomen sutured as was the overlying skin. The procedure was bilateral and accomplished while animals were under ether anaesthesia. Age-matched controls, sham-operated, and sham-treated (vehicle only) animals were included in the experiment. A total of 95 animals were involved in the experiment; at each time point, 10 treated animals, three age-matched controls, three sham-operated, and three sham-treated animals were examined. At intervals of 1, 3, 6, 12, and 18 months after treatment, animals were killed by cervical dislocation and the peritoneum opened by midline incision. Gross changes were noted; the ovaries and adherent tissue

were then fixed for light (buffered formalin) or electron (3% glutaraldehyde in phosphate buffer) microscopy. Histological examination of ovaries and associated tissue was performed on talc-treated and control animals 12 months after treatment.

To demonstrate the presence of talc in material stained (haematoxylin and eosin) for light microscopy, the sections were subjected to the Henderson replication technique (Henderson & Griffiths 1972) followed by evaluation of replicates by electron microscope microanalysis (Henderson *et al.* 1975). Identification of particulate material seen by electron microscopy in thin sections was also accomplished with electron microscope microanalysis.

## Results

Autopsy revealed that one or both ovaries from rats treated with talc by intrabursal injection were macroscopically cystic in appearance (Fig. 1); no gross changes were seen in any of the control animals. Gross



Fig. 1. Ovaries and associated genital tissue of the rat after talc treatment by intrabursal injection (see materials and methods). Animal was killed and autopsy performed 6 months after treatment. Note the apparent cyst formation and ovarian remnant just visible through the left bursa.

(buffered formalin) lehyde in phosphate stological examin- sociated tissue was ed and control ani- cement.

presence of talc in atoxylin and eosin e sections were sub- n replication tech- fiths 1972) followed es by electron micro- nderson *et al.* 1975). ate material seen by ain sections was also ron microscope mic-

one or both ovaries talc by intrabursal scopically cystic in gross changes were nrol animals. Gross



iated genital tissue of the by intrabursal injection ods). Animal was killed months after treatment. formation and ovarian ough the left bursa.

changes did not appear to be time-dependent as animals examined at 1, 3, 6, 12, and 18 months after treatment (10 treated animals and three each from the various control groups per time point) had similar numbers (> 50%) of grossly altered ovaries. Although oestrous cycles, as assessed by vaginal smears on sequential days, were irregular in talc-treated as well as control animals (especially apparent in older animals) day-to-day changes in the smears of individual animals indicated, however, the ovaries were still producing physiological concentrations of steroid hormones.

Histological examination showed that the cystic structures were not derived from the ovary but were due to distension of the bursal sac. The ovarian tissue was relatively decreased in amount and often spread as a thin remnant along the inner surface of a portion of the distended bursa.

The ovarian surface epithelium was, for the most part, formed by a single layer of low cuboidal cells in both injected and control cases. Focal multilayering of the ovarian surface epithelial cells, often in association

with small inclusion cysts, was seen with some frequency in the injected cases but was noted with an equal frequency in the control ovaries. In four of the injected ovaries, but in none of the control cases, small, focal areas of papillary change were seen in the surface epithelium (Fig. 2). The papillae were small and usually had a connective-tissue stromal support: some papillae appeared to be purely cellular but this was almost certainly due to cross-sectioning. The epithelium covering the papillae was regular with no evidence of cytoplasmic or nuclear atypia; mitotic figures were not seen. There was no correlation between the presence of intra-ovarian foreign body granulomas (*vide infra*) and the presence of these papillary areas and where both abnormalities were present in the same ovary they bore no topographical relationship to each other.

No evidence of cellular atypia or of mitotic activity was seen in the nonpapillary areas of the surface epithelium of the injected ovaries and in no ovary was there any evidence of frank neoplasia.

Foreign body granulomas, without any



Fig. 2. An area of papillary change in the surface epithelium of a rat ovary exposed to talc by intrabursal injection. H & E  $\times$  45.



**Fig. 3.** Surface epithelium of a rat ovary 6 months after talc administration. A number of talc particles can be seen in one epithelial cell, demonstrating their sheet-like (1) and fibre-like (2) forms; the X-ray analysis confirms their elemental composition (inset). In order to retain the talc particles during sectioning, the tissue is cut thicker than for normal morphological assessment, which accounts for a certain loss of definition ( $\times 6000$ ). Analytical spectrum from sheet crystal (inset) shows the 3:1 silicon to magnesium ratio characteristic of talc. KV 80, Beam current  $0.03 \mu\text{A}$ , 20-s count.

surrounding inflammation, were seen in five of the injected ovaries, usually in the cortical areas, and similar lesions were not uncommonly noted in the supracapsular fat and in the connective tissue matrix of the capsule. Birefringent crystals within these granulomas were judged to be talc by polarized light microscopy and this identification was readily confirmed by the Henderson replication technique (Henderson & Griffiths 1972) coupled with electron microscopic microanalysis (Henderson *et al.* 1975).

Electron microscopy revealed a heterogeneously sized population of particles deeply embedded in the ovarian tissue, and on rare occasions small particles were seen within individual surface germinal epithelial cells (Fig. 3).

### Discussion

In rats, intrabursal talc injection was followed by changes in the ovary and its associated tissues. Rats were chosen for this study because it was hoped that the near inclusion of the ovaries within a bursa in this species offered an opportunity for long-term exposure of the ovary to the mineral dust, once the dust was injected intrabursally. Unfortunately, bursal distention occurred as an unforeseen complication, this probably resulting from talc-induced fibrosis and obliteration of the small channel which normally allows communication between the cavity where the ovary lies and the peritoneum; the subsequent distension was almost certainly due to an accumulation of follicular fluid within the confined and enclosed space thus artificially created.

Despite the complexities introduced by the bursal distension it is of particular interest that papillary changes were seen in the surface epithelium of a proportion of the injected ovaries, for it is from this epithelium that most ovarian epithelial neoplasms, both benign and malignant, are thought to arise (Scully 1977). The papillary changes seen in this epithelium did not appear to be a reaction to an inflammatory process and

were somewhat reminiscent of those noted by Graham & Graham (1967) in the ovaries of asbestos-treated guinea pig. Whether these papillae represent the first stage in the development of a surface papillary epithelial neoplasm is, of course, a moot point but such lesions are, in the human ovary, certainly regarded as precursors of surface papillomas (Fox & Langley 1976).

It is clearly tempting to attribute the papillary change in the surface epithelium of injected ovaries to the direct effects of exposure to talc but an alternative explanation has to be considered. Ovarian epithelial neoplasms in humans contain steroid hormone receptors which are probably due to retention of a control system present in the normal cells from which such tumours arise (Holt *et al.* 1981; Hamilton *et al.* 1981). Steroid hormone receptors have recently also been reported as being present in the rat ovarian surface epithelium grown *in vitro* (Hamilton *et al.* 1982, 1983) and it is therefore possible that the changes seen in the surface epithelium of the injected rat ovaries were the result of long term (rather than intermittent) exposure to the high concentration of steroid hormones present in the entrapped follicular fluid within the distended bursa.

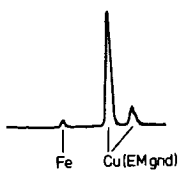
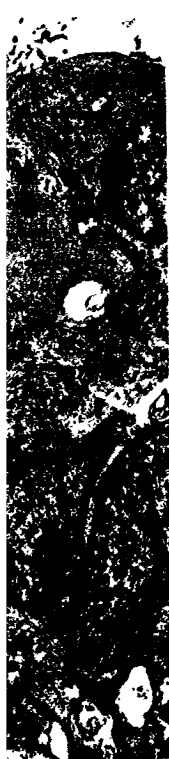
Hopefully future studies will clarify whether one or both of these possibilities has relevance to the malignant process in the ovarian surface epithelium.

### Acknowledgments

The authors wish to thank the secretaries of the Medicine Branch, NCI, for careful typing of this manuscript. One of us (T.C.H.) was supported by the Medical Research Council, UK (grant no. G977/505) during a portion of the time this work was in progress.

### References

- ANONYMOUS (1977) Cosmetic talc powder. *Lancet* **i**, 1348-1349.
- CRAMER D.W., WELCH W.R., SCULLY R.E. & WOJ-



number of talc particles can vary; the X-ray analysis during sectioning, the results for a certain loss of silicon to magnesium

- CIECHOWSKI C.A. (1982) Ovarian cancer and talc—a case-control study. *Cancer* 50, 372-376.
- FATHALLA M.F. (1972) Factors in the causation and incidence of ovarian cancer. *Obstet. Gynec. Survey* 27, 751-768.
- FOX H. & LANGLEY F.A. (1976) *Tumours of the Ovary*. London: Heinemann.
- GRAHAM J. & GRAHAM R. (1967) Ovarian cancer and asbestos. *Environ. Res.* 1, 115-128.
- HAMILTON T.C. & DAVIES P. (1983) Hormone relationships in ovarian cancer. *Reviews on Endocrine Related Cancer* 14, 19-22.
- HAMILTON T.C., DAVIES P. & GRIFFITHS K. (1981) Androgen and oestrogen binding in cytosols of human ovarian tumours. *J. Endocr.* 90, 421-431.
- HAMILTON T.C., DAVIES P. & GRIFFITHS K. (1982) Oestrogen receptor-like binding in the surface germinal epithelium of the rat ovary. *J. Endocr.* 95, 377-385.
- HAMILTON T.C., DAVIES P. & GRIFFITHS K. (1983) Steroid-hormone receptor status of the normal and neoplastic ovarian surface germinal epithelium. In *Factors Regulating Ovarian Function*. Eds G.S. Greenwald & P.F. Terranova. New York: Raven. pp. 81-85.
- HENDERSON W.J., JOSLIN C.A.F., TURNBULL A.C. & GRIFFITHS K. (1971) Talc and carcinoma of the ovary and cervix. *J. Obstet. Gynaec. Br. Commonw.* 78, 266-272.
- HENDERSON W.J. & GRIFFITHS K. (1972) Shadow casting and replication. In *Principles and Techniques in Electron Microscopy: Biological Applications*. Ed. M.A. Hayat. New York: Van Nostrand Reinhold.
- HENDERSON W.J., EVANS P.M.D., DAVIES D.J. & GRIFFITHS K. (1975) Analysis of particles in stomach tumours from Japanese males. *Environ. Res.* 9, 240-249.
- HENDERSON W.J., HAMILTON T.C. & GRIFFITHS K. (1979) Talc in normal and malignant ovarian tissue. *Lancet* i, 499.
- HOLT J.A., LYTTLE R., LORINCZ M.A., STERN S.D. PRESS M.F. & HERBST A.L. (1981) Estrogen receptor and peroxidase activity in epithelial ovarian carcinomas. *J. natn. Cancer Inst.* 67, 307-317.
- KATSNELSON B.A. & MOKRONOSAVA K.A. (1979) Non-fibrous mineral dusts and malignant tumors. *J. occup. Med.* 21, 15-20.
- LINGEMAN C.H. (1974) Etiology of cancer of the human ovary: a review. *J. natn. Cancer Inst.* 53, 1603-1618.
- LINGEMAN C.H. (1983) Environmental factors in the etiology of carcinoma of the human ovary: a review. *Am. J. ind. Med.* 4, 365-379.
- LONGO D.L. & YOUNG R.C. (1979) Cosmetic talc and ovarian cancer. *Lancet* ii, 349-351.
- ROE F.J.C. (1979) Controversy: cosmetic talc and ovarian cancer. *Lancet* ii, 744.
- SCULLY R.E. (1977) Ovarian tumours—a review. *Am. J. Path.* 87, 686-720.