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**COMMITTEE ON THE CARCINOGENICITY OF CHEMICALS IN FOOD,
CONSUMER PRODUCTS AND THE ENVIRONMENT**

“Tissue Organisation Field Theory” of Carcinogenesis

Introduction

1. Last April, a literature review by JA Newby and V Howard was published as a preview article in the J Nutritional and Environmental Medicine (1, attached at Annex 1). This claimed that it is feasible that chemical environmental contaminants, in particular synthetic pesticides and organochlorines with endocrine-disrupting properties, could be major factors in cancer aetiology. The paper generated some press and parliamentary interest at the time. It also referred to an alternative hypothesis to that of gene mutation for cancer development. This is termed the “tissue organisation field theory” (TOFT). This paper presents further information on this hypothesis for Members’ consideration.

Tissue organisation field theory (TOFT)

2. This hypothesis is being promulgated mainly by Professors A Soto and C Sonnenschein of Tufts University, US although it is said to be based on ideas which first originated at the end of the 19th century (2). In summary, it assumes that proliferation is the default state of cells, as it is for unicellular organisms or in the developing embryo (by default state, they mean the state under which cells are found when they are freed from any active control) (3, attached at Annex 2). Therefore, in normal tissues, there are biochemical systems in place to repress proliferation. Sporadic cancers arise when pathogens or carcinogens disrupt the normal biological interactions between different cellular layers. For example, a disruption in communication between the epithelium and stroma might prompt disoriented epithelial cells to mistakenly revert to pro-growth patterns of behaviour. Because this reversion represents a change in behaviour, rather than a permanent change, it is proposed that the epithelium, at least initially, can revert to normal behaviour. They consider that this is supported by the observation of spontaneously regressing tumours. The mutations present in neoplastic cells are considered to be secondary occurrences, not the initiating event that gives rise to neoplasia (2,3). [There is no reference to the fact that, under conventional thinking, some cancers are considered to arise via non-genotoxic mechanisms].

3. Sonnenschein and Soto propose that the stroma is the primary target of carcinogens (4). In support of this, they cite 3 recent studies which are outlined below.

Maffini et al (2004), J Cell Science 117, 1495-1502 (5, attached at Annex 3).

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4. In this study, the mammary epithelium of virgin female Wistar-Furth rats (a tumour susceptible strain) was surgically removed from the 4th and 5th inguinal mammary glands (MG) at 21 days of age. The excised epithelium was whole-mounted and observed microscopically to ensure that the ductal tree was removed in its entirety and only a small portion of the fat pad remained attached to it. The rats were then divided into four experimental groups of 10-14 rats each and treatment proceeded as follows:

- At 52 days of age, Groups 1 and 2 received a single i.p. dose of 50 mg NMU/kg bw and Groups 3 and 4 received a single i.p. dose of vehicle only.
- At 57 days of age, 50,000 mammary epithelial cells, from primary cultures of explanted tissue of mature virgin female rats of the same strain, were injected into the cleared fat pads as follows: Groups 1 and 4 received epithelial cells which had been treated *in vitro* with vehicle, Groups 2 and 3 received cells treated 5 days before transplantation with 50 ug/ml NMU.
- The rats were palpated weekly from one month after transplantation with epithelial cells. Thoracic glands were used as internal controls for NMU and were also palpated. Animals were sacrificed when inguinal tumours reached 1 cm in diameter or at 9 months after transplantation, whichever came first.
- Positive and negative control groups of rats (Groups 5 and 6) were injected at 52 days of age with NMU or vehicle only, respectively.

5. Only those animals whose stroma was exposed to NMU developed neoplasms, regardless of whether or not the transplanted mammary epithelial cells were exposed to the carcinogen (Table 1).

Table 1: Tumour development in rats injected with NMU and/or mammary epithelial cells (after Maffini *et al*, 2004)

Group	1	2	3	4	5	6
Treatment	NMU+ cells treated with V	NMU + cells treated with NMU	V + cells treated with NMU	V + cells treated with V	Pos control	Neg control
No. rats with tumours/no. alive at 9 months	10/13	6/8	0/10	0/6	6/6	0/6
%	77	75	0	0	100	0

V: vehicle

6. The authors also analysed DNA for the presence of the codon 12 GGA to GAA mutation in the Ha-*ras*-1 as a marker of tumour origin. This marker was chosen because it has been claimed that NMU induces this particular point mutation in the Ha-*ras*-1 gene of mammary epithelial cells. No correlation could be established between NMU-induced

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Ras mutations in either the mammary epithelium or stroma and tumour formation. 2/11 neoplasms from Group 1, 1/6 from Group 2 and 1/7 from Group 5 lacked the mutation whereas DNA extracted from all stroma samples taken from animals treated with vehicle (Groups 3 and 4) showed the mutation. The incidence of mutated Ras gene was not significantly different between animals that were or were not exposed to NMU ($p=0.604$).

Barclay et al, (2004), Endocrinology 146:13-18 (6).

7. This study compared the abilities of stromal cells from the prostate to induce the growth of a human prostatic epithelial cell line (BPH-1) in vivo. Stromal cells were isolated either from the normal peripheral zone (PZ-S), from tissue with benign prostatic hyperplasia (BPH-S), or from prostate cancer tissue (CA-S), and were recombined with BPH-1 cells. The recombined cells were then grafted under the renal capsules of nude mice. The results showed that grafting stromal cells (from any histology) alone, or BPH-1 cells alone, produced no visible grafts. Recombining PZ-1 cells with BPH-1 cells also produced no visible grafts ($n=15$). However, recombining BPH-S cells with BPH-1 cells produced small, well-organised and sharply demarcated grafts approximately 3-4 mm in diameter ($n=9$); and recombining CA-S with BPH-1 cells generated highly disorganised grafts which completely surrounded the host kidney and invaded into adjacent renal tissue, demonstrating induction of an aggressive phenotype ($n=7$).

8. The authors comment that their results support work from a number of different epithelial tumour systems which demonstrates that the tumour microenvironment, and specifically, epithelial-mesenchymal interactions are critically important for tumorigenesis and that this indicates that more attention should be given towards the nature of cancer associated stroma. They do not specifically refer to the TOFT but, in an editorial, Sonnenschein and Soto state that the paper provides compelling evidence in favour of the TOFT (7).

Maffini et al (2005), Am J Pathology 167, 1405-1410 (8, attached at Annex 4).

9. This study aimed to explore whether age and parity affects the ability of the stroma to support or repress tumour development.

10. The mammary epithelium was surgically removed from the 4th and 5th abdominal-inguinal MG of 10-day-old rats. The left abdominal-inguinal MGs were left intact and considered internal controls. The rats were then separated into 2 groups. One group contained twice-parous rats. The other consisted of virgin females of either 24, 52, 80 or 150 days of age. These ages were chosen for the following reasons. The first two time points are when ductal invasion of the stroma takes place in the intact gland: the beginning of ductal invasion occurs at 24 days of age and evident ductal growth is underway at 52 days of age. The latter age was also said to represent the window of maximum vulnerability to chemical carcinogens in tumour-susceptible strains of rat. At 80 days of age, the ducts reach the edge of the fat pad and at 150 days of age, the MG of a virgin animal is considered an organ where no major tissue remodelling is observed.

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11. The right side cleared fat pad of each rat was inoculated with 5×10^4 mammary tumour cells prepared from tumours induced in rats of the same strain by i.p. injection with NMU. Starting one month after the cell inoculation, all rats that received a cell transplant were palpated weekly. Animals were sacrificed when tumours reached 1 cm in diameter or 6 months after cell transplant, whichever occurred first.

12. The transplantation of mammary tumour cells into the cleared fat pads gave rise to ductal outgrowths which were phenotypically normal at the time of harvesting i.e. 6 months after inoculation. Tumour development in the host animals inversely correlated with the age of the host (see Table 2). The tumours were classified mainly as carcinomas.

Table 2. Outcome of Neoplastic Epithelial Cell Injection into Hosts at Different Ages and Parity Status (from Maffini et al, 2005)

Host age	Initial no.	Final no.	Tumors	Outgrowths
Twice parous	7	5	0/5 (0%)	5/5 (100%)
150 days old	11	11	2/11 (18.2%)	11/11 (100%)
80 days old	11	10	5/10 (50%)	7/10 (70%)
52 days old	10	8	8/8 (100%)*	7/8 (87.5%)
24 days old	9	8	6/8 (75%)†	5/8 (62.5%)

*Statistically different from twice-parous and 150-day-old host groups.

†Statistically different from twice-parous and 150- and 80-day-old host groups.

13. To recognise the tumour cells which were injected into the host's cleared fat pads, the authors used the codon 12 GGA to GAA mutation in the Ha-*ras*-1 as a marker of tumour origin. All of the donor tumours carried this mutation and it was observed in both types of secondary outcomes, namely, tumours or normal ductal development, which the authors consider confirms their tumour origin.

14. The authors concluded that the tumour development pattern suggested a parallel to the usual pattern of age- and reproductive state-dependent susceptibility and resistance to carcinogens. They suggest that, as susceptibility to carcinogenesis decreases, the ability of the stroma to reprogram neoplastic epithelial cells increases and thus, the neoplastic phenotype is context-dependent.

Questions for the committee

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15.1 The committee is asked for comments on the Tissue Organisation Field Theory of carcinogenesis and whether it considers the experimental work outlined above supports this hypothesis or not.

15.2 Does the committee have any comments on the paper by Newby and Howard?

Secretariat
May 2006

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5. Maffini MC, Soto AM, Calabro JM, Ucci AA and Sonnenschein C (2004). The stroma as a crucial target in rat mammary gland carcinogenesis. J Cell Science 117: 1495-1502.
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7. Sonnenschein C and Soto AM (2005). Are Times a' Changin' in Carcinogenesis? Endocrinology 146(1): 11-12.
8. Maffini MC, Calabro JM, Soto AM and Sonnenschein C (2005). Stromal regulation of neoplastic development. Am J Pathology 167: 1405-1410.

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Annex 1 to CC/06/10

Newby JA and Howard V (2006). Environmental influences in cancer aetiology. J Nutritional and Environmental Medicine, 1-59: Preview article.

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Annex 2 to CC/06/10

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Annex 3 to CC/06/10

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Annex 4 to CC/06/10

Maffini MC, Calabro JM, Soto AM and Sonnenschein C (2005). Stromal regulation of neoplastic development. Am J Pathology 167: 1405-1410.

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