Altered Morphology and Behaviour of Kidney Fibroblasts in vitro, Following in vivo Treatment of Rats with a Carcinogenic Dose of Dimethylnitrosamine

Based on the original observation by MAGEE and BARNES[1], SWANN and MCLEAN[2] demonstrated that priming with a protein deficient diet increased the LD_{50} dose of dimethylnitrosamine (DMN) to rats and that renal tumours appeared in all surviving rats given 60 mg/kg. Subsequently[3] it has been shown that a diet of sucrose and water given 3 days prior to DMN has the same effect and that 100% of rats under either of these dietary conditons, develop renal tumours of the mesenchymal type. HARD and BUTLER[4,5] have traced sequentially, the development of this tumour. A diffuse increase in mononuclear cells occurs within the cortical interstitium by 7 days which then rapidly regresses but at a dose of 60 mg/kg hypercellular foci associated with periglomerular areas never disappear. The hypercellular foci consist predominantly of inflammatory and immunological cells, but from 3 weeks, very occasional abnormal cells which possess ultrastructural characteristics of the malignant cells comprizing the final tumour are found. The growth of kidney cortex cells in vitro taken from rats treated with DMN has been studied. BARKER and SANFORD[6] have described the cytological changes which appear to be associated with neoplastic transformation of cell lines grown in tissue culture. Using these criteria, together with the known morphological changes which take place in affected cells in vivo during the development of DMN-induced renal mesenchymal tumour[5], we compared the cytological characteristics of kidney cortical tissue grown from control rats, with that from rats given DMN, 7 days prior to explantation. The tissue was cultured at this time as it coincided with the peak of acute toxic injury in the renal cortex.

Porton strain Wistar male rats, 6 weeks old, were maintained on sucrose granules and water for 3 days and injected i.p. with 60 mg/kg DMN. 7 days later the renal cortex from these and from untreated rats, was stripped of capsule, cut into 2 mm cubes and washed 3 times in Ca-Mg-free phosphate-buffered saline (PBS). The tissue was then agitated for 10 min in 0.1% trypsin in PBS at room temperature, the pieces allowed to settle and the supernatant discarded. Fresh trypsin was added and after a further 10 min agitation, supernatant was collected and placed in a conical centrifuge tube containing 0.5 ml of growth medium 199 including 10% calf serum. The process was repeated 4 to 5 times and the resultant cell suspensions from an individual rat were pooled. Following centrifuagtion at 600 *g* for 10 min, the cell pellets were resuspended in growth medium and the volume adjusted to a concentration of 5×10^5 cells per ml. The suspensions were dispensed in 4 ml aliquots into 30 ml plastic culture bottles. The cultures were incubated at 37 °C in a 5% CO_2 in air mixture. They were examined frequently by phase-microscopy, and at various periods, the monolayers were fixed intact for electronmicroscopy, with 2% OsO_4 and embedded in Epon 812. The fixation, dehydration and embedding processes were all performed within the culture bottles so that the location and disposition of particular cell clusters, detected by light microscopy, were retained. The selected areas were sectioned by ultramicrotome, in the plane of the monolayer.

Prior to fixation, the control cultures consisted mainly of groups of epithelial cells surrounded by fibroblastic cells (Figure 1) while those from DMN treated rats showed the following changes. Within 2 weeks of culture, clusters of fibroblastic cells were present in which cells grew over one another and lacked the uniform growth pattern of normal cells. Within these clusters there was considerable pleomorphism of cells and their nuclei, while nucleoli were very conspicuous. Some cells were multinucleate, while others were associated with abnormal division. Small cells with retracted cytoplasm were seen, and in many cases, these appeared to be detaching from the cytoplasm of larger, sometimes multinucleate cells within the clusters (Figure 2). Some piling up did occur in control cultures due to overcrowding, but these sites ultrastructurally were associated with cell death.

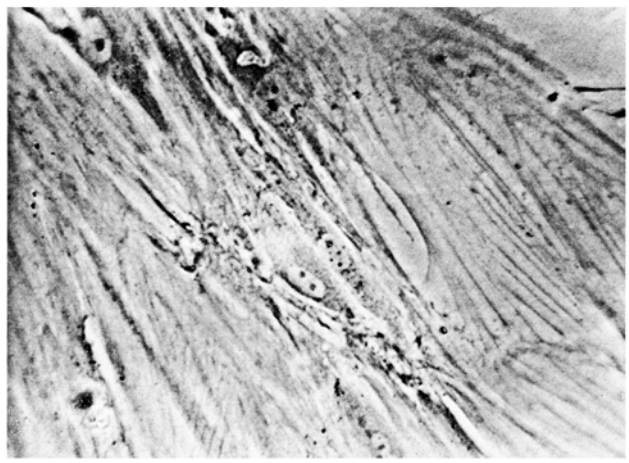

Figure 1. Culture of renal cortical cells from an untreated rat 14 days after initiation of culture showing a monolayer of spindle cells. × 200.

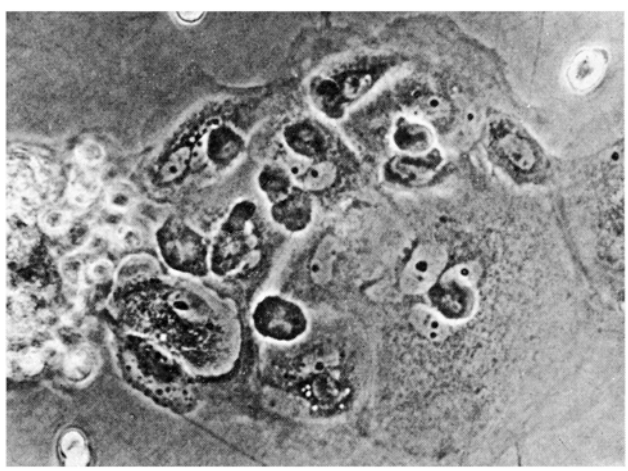

Figure 2. Culture of renal cortical cells 7 days following treatment with DMN and 14 days after initiation of the culture. The clusters of cells show pleomorphic cells and nuclei with prominent nucleoli. × 200.

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[2] P. F. SWANN and A. E. M. MCLEAN, Biochem. J. *107*, Procs 14P (1968).
[3] G. C. HARD and W. H. BUTLER, Cancer Res. *30*, 1796 (1970).
[4] G. C. HARD and W. H. BUTLER, Cancer Res. *30*, 2806 (1970).
[5] G. C. HARD and W. H. BUTLER, Cancer Res. *31*, 337 (1971).
[6] B. E. BARKER and K. K. SANFORD, J. natn. Cancer Inst. *44*, 39 (1970).

Cell clusters showing the above abnormal features were selected by light microscopy, prepared for electronmicroscopy at $2^1/_2$, 4 and $6^1/_2$ weeks, and their ultrastructure compared with that of representative areas within normal cultures from control animals. Heterogeneity of size and shape of cells was confirmed. Nuclei were enlarged and more frequent, exhibiting either hypertrophy or bizarre fragmentation of the nucleolar components or both. The cytoplasm of many treated cells was filled with sheets of microfilaments conferring on them the appearance of smooth muscle. This was in contrast to normal cells in which microfilaments appeared as relatively narrow bundles usually along the cell periphery. Some mitochondria in abnormal cells were of very irregular shape in contrast to the uniformly rod-like organelles of cells in untreated cultures.

The supernatant from the treated rat cultures yielded a population of cells which readily attached to fresh culture dishes and settled to form multinucleate, polygonal cells and cells with pleomorphic nuclei.

Increase in cell size, nuclear size and number, and abnormality of mitochondria and nucleoli are features characteristic of transformed fibroblast-like cells which initiate renal mesenchymal tumours and which may be found in persisting inflammatory lesions in vivo as early as 3 weeks after DMN treatment[5]. All of these features except mitochondrial abnormality are also included in the criteria listed as manifestations of neoplastic transformation of cells in tissue culture[6]. Abnormal cells with cytoplasm filled with microfilaments are also characteristic of early lesions preceding mesenchymal tumour development[5] whilst vascular smooth muscle is a feature of the final tumour[7]. The obseravtions suggest that the cytological characteristics seen in some groups of cells within renal cultures taken from rats treated with a carcinogenic dose of DMN may be due to the unimpeded in vitro development of cells transformed in vivo.

Zusammenfassung. Eine Woche nach Behandlung mit Dimethylnitrosamin wurden an Nierenrindenzellen von Ratten Fibroblasten mit veränderter Morphologie und Verhalten festgestellt, welche sich zu mesenchymalen Nierentumoren entwickelten.

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[7] G. C. Hard and W. H. Butler, Cancer Res. *31*, 348 (1971).
[8] University of Cambridge, Department of Pathology, Cambridge (England).

Different Incidence of Breast Carcinomas or Fibroadenomas in Daunomycin or Adriamycin Treated Rats

Experimental tumours induced by antitumour drugs are well known[1-3]. Informations concerning the oncogenic properties of the antineoplastic antibiotic daunomycin (Daunorubicine)[4] have been incidentally reported in the literature: fibrosarcomas have been noticed at injection site in about 25% of treated rats[5]; tumours of various organs, including mammary glands, kidney, uterus, vagina, lung have been found in Sprague-Dawley rats receiving high doses of the antibiotic[6].

In a Sprague-Dawley SPF strain of rats (Charles River, France) with a very low natural incidence of tumours, we tried to assess the carcinogenicity of both daunomycin and adriamycin – a new antitumour antibiotic of anthracyclin group[7] – whose chronic toxicity data have been already published[8,9].

Among findings so far obtained, we report here those concerning female rats treated i.v. with a single high dose of daunomycin or adriamycin. High incidence of mammary tumours was observed in such animals in a relatively short time. The first daunomycin-induced tumour appeared 94 days following the injection and the mean induction time was of 121 days; the first adriamycin-induced tumour appeared after 156 days and the mean induction time was 223 days. Besides this, in daunomycin-treated rats we

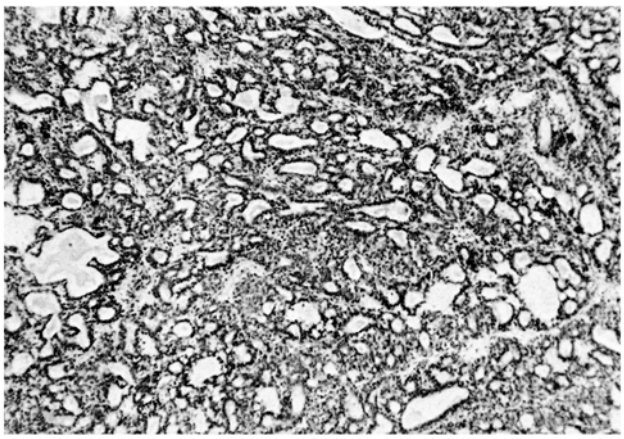

Fig. 1. Daunomycin – Mammary tissue: adenocarcinoma. Hem.-eos. ×80.

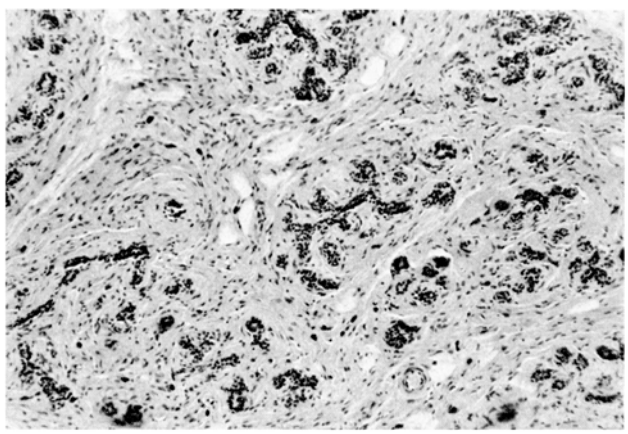

Fig. 2 Adriamycin – Mammary tissue: fibroadenoma. Hem.-eos. ×80.