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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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

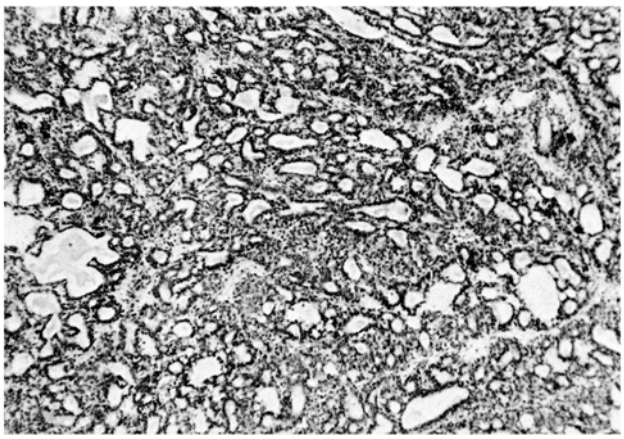

Fig. 1. Daunomycin – Mammary tissue: adenocarcinoma. Hem.-eos. ×80.

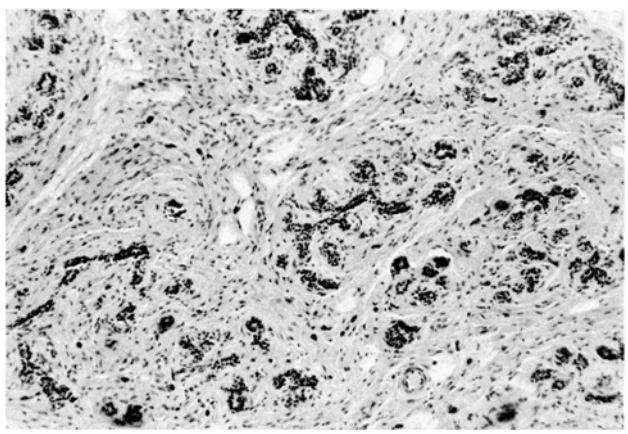

Fig. 2 Adriamycin – Mammary tissue: fibroadenoma. Hem.-eos. ×80.

Incidence of mammary tumours in rats during one year experiment

Treatment	Dose (mg/kg)	No. of rats	Rats dead during the experiment		Rats sacrificed at the end of the experiment		Total of tumour-bearing rats (A+B)	Tumour histology (A+B)	
			Without tumour	With tumour A	Without tumour	With tumour B		adenocarcinomas	fibroadenomas
Daunomycin	12.5	25	12	2	0	11	13	11	2
Adriamycin	8.–	25	17	1	1	6	7	1	6
Controls	–	25	0	0	25	0	0	0	0

observed 1 rabdomiosarcoma of the thigh and 1 polypus of the uterus; in the adriamycin group 1 meningioma and 2 uterine polypi were noticed. No tumours were found in the control animals. 14 of the 25 rats treated with daunomycin and 18 of the 25 rats treated with adriamycin died, due to severe renal damage or bone marrow aplasia, known to be induced by high doses of such antibiotics[8,10]. No relationship was found between death and presence or absence of tumours; in fact many rats died before tumour occurrence, particularly in the adriamycin group.

After 1 year of observation, all survivors and control rats were killed and examined carefully. Number of treated and control rats, doses, number of rats dead or sacrificed and breast-tumour incidence are plotted in the Table. In each tumour-bearing rat only 1 breast tumour and no metastases were found on extensive autoptic and histological examination.

Two findings are to be emphasized, i.e. the very high incidence of tumours in rats surviving until the end of the experiment, and the different histology of daunomycin tumours (mostly adenocarcinomas, Figure 1) in respect to adriamycin-ones (mostly fibroadenomas, Figure 2). The latter finding seems of high relevance, due to the close chemical similarity of the 2 antibiotics, which only differ in the presence of an hydroxy group linked to the C_{14}, present in adriamycin and absent in daunomycin.

Riassunto. La daunomicina (Daunorubicina) e l'adriamicina, antibiotici antitumorali, provocano dopo un'unica somministrazione endovenosa, nel ratto Sprague-Dawley di sesso femminile, l'insorgenza a distanza di alcuni mesi di tumori mammari, nel primo caso prevalentemente adenocarcinomi, nel secondo caso prevalentemente fibroadenomi.

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Temporary Retardation of Cartilage Growth in Mice after External β-Irradiation[1]

Partial whole-body irradiation of mice with external ^{90}Sr-^{90}Y applicators may provide a way to control both the dose rate and time of exposure of skeletal tissues to β-rays. In this way, toxicity information can be obtained which is impossible to resolve by the use of internal emitters which deposit nonuniformly and are subject to a changing distribution with time owing to internal remodelling. This method has already been employed by BRUES et al.[2,3] to study skin carcinogenesis and the epidermal cell population kinectics in mice after irradiation with high doses at a slow rate (75 rads/h). We have examined the skeletons from some of these mice exposed to body surface doses of 5000 to 7200 rads, where the respective dose rates to the knee joint tissues and marrow were estimated to be of the order of 68 and 20–30 rads/h. The data indicate that cartilage growth was temporarily stunted after these high doses, but that recovery was able to occur.

Materials and methods. The animals were drawn from 3 genetic strains of male C57 mice, black (C57 BL/6 ANL [ANL 66]), hairless white and haired analogues. At 4 months of age, they were exposed in a total-body surface β-irradiator (^{90}Sr-^{90}Y) designed by AUERBACH and BRUES[4]. The ^{90}Sr-^{90}Y source was in the form of ceramic microspherules embedded in polyurethane sheets. The sheet formed the inner lining of a 4″ long aluminum tube placed inside a wooden box, which was sealed by a 5/16″ thick fixed aluminum shield at one end and a similar but move-