The Diploid Deoxyribonucleic Acid (DNA) Content of Basal Cell Carcinomas in Man

Results obtained in previous studies of a number of carcinomas have revealed that such tumors consist of cells with aneuploid DNA values[1-8], in contrast to benign tumors, whose cells always have a diploid DNA content[6], corresponding to a diploid set of chromosomes. Thus, a criterion for the classification of benign and malignant tumors according to whether their DNA content is aneuploid or diploid has come into existence. However, it is not always true that a diploid set of chromosomes or a diploid DNA content validates the categorization of a tumor as benign and thereby excludes the possibility of the cells being neoplastic[6,9,10]. The present work was intended to investigate the possibility of a relationship between the amount of DNA and what is suspected to be the behavior of the lesions. Basal cell carcinomas were of special interest because they rarely metastasize, unlike other malignant tumors. Surprisingly enough, these carcinomas consist of cells whose DNA content is diploid and this led us to investigate a number of such tumors.

The sectioned material was stained by Feulgen's reaction[11], and the period of hydrolysis in 1 *N* NCL at 60 °C was kept constant to ensure uniformity. The DNA was measured in a microspectrophotometer operated by the 2 wave length method of PATAU[12], using the correction tables of MENDELSOHN[13] in the calculations. Only metaphase plates, which can safely be considered to yield the basic DNA content, were used for these measurements. Lymphocytes were measured in corresponding sections to give a reference for the diploid values of DNA. These results, however, could not be supplemented by chro-

Incidence of mitotic irregularities in basal cell carcinomas

Specimen No.	No. of cells counted	Mitotic irregularities (%)			Total
		Bridges	Fragments	Multipolar mitosis	
677	112	0.8	2.6	–	3.4
477	108	2.7	1.8	–	4.5
212	132	1.5	4.5	–	6.0
384	88	1.1	3.4	–	4.5
1667	110	1.8	4.5	–	6.3
1596	105	2.8	1.9	–	4.7
2243	132	2.2	2.2	–	4.4
1756	114	1.7	3.5	0.8	6.0
1706	115	1.7	2.6	–	4.3
3255	120	2.5	4.1	–	6.6
2380	115	3.4	5.2	–	8.6
3264	124	1.6	4.0	–	5.6
1291	104	5.7	3.8	–	9.5
6328	112	7.1	2.6	0.8	10.5
3071 B	101	4.9	6.9	0.9	12.7
3071 D	110	6.3	6.3	–	12.6

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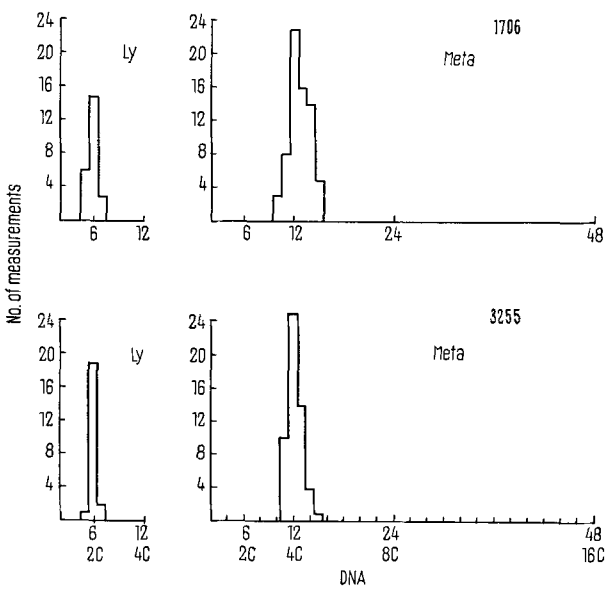

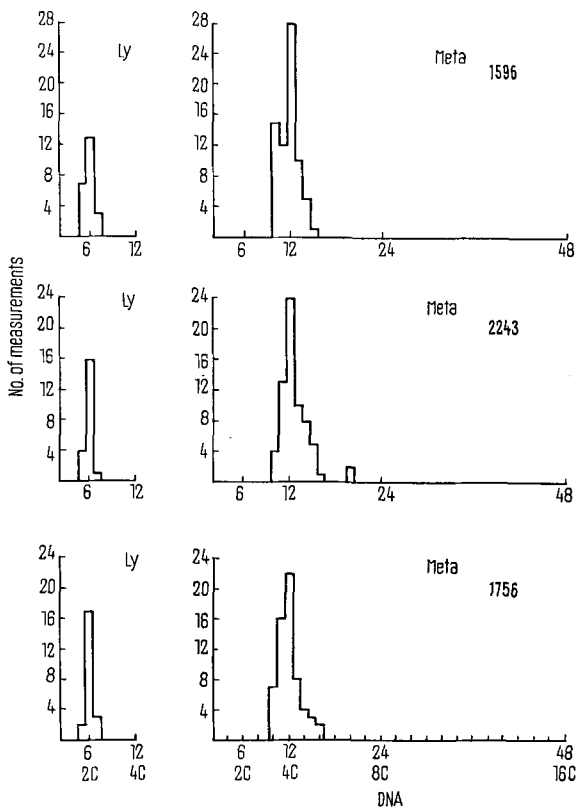

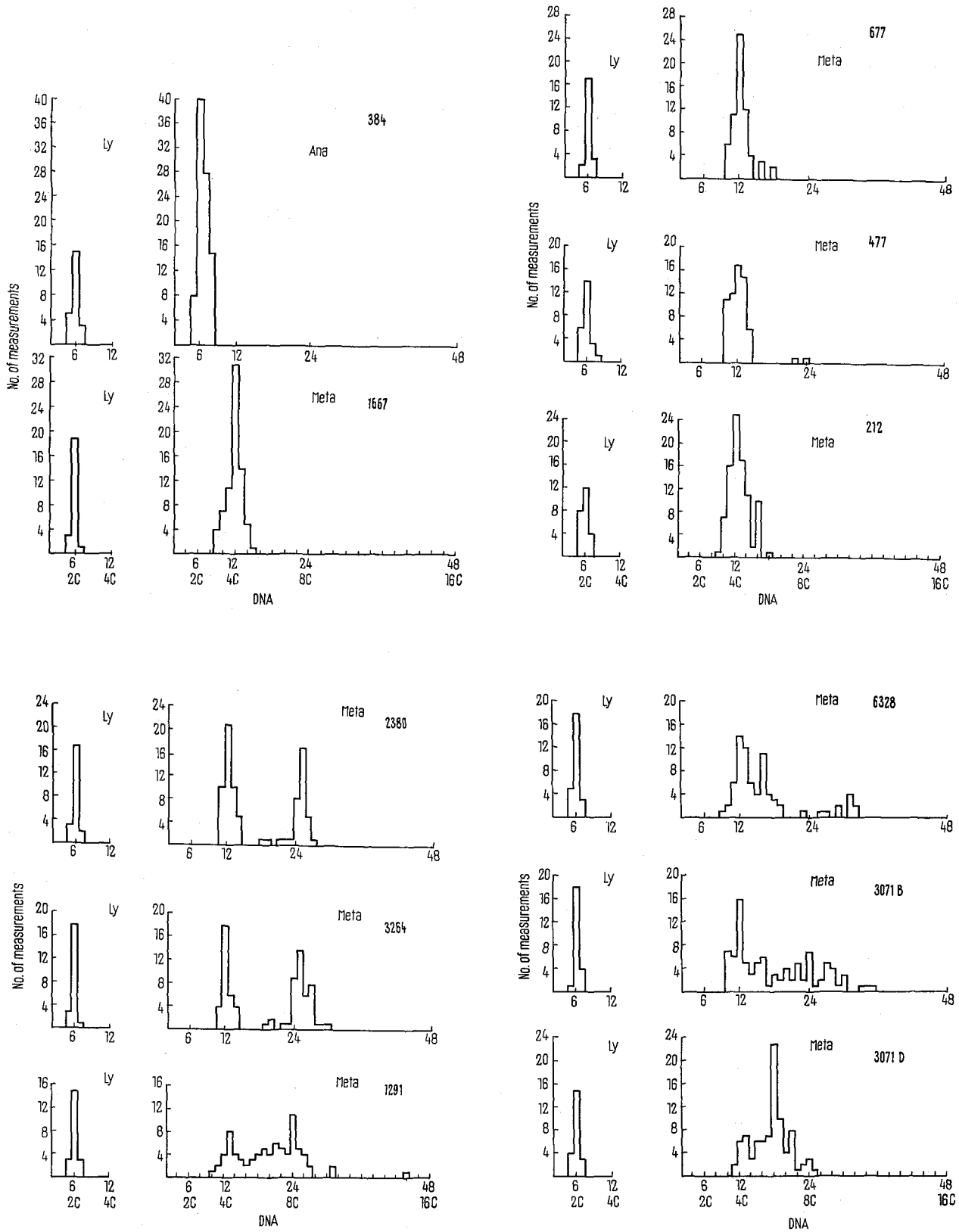

The deoxyribonucleic acid (DNA) content of anaphase and metaphase plates of the basal cell carcinomas. The cells of most carcinomas (Nos. 384, 1667, 677, 477, 212, 1596, 2243, 1756, 3255) contain a diploid amount of DNA No. 2380 and No. 3264 have DNA values in both diploid and tetraploid ranges. The other carcinomas (Nos. 1291, 6328, and 3071 B) showed a small number of aneuploid cells in addition to diploid nuclei, but the population is not as heterogenous as that of carcinomas in situ. No. 3071 D is a case of Baso-squamous cell carcinoma revealing a fast-growing aneuploid stem line. All DNA measurements are checked by measuring lymphocytes from the corresponding sections.

mosome counts because of the difficulty in using colchicine pre-treatment on human material.

Histologically, basal cell carcinomas have highly proliferated basal cell layers, with the peripheral cells showing a palisade arrangement. Their DNA content falls strictly in the diploid (Nos. 1706, 3255, 1596, 2243, 1756, 384, 1667, 677, 477, 212) or the diploid and tetraploid range (Nos. 2380, 3264). A number of abnormal mitoses in the form of chromosomal fragments and bridges were also observed and counted. The percentage of such abnormalities varies from 3.4–8.6% (Table). In some carcinomas, the basal cell masses do not show a complete layer of palisade cells at the periphery, but instead, haphazard aggregates of basal cells are seen lying deep within the corium. The DNA content of the nuclei of such carcinomas (Nos. 1291, 6328, 3071 B) is essentially diploid, but there are also a number of aneuploid cells, so the cell population is slightly heterogenous with regard to its DNA content. Mitotic irregularities in these lesions range from 9.5–12.7% (Table). One tumor (No. 3071 D) which had been biopsied from the same person as tumor No. 3071 B, showed cells which were histologically squamoid lying close to the fragmented masses of the basal cell layer of the corium. The nuclei of this lesion showed a highly proliferative aneuploid stem line, which outnumbered the diploid and the tetraploid nuclei. This appeared to be a case of baso-squamous cell carcinoma.

Therefore, it may be concluded, that most basal cell carcinomas have a diploid content of DNA. However, where the basal cell masses pass deep into the corium, the population tends to have a heterogenous DNA content. Simultaneously, these tumors show a progressive increase in the percentage of mitotic abnormalities. Lesions which are completely diploid may regress, as do most of the basal cell carcinomas, but the small percentage of lesions which have a mosaic composition could become malignant. A change in the environment of the tumor, such as by exposure to radiation[3,14] or antimetabolites[15,16], may help to select an aneuploid stem line out of the heterogenous population of the tumor[17].

Zusammenfassung. Der Desoxyribonukleinsäuregehalt ist bei Basalzellkarzinomen diploid im Tumor selbst, weicht jedoch bei invadierenden Stellen nach Diploidie ab.

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Cytogenetic Changes Induced by 1-(N^1-Methylhydrazinomethyl)-N-Isopropyl Benzamide in Ehrlich Ascites Tumor Cells

Dramatic anti-tumor effects of certain derivatives of methylhydrazine have been described in experimental animal tumors[1] and clinically, in Hodgkins disease refractory to other chemotherapeutic agents[2,3]. Neither the active molecular species nor the mechanism of action of this relatively new class of cytotoxic agents is known, although it is possible that their action involves the oxidation[4] and alkylation[5] of cellular constituents. RUTISHAUSER and BOLLAG[6] have observed inhibition of mitosis and the appearance of various cytogenetic aberrations in Ehrlich ascites tumor cells from animals treated with 1-methyl-2-benzylhydrazine (MBH). The primary cytological changes observed by these workers were non-specific chromatid breaks accompanied by large numbers of reunions and triradial rearrangements. BOLLAG[7] has also reported the development of resistance in Erlich ascites cells after treatment with *p*-(N^1-methylhydrazinomethyl)-N-isopropyl benzamide (MIH). However, to our knowledge, cytogenetic and biochemical studies of tumor lines resistant to methylhydrazine derivatives have not been reported. Such experiments are in progress in our laboratory at the present time. In this paper we report the results of initial experiments in which the treatment of tumor cells with MIH over several transplant generations has led to the development of a resistant line. In contrast to previously reported resistant cells, this new tumor line is characterized cytologically by the appearance of increased numbers of multinucleated cells, and with the appearance of 2 additional metacentric chromosomes in all scoreable cells. By appropriately altering the dosage schedule, an identical cytological picture could be produced in a single transplant generation.

Ehrlich ascites tumor cells were harvested 6–7 days after inoculation from the peritoneal cavity of mice weighing about 25 g. The cells were incubated with colchicine (*0.2 µg/ml) in vitro for 2 h in order to arrest them in* metaphase. The percentage of mitoses in 1000 counted cells was recorded after staining with Wright's stain. Treatment of tumor-bearing animals with a single s.c. dose (200 mg/kg) of MIH resulted in a rapid fall of the mitotic index (Table). It may be noted that no inhibition of mitosis was observed 4 h after the administration of MIH. But, the effect of the drug became maximal between 4 and 8 h. This inhibition persists for a period of

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