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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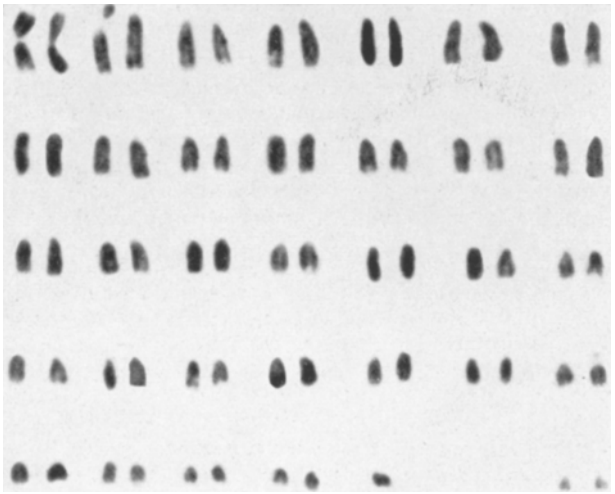

Fig. 1. Karyotype of an Ehrlich ascites tumor cell after MIH treatment through 5 transplant generations. Note the 2 additional metacentric chromosomes in the top row at the left.

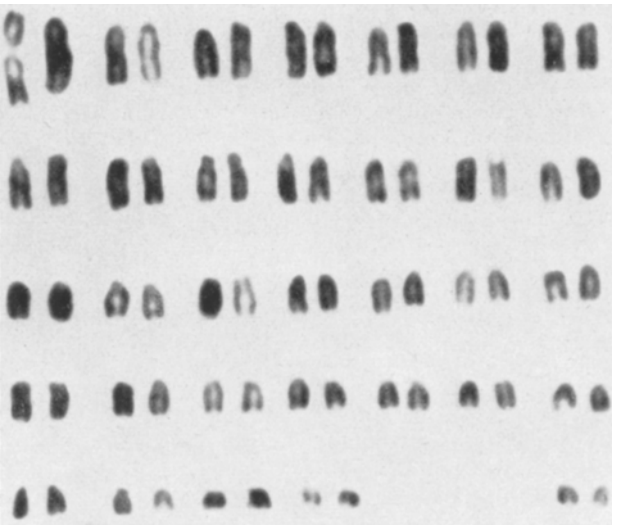

Fig. 2. Karyotype of an untreated Ehrlich ascites tumor cell. Note the single metacentric chromosome in the top row at the left.

The effect of MIH on the mitotic index of Ehrlich ascites tumor cells

h after treatment	Mitotic index in % Control	MIH (200 mg/kg)
4	5.5	5.0
8	3.8	0.5
24	3.5	0.2
48	3.5	0.8
72	3.5	1.5

about 48 h, and about 72 h after treatment the number of cells in mitosis begins to increase toward the control level. These results are similar to, but more marked than those reported in cells after treatment with MBH[6]. Increased numbers of multinucleated giant cells were found at the height of mitotic arrest, but chromatid breaks, translocations, and rearrangements were infrequently seen.

Striking chromosomal aberrations were observed, however, after tumor-bearing animals were treated with MIH over 5 transplant generations. During each passage the animals received a total of 600 mg/kg of MIH divided into 4 daily doses of 200, 200, 100 and 100 mg/kg. At the end of this treatment period the cells observed were characterized by the appearance of 2 additional metacentric chromosomes. This is illustrated in Figure 1. The new chromosomes were observed consistently in all scoreable cells. The karyotype of the tumor cells unexposed to MIH exhibit only a single metacentric chromosome and a distinctive medium-sized chromosome with 2 satellites. This is illustrated in Figure 2. The karyotype of bone marrow cells from MIH-treated animals remained unchanged. The new and large metacentric chromosomes probably result from the translocation of small-and medium-sized chromosomes secondary to damage around the centromere and to faulty reunion. Coincident with the appearance of the metacentric chromosomes, the tumor line becomes resistant to the effects of MIH and mitotic inhibition is no longer observed. Comparative biochemical studies of the sensitive and resistant tumor line will be published elsewhere.

It is of further interest that identical chromosomal abnormalities were also produced in a single transplant generation by altering the schedule of MIH administration. For example, if MIH (200 mg/kg) was administered to animals every fourth day, rather than on consecutive days, 2 translocations in 80% of the scoreable cells were observed before the first passage (in about 8–10 days). The total dose administered to the animals in these experiments was 600 mg/kg. This is the same dose that when administered to the animals on consecutive days required 5 such treatments during separate passages of the tumor before complete conversion to the new cell type and the development of resistance. These experiments demonstrate that the rapidity with which resistance to MIH develops, at a given dosage level, is dependent on the dosage schedule, and they have obvious relevance to problems of clinical resistance.

Zusammenfassung. Trägermäuse von Ehrlich-Ascites-Tumoren zeigen nach Behandlung mit 1-(N^1-Methylhydrazinomethyl)-N-Isopropyl-Benzamid (MIH) eine sofortige Zellteilungshemmung. Diese lässt sich nach s.c. MIH-Injektion von 200 mg/kg bis auf 72 h ausdehnen. Der MIH-Behandlung widerstehende Zellen (nach 5 Tagen) waren durch 2 neue metazentrische Chromosomen ausgezeichnet.

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