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Tumor Etiology and Chromosome Pattern

Abstract. *Fibrosarcomas induced in Chinese hamsters and rats by Rous sarcoma virus and 7,12-dimethylbenz(a)anthracene are associated with nonrandom chromosome variation. Although histologically indistinguishable, the tumors induced by the virus or chemical in each host species are characterized by completely different karyotypic patterns.*

Nonrandom karyotypic patterns in tumors may be largely dependent on the inducing agent, with different agents predisposing to different patterns (*1*). This has been observed in tumors of two host species, Chinese hamsters and rats, after induction with two oncogenic agents, Rous sarcoma virus (RSV) and 7,12-dimethylbenz(a)anthracene (DMBA).

In 42 primary sarcomas induced by RSV in an inbred strain of Chinese hamsters, Kato analyzed 32 hyperdiploid stem- and sidelines (*2*). Among them, 29 had one or more additional chromosomes of chromosome pairs No. 5, 6, or 10. In 20 of the 24 trisomic stem- and sidelines, the only abnormality was the addition of a chromosome of one of these three pairs; this is obviously a nonrandom pattern.

In five out of six primary sarcomas induced by DMBA in the same inbred Chinese hamster strain, we found a different chromosomal pattern, characterized primarily by additional chromosomes of pair No. 11. This was found in four diploid or near-diploid tumors and in one near-triploid, with one diploid tumor showing no deviation from the normal Chinese hamster karyotype (*3*).

In the rat, the RSV-induced sarcomas exhibited one of the most striking nonrandom patterns ever observed, involving a characteristic three-step karyotypic evolution with additions, in turn, of one medium-sized t (*4*), one st_3, and one st_5 chromosome (Fig. 1). The establishment of this pattern was based on the analysis of stemlines, sidelines, and single deviating cells in 80 primary and 20 metastatic tumors, including sequential passages of a number of parallel sublines from two of the primary tumors (*1*, *5*).

Chromosome analysis of 12 primary DMBA-induced sarcomas in the same inbred rat strain revealed a completely different karyotypic pattern (*6*). Among these sarcomas were ten diploid or near-diploid tumors, in which trisomy for the longest t chromosome, t_1, was the first characteristic feature, the second being the addition of an m chromosome (Fig. 1). The chromosomes most often involved in the evolution of the RSV tumors were participating only exceptionally in the variation of the DMBA tumors. Gains of the t_1 chromosome and of one m chromosome were leading features also in rat leukemias induced by DMBA and by the closely related hydrocarbons 6,8,12- and 7,8,12-trimethylbenz(a)anthracene (*7*).

These results throw light on the interaction during oncogenesis of the inducing agents with the hereditary apparatus of the cell. If the chromosomal changes are indicators of the underlying variation in the genic material on the molecular level, it appears that with different oncogenic agents essentially different pathways must be involved. Our observations may explain the ap-

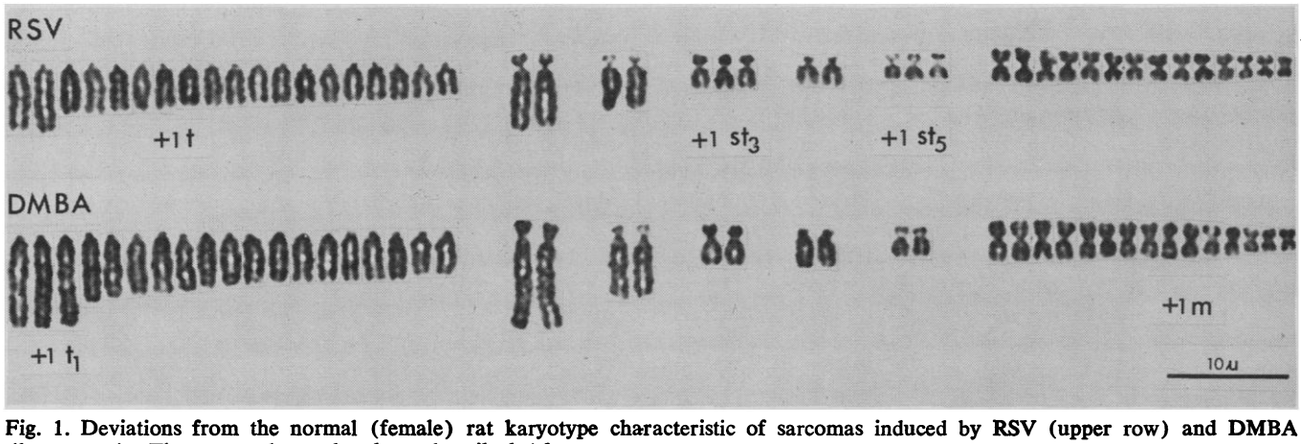

Fig. 1. Deviations from the normal (female) rat karyotype characteristic of sarcomas induced by RSV (upper row) and DMBA (lower row). The nomenclature has been described (*4*).

parent lack of chromosomal patterns noticeable in most classes of tumors. Just as the present RSV and DMBA tumors are indistinguishable histologically but readily distinguishable chromosomally, other tumors, histologically well-defined, may be composed of many etiologically and chromosomally diverse entities; this mixture of karyotypes would obscure the recognition of specific chromosomal patterns.

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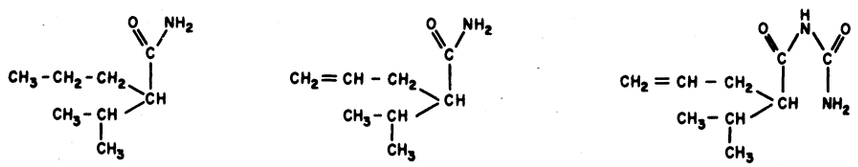
Destruction of Cytochrome P_{450} by Secobarbital and Other Barbiturates Containing Allyl Groups

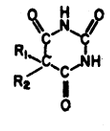
Abstract. *Administration of certain commonly used barbiturates containing allyl groups, such as secobarbital, allobarbital, or aprobarbital to rats treated chronically with a microsomal enzyme inducer causes a rapid destruction of the liver microsomal hemoprotein that serves as the terminal oxidase for drug metabolism. In contrast, barbiturates without an allyl group do not have this effect. The decrease in this hemoprotein, cytochrome P_{450}, by the barbiturates containing an allyl group could also be demonstrated in an in vitro liver microsomal system requiring reduced nicotinamide adenine dinucleotide phosphate. These results suggest that the barbiturates containing an allyl group are converted to a metabolite that leads to the destruction of cytochrome P_{450}.*

Several groups of structurally unrelated compounds produce, in rodents, an experimental porphyria that resembles hepatic porphyria in humans (*1*, *2*). These compounds stimulate the formation of porphyrins in liver cells in vivo and in vitro (*1*, *3*), presumably as a result of the increased synthesis of δ-aminolevulinic acid (δ-ALA) synthetase, the rate-limiting enzyme in porphyrin and heme biosynthesis (*1*, *4*). Although the exact mechanism of the chemically induced increase in δ-ALA synthetase is not known, it may result from an interference by these compounds with the feedback control exercised by heme on the enzyme (*1*). One of these compounds, allylisopropylacetamide (AIA), a barbiturate-related derivative, causes an initial rapid decrease in the concentration of cytochrome P_{450}, while exerting little or no effect on other enzymes associated with liver microsomes (*5*, *6*). This decrease is due to the destruction of cytochrome P_{450} heme with the resultant accumulation of the heme breakdown products in the liver (*5*, *6*). These products, which impart a green-brown color to liver microsomes, may be nonphysiological isomers of biliverdin (*7*). Preliminary evidence indicates that a metabolite of AIA is required for this breakdown of cytochrome P_{450} heme (*5*). Cytochrome P_{450} is the terminal oxidase in the metabolism of a wide variety of substrates such as insecticides, steroids, drugs, and chemical carcinogens (*8*). Treatment of rats with various compounds that induce liver microsomal cytochrome P_{450} results in an increase in the rate of breakdown of cytochrome P_{450} by AIA (*5*). Kaufman *et al.* (*9*) have demonstrated an increased in vivo metabolism of AIA following treatment of rats with phenobarbital (PB), an inducer of microsomal enzymes. Because epoxides are intermediates in the oxidative metabolism of certain olefins and aromatic compounds (*10*), we initiated studies to determine if the allyl group of AIA, also capable of oxidation to an epoxide, was required for this breakdown of cytochrome P_{450} heme. We therefore studied the effect of a number of barbiturates and related compounds containing allyl and alkyl groups on liver microsomal cytochrome P_{450}.

Adult male Long-Evans rats (170 to 180 g) had free access to a commercial diet and water. Sodium phenobarbital dissolved in 0.9 percent NaCl was administered intraperitoneally, at a daily dose of 75 mg per kilogram of body weight for 3 days, to stimulate the synthesis of liver microsomal hydroxylase (*8*). On the fourth day, the test compounds were administered subcutaneously 1 hour before the animals were killed. Liver microsomes were prepared in 0.25*M* sucrose, suspended in 1.15 percent KCl, and centrifuged. The final microsomal pellets were layered with 3 ml of 0.1*M* potassium phosphate buffer (*p*H 7.4), and stored, frozen, for 1 to 5 days before being used. For in vitro metabolism studies, the incubation mixture, in a total volume of 7.5 ml, consisted of microsomes equivalent



Isopropylvaleramide | Allylisopropylacetamide | Allylisopropylacetylcarbamide



Barbiturate	R_1	R_2
Aprobarbital	allyl	isopropyl
Probarbital	ethyl	isopropyl
Secobarbital	allyl	1-methylbutyl
Pentobarbital	ethyl	1-methylbutyl
Allobarbital	allyl	allyl
Barbital	ethyl	ethyl

Fig. 1. Structures of several barbiturates and barbiturate-related compounds.