POLYPLOIDY AND CANCER

The Desoxypentosenucleic Acid Content of Nuclei of Normal, Precancerous, and Neoplastic Rat Tissues

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INTRODUCTION

Certain authors have reported that the desoxypentosenucleic acid content of tumor tissue is higher than that of homologous normal tissues (8, 19), and further, that the content of this nucleic acid is increased in liver during the process of cancer induction in this organ (4, 8, 12, 15). Desoxypentosenucleic acid (DNA) is confined entirely or almost entirely to the nucleus in neoplastic and non-neoplastic cells (4, 14, 15). It follows that the number of nuclei in a given volume, or their average DNA content, or both, must be increased, in tumors and in tissues showing precancerous changes. The first explanation is in accordance with histological findings (4, 15). However, it has been reported recently that in certain normal mammalian tissues the quantity of DNA per nucleus is a constant independent of the cell type (3, 21, 22) and nearly identical in various species (22). Similarly, constant values for DNA per nucleus in certain cell types have been reported independently by Mirsky and Ris (13). Therefore, it was of interest to determine whether exceptionally large quantities of this nucleic acid appeared in individual nuclei as a result of induction of tumors. Accordingly, studies were undertaken to determine the average DNA content of nuclei in normal tissues of the rat, in rat tissues undergoing precancerous changes, and in tumors produced in this species.

Methods

Male albino rats of the Holtzman, Sprague-Dawley strain weighing 200 to 300 gm., were used, except as otherwise indicated. For induction of tumors, rats were placed on a basal semisynthetic diet, previously described (8) to which was added either 0.06 per cent 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB) or 0.04 per cent (7) 2-acetylaminofluorene (AAF). Hypertrophic livers, free of visible tumors, were obtained after feeding 3′-Me-4-DAB for some weeks, and after 5 months of feeding AAF. Livers were freed of blood by perfusion with cold physiological saline.

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In a room kept at 1°–2°C., the various tissues used were suspended in ice cold 2 per cent citric acid. Livers, tumors, and external orbital glands were homogenized in a Waring blendor until relatively few intact cells remained (1 to 6 minutes). A medium, described previously (4) containing 0.88 M sucrose, was used in one experiment as noted in Table I, but citric acid was used in the remainder of the experiments to facilitate isolation of intact nuclei (2). Spleens, thymuses, and newborn rat livers were

TABLE I

Average DNA Content of Nuclei of Various Tissues of Male Rats Fed 3'-Me-4-Dimethylaminoazobenzene (3'-Me-DAB) or 2-Acetylaminofluorene (AAF), or Not Treated with Carcinogens

Tissue	No. of rats	Carcinogen	DNA per nucleus micrograms × 10^4
Liver	3	None	6.1
" *	2	"	5.9
"	3‡	3'-Me-DAB	6.2
"	3‡	"	5.6
"	3§	"	6.0
"	2	AAF	6.4
"	2	"	5.3
Thymus	1	None	6.3
Spleen	1	"	6.5
Tumor of:			
Liver	3	3'-Me-DAB	7.5
"	3	"	8.1
"	1	"	7.9
"	1	AAF	6.0
Face (carcinoma)	1	"	5.9
Liver‖	3	None	8.0
Thymus‖	3	"	6.1
Spleen‖	3	"	6.0
External orbital gland	3‖	"	21.3
Liver ¶	10	"	9.4

* Suspended in 0.88 M sucrose medium.
‡ Carcinogen fed 6 weeks.
§ Carcinogen fed 10 weeks.
‖ Over 1 year old, Wistar strain.
¶ Newborn, Wistar strain.

ground gently with a mortar and pestle and a suspension of the tissue in 2 per cent citric acid solution was prepared. Where necessary, the suspensions were strained through four layers of cheese-cloth, to remove any remaining clumps of cells. Approximately 1000 unstained nuclei of each suspension were counted on hemocytometer slides. From aliquots of the suspensions, containing known numbers of nuclei, about 70 per cent of the nuclei were sedimented in a small clinical centrifuge developing a field approximately 500 × *g*. The sediment was washed with 2 per cent citric acid solution and the supernate combined with that from the first run. Nuclei were

then counted in the combined supernates and the number of nuclei in the washed sediment obtained by difference. This method of isolating a known number of nuclei was developed to minimize errors due to contamination of the isolated nuclei with any chromatin threads from ruptured nuclei, or with the Feulgen-positive bodies of tumor cytoplasm described by Price *et al.* (14). It was considered desirable to avoid counting the isolated nuclei directly, because of the slight clumping tendency of the centrifuged nuclei.

The DNA of the isolated nuclei was determined by the method of Schneider (18). Multiple determinations of the amount of DNA per nucleus in a given tissue homogenate agreed within 5 per cent. Nuclei counts were reproducible with variations of less than 5 per cent.

RESULTS

Calculated average values for DNA per nucleus, on nineteen samples of rat tissue representing numerous tissue types, are given in Table I. It appears that there were no significant differences between values for any of the tissues studied except for the liver tumors produced by 3′-Me-DAB, livers of newborn rats, and liver and external orbital glands of rats over 1 year of age.

DISCUSSION

The average value of approximately 6.0×10^{-6} micrograms of DNA per nucleus, for the tissues giving closely similar values, is in very good agreement with the results of Vendrely and Vendrely (22) for various organs from mammals of nine different species. These authors have concluded (22) that the figure is almost identical for somatic cells of all mammals, the value for haploid cells being half that for somatic cells (21). With the exceptions noted, differences between values for rat tissues here reported are no greater than those between values for four bovine organs (liver, thymus, kidneys, and pancreas) reported by Vendrely and Vendrely (21).

The exceptionally high value for external orbital glands and the somewhat higher values for livers of older rats are presumably due to appearance of greater numbers of tetraploid, octaploid, and even higher polyploid nuclei in these tissues, with advancing age (9, 20). The large polyploid nuclei in these tissues can be recognized microscopically and presumably contain more DNA than diploid nuclei in accordance with their greater numbers of chromosomes, as shown by Ris and Mirsky (17). Similarly, nuclear measurements reported by Langer (10) indicate that azo dye-induced liver tumors in rats, like a number of other types of tumors (1, 5, 6, 20), contain relatively large numbers of polyploid nuclei. Rat livers showing precancerous changes caused by feeding azo dyes (10), as well as cirrhotic human livers (1), contain increased numbers of large nuclei, but this alteration would not necessarily be reflected by increased average values for DNA per nucleus in the livers, because of the concurrent proliferation of bile duct epithelial cells, which have small nuclei

(1, 4). Mark and Ris (11) have reported that the smallest spherical nuclei in normal rat liver, and in hepatomas and cholangiomas induced by a carcinogenic azo dye, contain almost identical quantities of DNA; but they state that certain larger tumor nuclei might have contained different amounts of DNA. Relatively large, presumably polyploid, nuclei were numerous in all the suspensions of tissues giving exceptionally high values in the present investigations, including the suspension of livers of newborn rats, while nuclei from spleen and thymus with rare exceptions appeared to have a uniform size.

The data afford no evidence that the amount of DNA in each set of chromosomes is not a constant, in all normal, precancerous, or tumor cells of the rat. Theoretically, the relations between diploid and polyploid cells of a given type are analogous to those between monomeric, dimeric, and polymeric molecules (9). Therefore the DNA content of an organ or tumor may be considered to be a measure of the number of its diploid cell units. Polyploid nuclei probably arise by interrupted mitosis (20). The quantity of DNA in a nucleus also increases preparatory to cell division, as is evident in regenerating liver (16). Thus, in general, any increase in DNA content of a tissue mass is to be attributed to the process of doubling of diploid units or of multiples thereof, either by completed or by interrupted mitotic cell division.

The findings of increased concentrations of DNA in tumors and in organs showing precancerous changes are essentially confirmations of histological observations of increased cellularity in these tissues, indicating loss of extracellular material or appearance of cells having relatively small cytoplasmic masses. Special cytological techniques are necessary for further elucidation of the relation between polyploidy and cancer formation.

SUMMARY

The average desoxypentosenucleic acid content of individual nuclei was determined for various normal and tumor tissues, and for livers showing precancerous changes, in the rat. With certain exceptions attributable to polyploidy, the values were practically indistinguishable from each other and from values reported for cell nuclei of other mammals. The amount of this nucleic acid in diploid cells of the rat appears to be a constant, nearly equal to 6×10^{-6} micrograms. Findings of increased concentrations of this nucleic acid in tissues showing preneoplastic or neoplastic changes therefore confirm histological observations of increased cellularity and polyploidy in such tissues.

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