# Distribution of Desoxyribose Nucleic Acid in Tumor Nuclei.* (20103)

SAUL BADER.† (Introduced by C. R. Moore.)
*From the Whitman Laboratory, University of Chicago.*

The relative amount of desoxyribose nucleic acid (DNA) in somatic diploid nuclei of non-dividing tissues has been shown to be a constant value. Nuclei with geometric multiples (1:2:4) of this value occur in some tissues (1,2). Since adult mouse and rat liver are known to be polyploid tissues(3,4), the geometric progression of DNA amounts in these tissues is probably a reflection of chromosome content(5). In several dividing tissues it has been shown that values occur intermediate between the diploid and twice the diploid amount of DNA. These values have been associated with the observation that in these tissues DNA synthesis occurs sometime in interphase preceding visible prophase. The mitotic index, and the rate and time of DNA synthesis presumably determine the number of such intermediate values(6). Tumor nuclei have been reported to contain increased amounts of DNA per nucleus as measured by both biochemical and absorption spectrophotometric methods(7,8). Chromosome counts of tumors have revealed polyploidy and aneuploidy to be present(3,9-11). It has been suggested that the increase in DNA content of tumor tissues and tumor nuclei is associated with a vaguely defined disturbance in "nucleic acid balance"(7,12). Recent reports have tended to refute this(5,13,14).

Average amounts of DNA, as determined biochemically, do not have the advantages offered by measurements of DNA in individual nuclei for interpreting growth or cell division. This is particularly the case in tissues where polyploidy is common. In the present study, the DNA content of individual nuclei of tumors and homologous normal tissues has been measured in Feulgen-stained sections.

*Methods.* The DNA content of nuclei of 5 tumors is presented. 1) A methylcholanthrene-induced squamous cell carcinoma in glandular epithelium of the left ventral prostate of the rat(15) is compared with normal prostate epithelium and precancerous squamous cell metaplasia. 2) The Cloudman S-91 melanoma carried in the dba mouse(16) and a benign skin papilloma of spontaneous origin in a $C_{57}$ mouse are compared with skin of a dba mouse. Neuroepidermal cells were not specifically measured as control for the melanoma. 3) A transplanted mammary carcinoma of spontaneous origin (15091a) in an A strain mouse and a mammary adenocarcinoma which arose spontaneously in a dba mouse are compared with normal dba mammary gland. All tissues were fixed in 10% neutral formalin and washed for 24 hours in running tap water. Embedded tissues were sectioned at 15 $\mu$. Relative amounts of DNA in individual nuclei of tumor and control tissues were measured by absorption microspectrophotometry as described by Swift(1) with light of 560 m$\mu$ wavelength from a Beckman monochromator. Tissues were stained in Feulgen reagent for one hour after 14 minutes hydrolysis in N HCl at 60°C. Whenever possible tissues to be compared were simultaneously fixed and embedded, and mounted on the same slide for staining. Slight differences between tissues in the distribution of diploid values are probably caused in part by variations in the preparation of tissues.

*Results and discussion.* The results can be characterized by 3 generalized observations. 1) the interphase nuclei of the tumor tissues examined contained relative DNA amounts which were roughly distributed into classes representing geometric multiples of the lowest class or diploid value (Class I). 2) All tumor tissues examined, with the exception of the benign skin papilloma, showed an increase in DNA amounts as compared with homologous control tissue, and 3) in most of the tumor tissues examined there was a striking occur-

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† Present address: La Rabida-Jackson Park Sanitarium, Chicago, Ill.

rence of intermediate values and/or trailing values following the highest DNA class.

DNA classes in multiples of the diploid value have been shown to occur in normal liver (Fig. 1), pancreas, and several other tissues(1.2). Chromosome counts of regenerating liver indicate that the distribution of DNA classes in this tissue is a reflection of the polyploid content of the nuclei(4). Chromosome counts and absorption photometric measurements on some ascites tumors have also revealed a parallelism between DNA content and polyploidy(8-10). The increase in DNA classes—tetraploid (Class II) and octaploid (Class III)—in the tumors measured is thus presumably the result of induced polyploidy in these tissues. Fig. 1, 2, and 3 show the increase in DNA amounts for the rat squamous cell carcinoma, the 2 mammary carcinomas, and the melanoma. The benign epithelial papilloma and the mammary adenocarcinoma show no increase in DNA classes since normal dba skin epithelium and mammary gland contain a tetraploid class. Tumors with unchanged DNA content have been reported(6.7).

How does polyploidy arise in these tumor tissues? Several mechanisms for the production of polyploidy have been described. 1) Beams and King(17) have observed in regenerating rat liver that many polyploid cells originate by nuclear fusion in binucleate cells during mitosis. Chromosomes of 2 diploid nuclei of such cells may be aligned on a single metaphase plate, thus giving rise to tetraploid nuclei. Polyploid cells may also be considered as arising by 2) endomitosis in which chromosome duplication occurs without a breakdown of the nuclear membrane, resulting in a nucleus with a doubled chromosome number, 3) mitotic anomalies in which there is nondisjunction of chromosomes at metaphase or anaphase through either "sticky" chromosomes or spindle abnormalities, and 4) the fusion of interphase nuclei as observed in the livers of thioacetamide injected rats(18). It

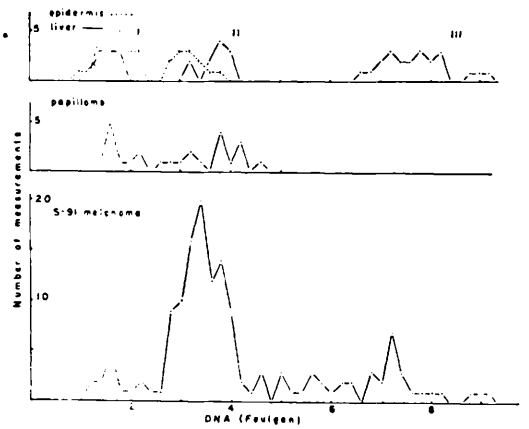


FIG. 1. Amounts of DNA in individual nuclei as determined photometrically on Feulgen-stained sections. Each unit is approximately equivalent to $3 \times 10^{-6}$ mg DNA. Top: Nuclei from normal $C_{57}$ strain mouse liver and dba strain epidermis. Middle: nuclei from a spontaneous benign skin papilloma in a $C_{57}$ mouse. Bottom: nuclei from Cloudman S-91 melanoma in a dba mouse.

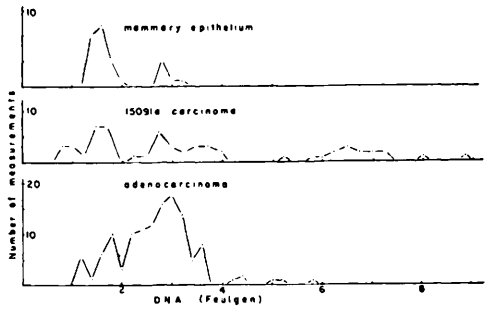


FIG. 2. Amounts of DNA in individual nuclei as determined photometrically on Feulgen-stained sections. Top: normal mammary epithelium from a lactating dba strain mouse. Middle: nuclei from a transplanted mammary carcinoma (15091a) in an A strain mouse. Bottom: nuclei from a spontaneous mammary adenocarcinoma in a dba strain mouse.

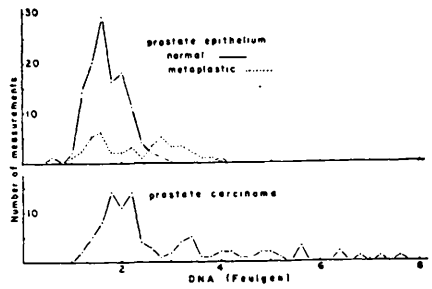


FIG. 3. Amounts of DNA in individual nuclei as determined photometrically on Feulgen-stained sections. Solid lines, top: nuclei from normal prostate epithelium (right side) of a male Sprague-Dawley rat. Bottom: nuclei of a methylcholanthrene-induced squamous cell carcinoma from the same animal (left side). Dotted lines: nuclei of a methylcholanthrene-induced squamous cell metaplasia of a male Sprague-Dawley rat.

is generally apparent that in the tumors studied the formation of polyploidy is concomitant with cell division, and no evidence of endomitosis has been obtained. In some cases, such as in the Cloudman melanoma, Class II nuclei are numerous and actively dividing, and thus seem largely the product of normal cell division.

Intermediate or trailing values in non-dividing mammalian tissues have been rarely encountered(1). Their increased occurrence in the interphase nuclei of dividing tissues and in the tumors examined are presumably largely due to DNA synthesis in preparation for division. Many mitotic figures were observed in the melanoma. In this tissue the occurrence of intermediate values is very striking. Chromosomal abnormalities described in tumor nuclei, such as stickiness, non-disjunction and displacement of chromosomes may also be responsible for some of the intermediate values in these nuclei(11). The differences observed in tumor tissues with respect to the presence and frequency of intermediate and trailing values may be a reflection of rate of growth. The trailing and intermediate values observed in the control prostate and metaplastic prostate epithelium are probably the result of mitotic activity.

The DNA content of some normal tissues of tumor bearing animals, as measured by chemical extraction methods, has been reported to increase(19). Studies in progress, of the DNA content of such tissues on a per nucleus basis as measured in Feulgen-stained sections, reveal that such changes may be due to polyploidy and/or cell number increases (20).

From the data presented here the distribution of DNA values is different in 4 of the 5 tumors from that of the normal controls. It should be emphasized, however, that the tumor values obtained closely resemble those from a number of normal growing tissues where mitosis and polyploidy are present, such as liver in young mice(1). The changes found thus are those to be expected from increased mitosis and polyploidy alone, and do not justify any hypothesis of nucleic acid disturbance(7) or "quantitative change in nucleic acid synthesis"(11), as specifically characteristic of tumor cells. Some of the intermediate DNA values found may be associated with aneuploidy rather than DNA synthesis, although small variation in chromosome number also occurs in normal tissues(21). In the control tissues, variations in DNA values about the class mean are undoubtedly caused to some extent by inaccuracies in the measuring technic(6) and possibly also to aneuploidy, although these variables have not been analyzed in the present case. Data from the tumor tissues are in general agreement with the hypothesis of DNA constancy per chromosome set(1,2,22). The changes in DNA of tumors seem best interpreted as only one of a number of manifestations of a basic alteration in cell processes. From the present evidence DNA change cannot therefore in itself be considered a causative factor in tumor formation.

*Summary.* The relative amounts of DNA per nucleus in individual nuclei of 5 different tumor tissues, measured by absorption microspectrophotometry in Feulgen-stained sections, are largely distributed into classes in a geometric progression (1:2:4). The tumors showed increases in DNA amounts as compared with homologous control tissue with the exception of a benign papilloma. The occurrence of intermediate values and increased DNA classes is considered to be an expression of changes in chromosome content associated with mitosis and polyploidy rather than a disturbance in nucleic acid synthesis.

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## Treatment of Nephrotic Syndrome with Interrupted ACTH or Oral Cortisone Therapy.* (20104)

KURT LANGE, LAWRENCE SLOBODY, AND RUTH STRANG.

*From the Departments of Medicine and Pediatrics, New York Medical College and the Research Unit, Metropolitan Hospital.*

In previous investigations(1,2) we have demonstrated that serum complement levels are persistently low in all cases of acute and subacute glomerulonephritis. We have also shown that in almost all cases with the nephrotic syndrome complement is low during the edematous phase, and that 24-48 hours prior to the onset of diuresis, serum complement rises to or toward normal. Conversely, 1 to 2 days prior to clinical relapses, complement falls below normal levels(3). This behavior of complement has been considered to be the result of a complement-binding antigen-antibody reaction *in vivo,* wherein the altered kidney and probably, more specifically, the altered basement membrane of the glomerulus, represents the antigen which provokes antibody formation in the body(1,2,3). The low serum complement levels in these diseases are apparently in no way related to urinary excretion of complement. In the nephrotic syndrome we were unable to find complement in the urine. Furthermore, the low complement levels are not related to the excretion of protein, for in spontaneous or ACTH induced remissions the degree of albuminuria often does not change significantly while complement levels rise sharply *prior* to the remission. No correlation between proteinuria and serum complement levels was demonstrated in four cases of Kimmelstiel-Wilson's Disease in which massive proteinuria and normal complement levels were found. In our determinations of serum complement, the technic of which has been reported previously(1,2), 166 normal subjects or patients with diseases other than glomerulonephritis or the nephrotic syndrome have placed the normal level of serum complement between one and 3 units. (Average, 1.78 units, standard deviation 0.587,† Fig. 1).

*Complement unit.* Complement was freshly prepared from commercial lyophilized guinea pig serum, diluted in steps of 5 from 1:5 to 1:50 and titration was carried out in the same manner as previously described(1) for the unknown serum. Guinea pig serum 0.1 cc was mixed with 0.1 cc inactivated human serum because guinea pig serum is known to have approximately twice the complementary activity of human serum. The dilution of

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† As the actual shape of the statistical distribution is unknown and the data are not homogeneous enough and numerous enough to determine this shape, a normal distribution function has been assumed for the sake of expedience. While comparisons between the means are not significantly affected by the shape of the distribution functions, no extrapolation beyond the range of $\pm$ one standard deviation appears permissible.