A MICROSPECTROPHOTOMETRIC STUDY OF THE DESOXYRIBOSE NUCLEIC ACID (DNA) CONTENT IN CELLS OF NORMAL AND MALIGNANT HUMAN TISSUES *

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Desoxyribose nucleic acid (DNA) is a constant and perhaps the most important nuclear constituent of all living cells. It is an essential part of chromatin and of the chromosomes, and is intimately concerned with the process of cell division. It would be reasonable to expect that the DNA content of tumor cells is different from that of normal cells. That such is the case is suggested by the increase in size and staining density of nuclei so frequently observed in tumors. To date, however, there are no chemical data to confirm or negate this concept.

This may seem surprising, but it can be explained by the lack of adequate chemical methods to attack such a problem. One must keep in mind that in order to answer the pertinent question whether the DNA content of a tumor cell is different from that of a normal cell, a special method is needed which permits the chemical analysis of a *single* cell. It is obvious that the conventional biochemical procedure which determines the DNA content in a mashed neoplastic tissue cannot give an answer because it yields only a value which refers to the fresh or dry weight of the tissue without discrimination between cellular and non-cellular material.

Even if biochemical analysis is done on a mass of isolated cells or nuclei (which in most tumors is nearly impossible because of the technical difficulties encountered in isolating nuclei of human tumors) the DNA content per cell is only an average value computed from the analysis of a mass of cells.[1] While such an average value may be representative for single cells in cell suspensions with a uniform DNA content, it is not significant for *the* single cell, nor does it reveal any variation from cell to cell if the suspension analyzed consists of cells with varying DNA content. The difficulties and pitfalls encountered in interpreting the biochemical results in suspensions of polyploid cells

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of liver have been demonstrated in previous studies[1] and discussed in detail.[2]

Fortunately enough, due to Caspersson's pioneering in 1936,[3] microspectrophotometric methods were developed which permit the quantitative estimation of chemical constituents of single cells or cell structures. By combining it with a photometric device, the microscope became a tool concerned with the chemistry of cells and tissues. The

TABLE I

Amount of DNA (Microspectrophotometry) in Cells of Normal Human Tissues

No. of cases	No. of nuclei measured	Range of ages	Sex	Tissue	Mean amount of DNA per nucleus in	
					Arbitrary units	Absolute amounts (10^{-9} mg.)
1	30	11 yrs.	F	Breast	3.25±0.09	6.50±0.18
1	30	51 yrs.	F	Cecum	2.58±0.05	5.16±0.10
1	30	62 yrs.	M	Kidney	3.17±0.04 5.43	6.34±0.08
5	140	3 mos. to 6 yrs.	M and F	Liver	2.82±0.08	5.64±0.16
8	150	13 to 86 yrs.	M and F	Liver	2.83±0.09 6.12±0.25 11.05±0.28	5.66±0.18 12.24±0.50 22.10±0.56
1	30	54 yrs.	M	Lung	3.02±0.07	6.04±0.14
1	30	65 yrs.	M	Lymphocytes	2.53±0.05	5.06±0.10
1	30	77 yrs.	F	Pancreas	2.59±0.17	5.18±0.34
4	168	1 to 68 yrs.	M and F	Skin	2.80±0.06	5.60±0.12
1	30	65 yrs.	M	Stomach	2.68±0.09	5.36±0.18
2	60	51 to 62 yrs.	M	Urinary bladder	3.12±0.15 5.68±0.23 9.80	6.24±0.30 11.36±0.46 19.60
21	630	23 to 45 yrs.	M	Spermatozoa	1.22±0.01	2.44±0.02

advantages are obvious; here for the first time, the appearance of a specific cell can be correlated directly *in situ* under the microscope with its chemical composition without destroying the cytologic and histologic architecture. Changes in structure, which of necessity must often be subjectively interpreted, can now be expressed in quantitative chemical terms.[4,5] Furthermore, the possibility of detecting quantitative changes in intracellular substances before the structural alterations in cells manifest themselves under the microscope (as for example, the decrease in the DNA content in sperm cells of infertile human males[6]) has not only opened completely new pathways for the study of disease, but shows also that the microspectrophotometric method may serve as a valuable diagnostic tool.

The purpose of the present study is an attempt to relate one of the most important chromosomal components, DNA, to the malignant transformation of cells. However, since very little was known about the DNA content of cells in normal human tissues prior to this report, a comparative extensive study on the DNA content of a variety of normal and malignant human tissues had to be carried out to establish a baseline.

For this study, results of the DNA measurements of nearly 2500

TABLE II

Amount of DNA (Microspectrophotometry) in Liver Cells of Normal Children and Adults

No. of cases	Ages	Sex	Cytologic appearance	No. of nuclei measured	Mean amount of DNA per nucleus in	
					Arbitrary units	Absolute amounts (10^{-9} mg.)
5	3 mos. to 6 yrs.	M and F	Normal, no mitosis	150	3.27±0.11	6.54±0.22
					2.70±0.06	5.40±0.12
					2.89±0.06	5.78±0.12
					2.41±0.12	4.82±0.24
					2.81±0.03	5.62±0.06
5	13 to 86 yrs.	M and F	Normal, no mitosis	150	2.65±0.09	5.30±0.18
					5.74±0.31	11.48±0.62
					11.69	23.38
					2.96±0.08	5.92±0.16
					6.26±0.37	12.52±0.74
					11.38	22.76
					3.45±0.21	6.90±0.42
					7.66	15.32
					2.31±0.09	4.62±0.18
					4.27±0.08	8.54±0.16
					9.20	18.40
					3.56±0.11	7.10±0.22
					7.09±0.23	14.18±0.46
					12.50	25.00

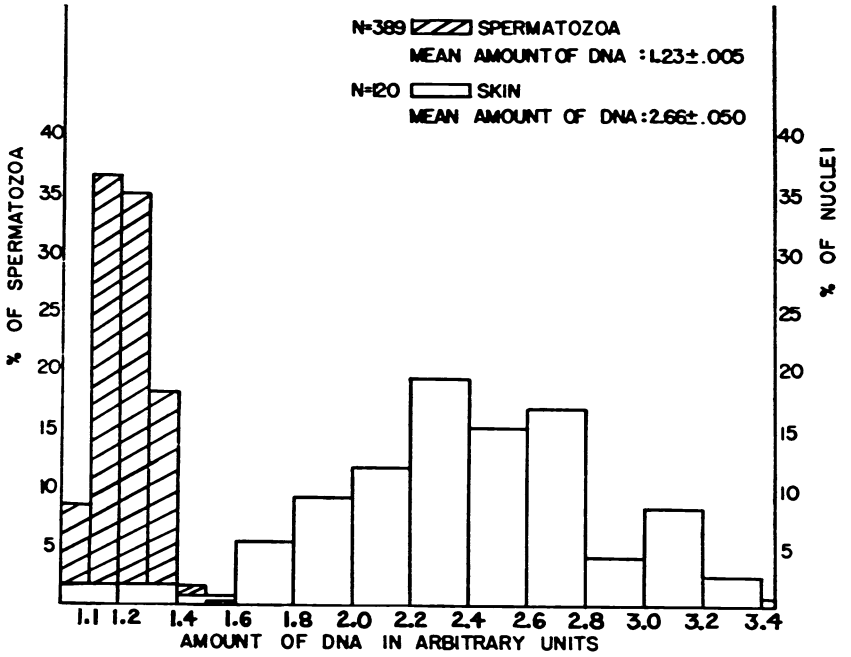
individual cells are presented, derived from 11 different tissues and from 76 individual cases. The validity of the quantitative microspectrophotometric methods in determining DNA in individual mammalian cells has been established in previous studies,[7] in the course of which it was found that the mean amounts of DNA in individual nuclei of beef tissues as determined by ultraviolet and Feulgen microspectrophotometry agreed closely with the results of DNA estimation done on the same cells by biochemical analysis.

MATERIAL AND METHODS

In order to carry out the investigation to be described, normal, precancerous, and cancerous tissues were secured from 76 human sub-

jects. Of these, 47 were normal, while 29 were patients with precancerous or malignant lesions. Whenever possible, surgical and biopsy material from Doctors Hospital, Cleveland, Ohio, was used. This was fixed, embedded, cut, and stained as previously described.[4] While, for some of the studies on normal tissues, post-mortem material had to be utilized,* it should be pointed out that due to the stable character of DNA, which does not change in amount for a considerable period after death, the results are not affected.

For the estimation of the amounts of DNA, the Feulgen reaction and microspectrophotometric analysis was used as previously de-



Text-fig. 1. Comparison of the amount of DNA (microspectrophotometry) in individual spermatozoa of fertile human males, and of individual normal human skin cells.

scribed.[4,8] All microspectrophotometric measurements were done on cells which appeared cytologically to be in interphase. For each tissue at least 30 individual nuclei were measured. The amounts of DNA per nucleus are expressed in arbitrary units as well as in absolute amounts. The former are given for the convenience of other workers in the field who use the same arbitrary units. The absolute amounts are based on previous studies[7,9] in which the biochemical analysis of DNA, when

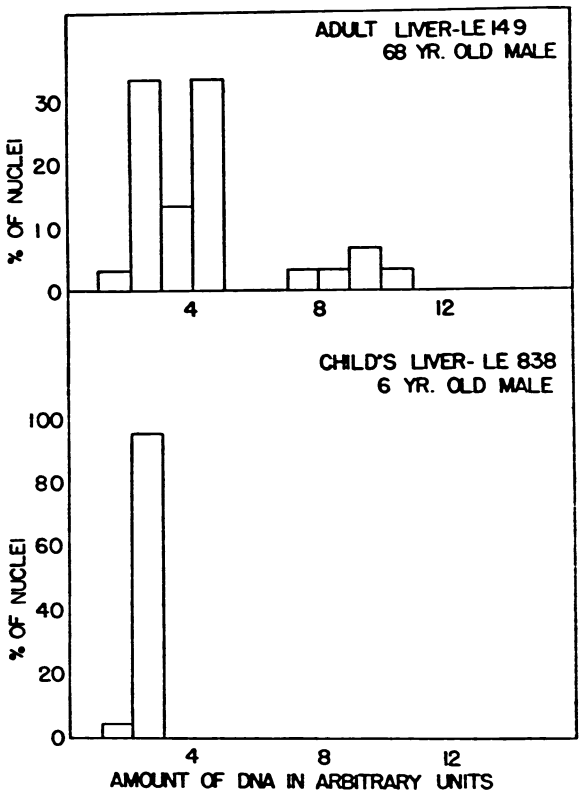
* We are indebted to Dr. Lester Adelson of the Institute of Pathology, Western Reserve University, and of the Cuyahoga County Coroner's Office for some of the post-mortem material used in this study.

compared with ultraviolet and Feulgen microspectrophotometric results, established that a factor of 2×10^{-9} mg. will convert arbitrary units to absolute amounts. In each case a statistical analysis was carried out and each mean value which was obtained on a number of nuclei larger than 10 is given with its standard error.

Results

The DNA Content of Cells of Various Normal Human Tissues

The results of the DNA measurements in 1358 individual nuclei of 11 different normal tissues in 47 individuals are presented in Table I.



Text-fig. 2. Individual DNA measurements (microspectrophotometry) in nuclei of liver in an adult and a child.

From this it is evident that there is a remarkable constancy of the DNA content in the somatic cells of the various human tissues examined. Each tissue contains cells with a basic mean DNA value of approximately 5.6×10^{-9} mg. (or approximately 2.8 arbitrary units) regardless of age, sex, or race of the individual. Furthermore, it can

TABLE III

Amount of DNA (Microspectrophotometry) in Nuclei of Precancerous Tissues and in Benign and Malignant Neoplastic Tissues

Case no.	Diagnosis	Age, sex	Tissue	No. of nuclei measured	Mean amount of DNA per nucleus in: Arbitrary units	Mean amount of DNA per nucleus in: Absolute amounts (10^{-9} mg.)
1 (Le 901)	Adenocarcinoma	22 F	Breast	30	3.69±0.08	7.38±0.16
2 (Le 902)	Adenocarcinoma	29 F	Breast	41	3.15±0.18	6.30±0.36
3 (Le 903)	Adenocarcinoma	33 F	Breast	30	3.09±0.10	6.18±0.20
4 (Le 904)	Adenocarcinoma	40 F	Breast	30	3.31±0.06 5.84±0.31	6.62±0.12 11.68±0.62
5 (Le 905)	Adenocarcinoma	49 F	Breast	30	3.75 6.64±0.17 9.73 14.55	7.50 13.28±0.34 19.46 29.10
6 (Le 906)	Adenocarcinoma	54 F	Breast	30	3.64 6.37±0.14 10.54	7.28 12.74±0.28 21.08
7 (Le 907)	Adenocarcinoma	59 F	Breast	30	4.06±0.16 8.30	8.12±0.32 16.60
8 (Le 908)	Adenocarcinoma	62 F	Breast	30	2.99±0.24 6.49±0.27 10.50	5.98±0.48 12.98±0.54 21.00
9 (Le 461)	Adenocarcinoma	67 F	Breast	40	2.78±0.07 7.00±0.27 12.06	5.56±0.14 14.00±0.54 24.12
10 (Le 909)	Adenocarcinoma	83 F	Breast	30	2.85±0.15 5.82	5.70±0.30 11.64
11 (Le 457A)	Adenocarcinoma	51 F	Cecum	38	3.03±0.15 6.70±0.19	6.06±0.30 13.40±0.38
12 (Le 428B)	Clear cell carcinoma	76 M	Kidney	55	5.21±0.16	10.42±0.32
13 (Le 852)	Adenoma of renal cortex	62 M	Kidney	30	2.80±0.07 5.97	5.60±0.14 11.94
14 (Le 450D)	Metastasis of pancreatic carcinoma	63 M	Liver	30	5.82±0.31	11.64±0.62
15 (Le 432)	Anaplastic bronchial carcinoma	69 M	Lung	28	3.13±0.15 6.45	6.26±0.30 12.90
16 (Le 916)	Partly differentiated adenocarcinoma	54 M	Lung	30	2.70±0.06 5.49±0.30 13.66	5.40±0.12 10.98±0.60 27.32
17 (Le 460A)	Metastasis of adenocarcinoma of stomach	65 M	Lymph node	30	4.40±0.34	8.80±0.68
18 (Le 452B)	Carcinoma	77 F	Pancreas (head)	40	2.43±0.11 6.03±0.19	4.86±0.22 12.06±0.38
19 (Le 450)	Carcinoma	63 M	Pancreas (head)	40	3.20±0.16 5.95±0.10	6.40±0.32 11.90±0.20
20 (Le 506)	Senile keratosis (Freudenthal)	67 F	Skin	48	5.3 ±0.27	10.6±0.54

TABLE III (Continued)

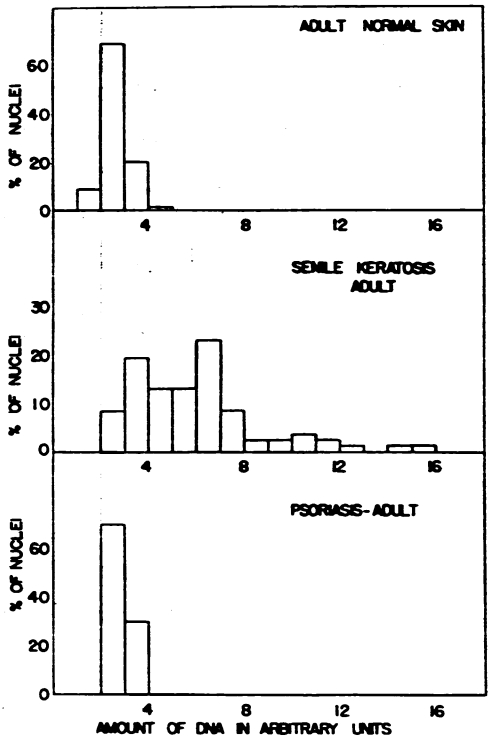
Case no.	Diagnosis	Age, sex	Tissue	No. of nuclei measured	Mean amount of DNA per nucleus in	
					Arbitrary units	Absolute amounts (10^{-9} mg.)
21 (Le 657)	Senile keratosis (Freudenthal)	68 M	Skin	35	6.60±0.60	13.20±1.20
22 (Le 634)	Senile keratosis (Freudenthal)	67 M	Skin	44	6.30±0.23	12.60±0.46
23 (Le 431B)	Diffusely growing undifferentiated carcinoma	66 M	Stomach	30	3.37±0.26 6.61±0.40	6.74±0.52 13.22±0.80
24 (Le 460B)	Adenocarcinoma	65 M	Stomach	30	3.43±0.21	6.86±0.42
25 (Le 426)	Seminoma	48 M	Testis	40	4.13±0.25 9.20	8.26±0.50 18.40
26 (Le 427)	Transitional cell carcinoma	51 M	Urinary bladder	30	6.13±0.51 12.55±0.49 17.68	12.26±1.02 25.10±0.98 35.36
27 (Le 467)	Diffusely growing transitional cell carcinoma	51 M	Urinary bladder	40	5.74±0.32 9.43±0.31 17.74	11.48±0.64 18.86±0.62 35.48
28 (Le 469)	Epidermoid carcinoma	62 M	Urinary bladder	58	3.72±0.15 6.62±0.21 11.07	7.44±0.30 13.24±0.42 22.14
29 (Le 917C)	Anaplastic glandular carcinoma of peri-urethral region (primary, ovary?)	66 F	Urinary bladder	30	3.95±0.20	7.90±0.40

be seen also that some of the tissues (for example: adult liver, kidney, urinary bladder) have, in addition, cells carrying amounts of DNA which are nearly exact multiples of the basic amount of DNA. The mean uniform basic DNA content in cells of human tissues with diverse metabolic activities and the occurrence of cells with multiple DNA values in some tissues are in full accordance with results on other animal tissues.[1,10-12]

Since previous comparative studies[8,9,13] established a direct relationship between counts of chromosomal numbers and DNA content of cells, it seems justified to consider the multiple amounts of DNA in some human tissues as an expression of multiple sets of chromosomes or, in other words, of polyploidy. A further confirmation of the relationship comes from the human sperm data[6] which, in accordance with their haploid chromosomal number, also show approximately half the DNA content found in the diploid somatic cells. Because the occurrence of multiple DNA classes (2DNA, 4DNA) was not observed in tissues of children, while they were always observed in the same tissues (*i.e.* liver) of adults, a study of the DNA content in normal livers

at different ages was made. A separate detailed report on the correlation of multiple DNA classes with age will appear elsewhere. A few examples pertinent to this study are presented in Table II. On the basis of the DNA data presented in this table, it can be seen that there is a definite relationship between the age of the individual and the occurrence of multiple DNA classes.

In the 5 children from 3 months to 6 years of age, only *one* DNA

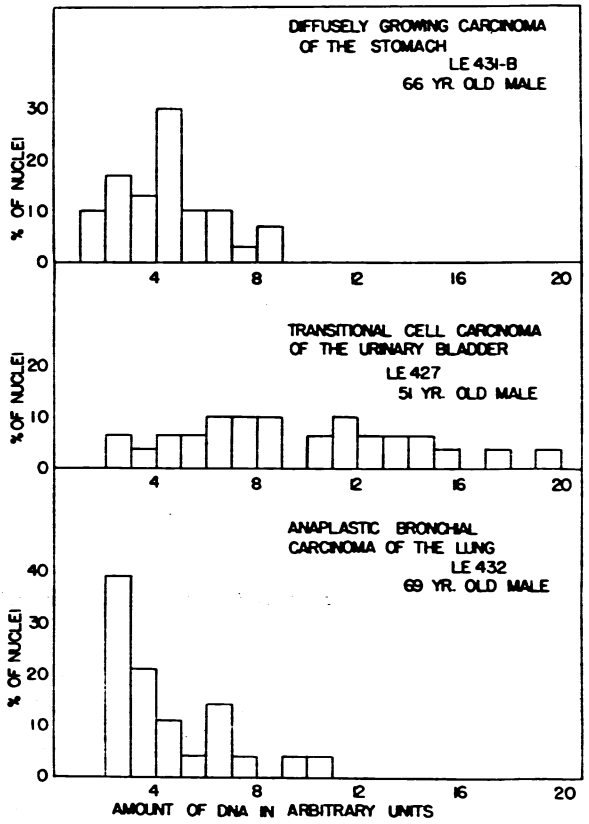


Text-fig. 3. Individual DNA measurements (microspectrophotometry) in nuclei of normal skin and in senile keratosis.

class, namely, the characteristic basic mean DNA value of approximately 5.6×10^{-9} mg., was observed while each of the 5 individuals from 13 to 86 years showed in addition one or two multiple DNA classes. It can also be noted from Table II for comparison, that only livers of children up to 6 years of age were selected. This was necessary because mitosis was encountered frequently in livers of children from 7 to 12 years. Since during mitosis the DNA content increases regularly in the interphase cells (as will be discussed later), demonstration of DNA classes is extremely difficult in mitotic tissues. Besides the

characteristic DNA content of each cell (1DNA ±, 2DNA ±, 4DNA ±), there is synthesized during mitosis an additional amount of DNA needed for the formation of the daughter cells. Since this quantity varies from cell to cell according to the stage of DNA synthesis (from 2DNA to 4DNA), a different amount is added to each cell which makes the establishment of DNA classes very complicated, if not impossible. Thus, for the comparison of DNA classes only non-mitotic tissues should be chosen.

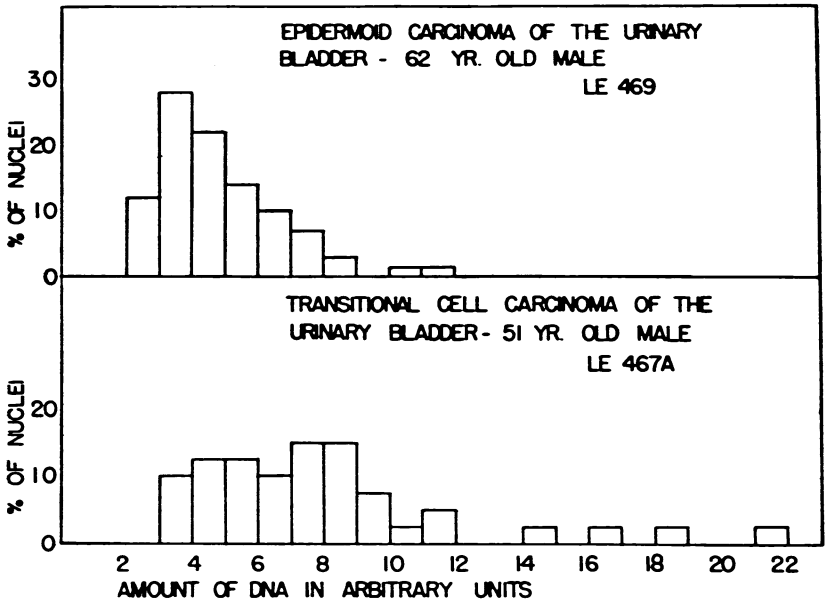
So far all DNA values on normal tissues have been given as *mean*



Text-fig. 4. Individual DNA measurements (microspectrophotometry) in nuclei of carcinomas in stomach, urinary bladder, and lung.

values per nucleus computed from the microspectrophotometric analyses of a number of individual cells. As pointed out repeatedly in previous studies,[1,2,9] such a mean DNA value, of necessity, gives no information as to the variation of the DNA content which may occur from cell to cell within the same tissue. In Text-figures 1 and 2 some

typical examples of the distribution of DNA values in individual cells of different normal tissues are presented. It is evident from the example shown (Text-fig. 1) that a great number of the cutaneous cells measured have DNA values which vary relatively little from each other and from the computed basic mean value, which lies between 2 and 3 arbitrary units as indicated by the peaks. However, it can be noted also that some of the cells have a DNA content which is significantly lower or higher than this basic value. A somewhat similar dis-



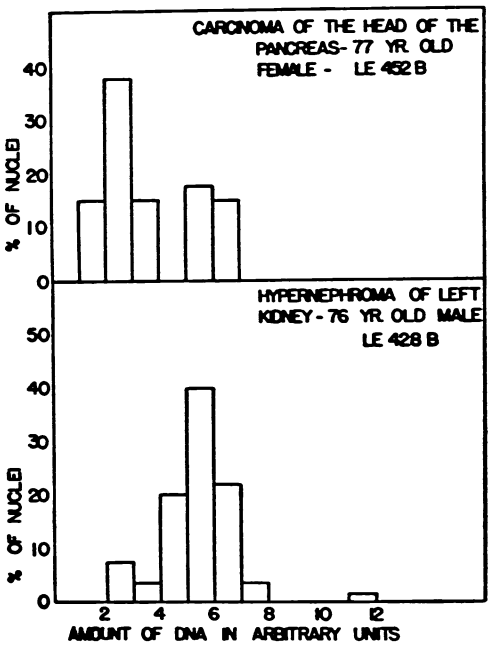
Text-fig. 5. Individual DNA measurements (microspectrophotometry) in nuclei of carcinomas of urinary bladder.

tribution curve is obtained if the individual DNA data of the haploid sperm cells are plotted. While in this instance the great majority of the spermatozoa contain nearly the same amount, there are also a certain number which have a deviating content of DNA.

The same holds true if the distribution of DNA is considered in a tissue with multiple amounts of DNA as shown in Text-figure 3. While the cells of the adult liver show definite peaks in their DNA amounts, between 2 and 3, 4 and 5, and 9 and 10, indicating polyploidy, somewhat smaller or larger DNA values may occur within each group. A very similar range of DNA values has been reported previously in animal and plant tissues and its possible biologic importance and relation to deviations in chromosomal numbers have been discussed.[1,2,7,9,14,15]

While it thus appears that the DNA content is not as constant in

each cell of a tissue as has been claimed by some workers,[11,12] the relative stability in the mean amount in cells of different tissues and of different individuals seems to be well established also for man (Tables I and II).



Text-fig. 6. Individual DNA measurements (microspectrophotometry) in nuclei of carcinomas in pancreas and kidney.

The DNA Content of Cells of Various Precancerous and Malignant Human Tissues

Using the relatively uniform mean DNA content in cells of normal tissues as a baseline, the question arises whether cells of precancerous or malignant tissues exhibit a similar constancy of DNA. The results of DNA measurements are presented in Table III.

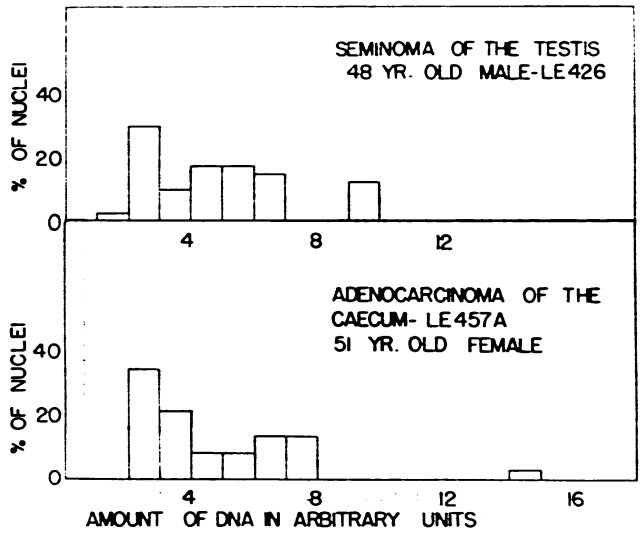
It can be seen from the data in Table III that the mean DNA values for the 29 tumors were not as constant and as uniform as for the 47 normal cases (Table I). These cases can be grouped into three categories:

The first group of tumors has DNA values which are either the same or only slightly higher than the basic value of the normal tissues (cases 2, 3, 4, 8, 9, 10, 11, 13, 15, 16, 18, 19, 23, and 24).

The second group has DNA values approximately 30 per cent higher than the basic normal DNA content (cases 1, 5, 6, 7, 17, 25, 28, and 29).

In the third group, cells with the basic DNA content are completely lacking and all the cells have as a lowest value a multiple (tetraploid) DNA content (cases 12, 14, 20, 21, 22, 26, and 27). The occurrence of such "tetraploid tumors" in humans is in conformity with tetraploid animal tumors.[9]

The differences between the DNA content of cells of normal and malignant tissues are even more obvious when the *individual data* for tumors and for normal cases are compared. As pointed out, the com-



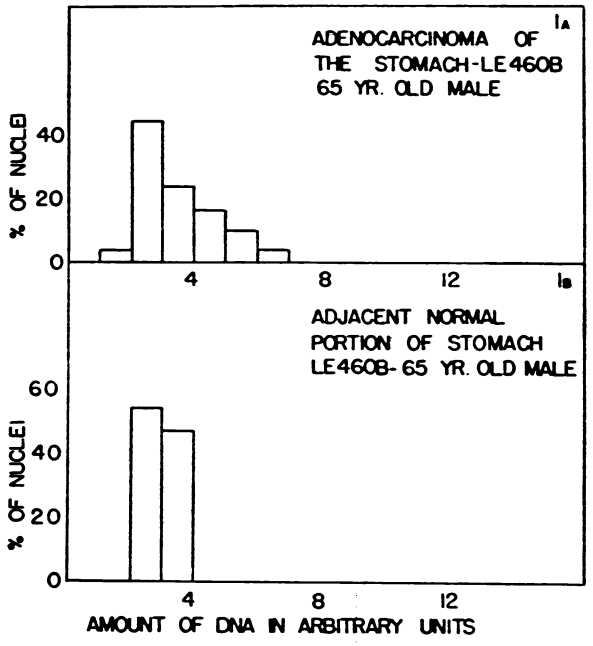
Text-fig. 7. Individual DNA measurements (microspectrophotometry) in nuclei of a seminoma of testis and carcinoma of cecum.

puted mean DNA value does not allow an appraisal of the variability of cells within a tissue.

In Text-figures 3 to 10, typical examples of the DNA distribution in individual cells of various types of tumors are presented. It can be seen that all of the primary tumors (and the so-called precancerous stages of senile keratosis) as well as the metastases have cells which show a marked variability of the DNA content. This spread of the DNA data and the frequent occurrence of DNA values intermediate between the multiple DNA classes is especially striking when compared with the relatively small variability of the DNA values in normal homologous cells (Text-figs. 8, 9, and 10). It can be noted also that the spread of the DNA data of metastatic nodules is even more extensive than that of the primary tumor.

The unequal amounts of DNA in the cells of malignant tissues have

been found so far in every tumor examined. However, since the tumors studied fall within a limited age group (namely, 48 to 77 years), the question arises whether in younger individuals the tumors show a similar variation of DNA in their cells. Such an investigation seems pertinent because, as pointed out previously, normal tissues (*i.e.*, liver) of young persons have cells with only one DNA class while the same normal tissue of older persons always has two or more DNA classes.



Text-fig. 8. Individual DNA measurements (microspectrophotometry) in nuclei of an adenocarcinoma of stomach and of adjacent normal portion of stomach.

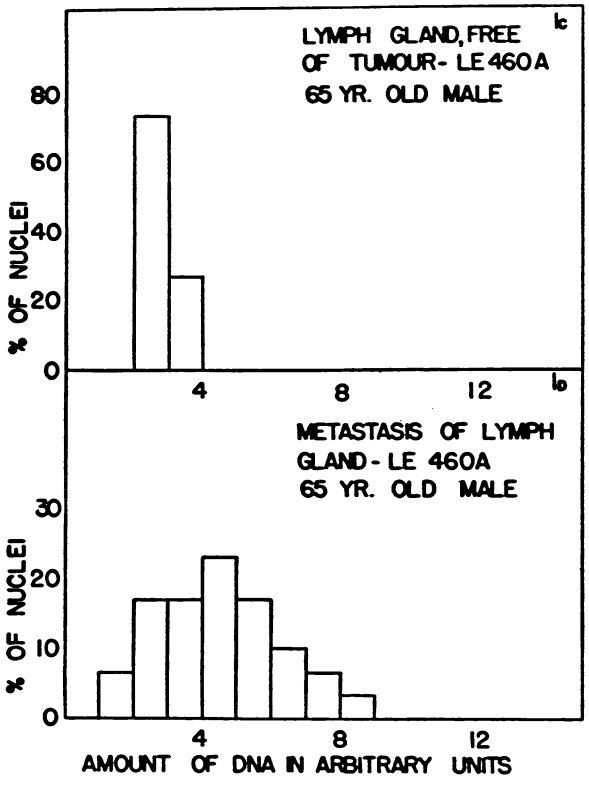
In order to examine the question, adenocarcinoma of the breast was chosen because it represents a type of tumor which occurs in a particularly wide range as to age. DNA was studied in 9 women from 22 to 83 years of age. The results of the individual DNA measurements are given in Text-figures 11 to 14. On the basis of the data obtained so far, it seemed that the breast tumors of the younger age group (22 to 33 years) show less variability from cell to cell and a lower DNA content than those of the older age group. All 6 cases between 40 and 83 years exhibited a more pronounced variation from cell to cell and higher DNA contents than the 3 cases of the younger age group. It is realized, of course, that a much larger number of cases is necessary before this relationship can be considered as established.

Discussion

On the basis of the preceding studies the findings may be summarized as follows:

Results for Normal Tissues

All normal tissues examined so far showed a very similar basic mean DNA value in the nuclei of their cells. This DNA content was approximately twice that of the sperm cell and was probably characteristic for cells with diploid chromosomal numbers.



Text-fig. 9. Individual DNA measurements (microspectrophotometry) in nuclei in a metastatic nodule in a lymph node from the adenocarcinoma of stomach (Text-fig. 8) and in an adjacent normal lymph node.

Some of the normal tissues carried cells with nearly exact multiples of the diploid DNA content, that is, tetraploid and octoploid amounts. In spite of the similarity of the mean DNA value in the different normal tissues, there was a certain degree of variation in the amount of DNA from cell to cell within each tissue.

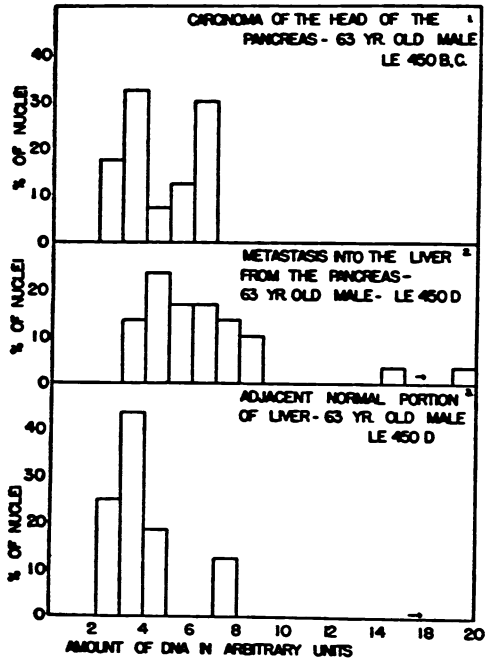
Results for Malignant Tissues

In contrast to the presence of a basic mean DNA content in *all* nor-

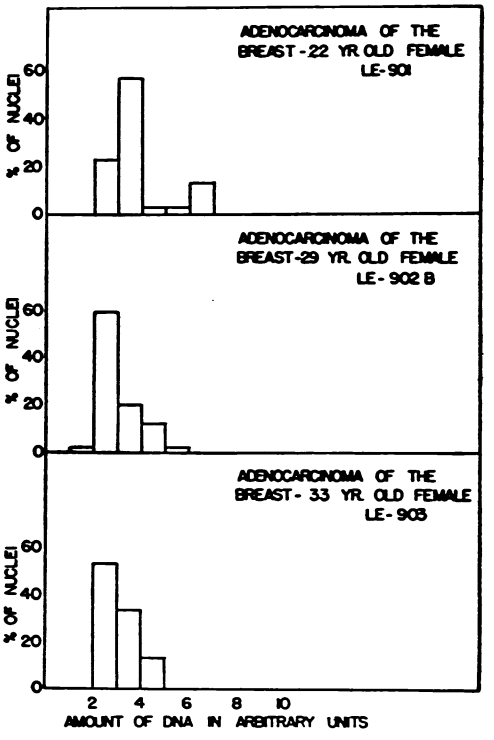
mal tissues, precancerous and malignant tissues often deviated from this basic amount. In such cases DNA values were either considerably higher (30 per cent) than the basic value, or showed as their lowest amount the double value.

In contrast to the relatively small variability of DNA from cell to cell within a normal tissue, *all* malignant cells showed a much wider fluctuation in their DNA content from cell to cell.

The findings of the relative stability and constancy of the DNA content in cells of a variety of normal human tissues and of different individuals is in good agreement with the observations on animal tissues. This constancy of the basic DNA content is indeed striking, espe-



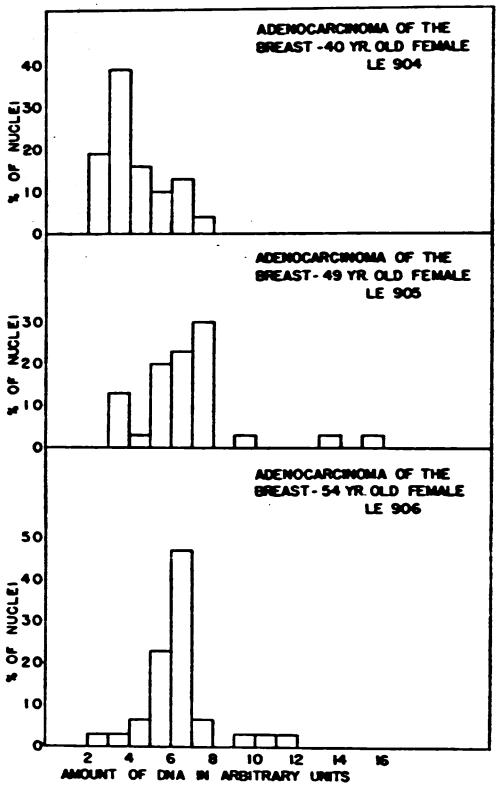
Text-fig. 10. Individual DNA measurements (microspectrophotometry) in nuclei of a carcinoma of pancreas, of a metastatic nodule of this carcinoma in liver, and of the normal liver.



Text-fig. 11. Individual DNA measurements (microspectrophotometry) in nuclei of adenocarcinoma of breast, ages 22 to 33 years.

cially if considered in the light of the diverse metabolic processes going on in the cells of the different tissues. However, if one takes into account the present-day concept that DNA is an integral part of only the chromosomes and that the chromosomal numbers, up to a certain degree, are more or less constant for a cell, then this constancy is not

too surprising. This apparent correlation between chromosomal number or mass and DNA content is further supported by the parallelism between chromosomal counts and DNA content, as, for example, in haploid germ cells[6] or tetraploid ascites tumors.[9] It thus appears that



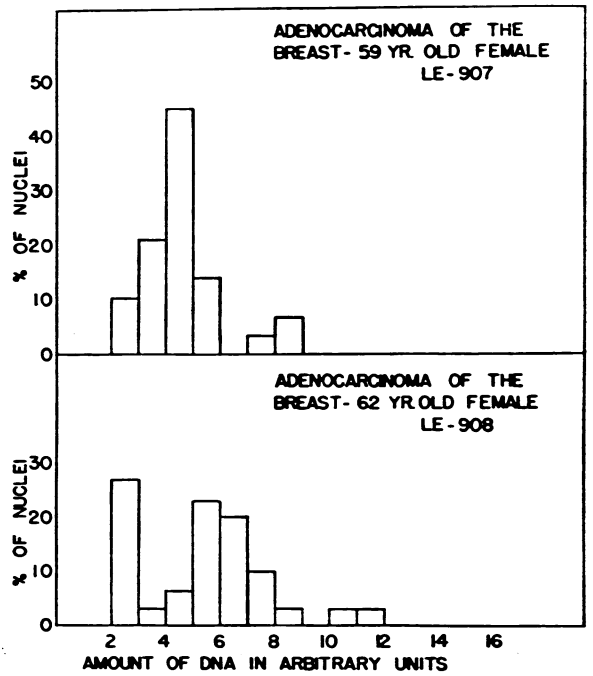
Text-fig. 12. Individual DNA measurements (microspectrophotometry) in nuclei of adenocarcinoma of breast, ages 40 to 54 years.

cells of normal tissues have an orderly pattern of DNA content linked closely to the chromosomes and, though there are some variations in some cells as indicated by the individual data, most of the tissue cells have a similar characteristic average amount.

This stability, which indicates a certain static behavior of the cell in regard to its chromosomal constituents, is actually to be expected in cells of a non-growing normal tissue. On the other hand, the marked fluctuation of DNA observed in the neoplastic tissue again indicates the close linkage of DNA to the chromosomal behavior. The characteristic feature of each tumor, namely, the formation of new cells and the occurrence of mitotic figures, *must* of necessity lead to changes in

the DNA contents. The scatter of the DNA data is just an indication of the DNA synthesis necessary for the formation of new cells.

Thus it appears that the deviation in DNA data in the tumors can be explained simply on the basis of growth and mitotic processes alone, without even considering the *malignant* character of the cells. This concept is well supported if one looks at the DNA data of cells of a *normal* mitotic human tissue. While mitosis is rarely encountered in adult human liver, we have observed mitosis in livers of children from

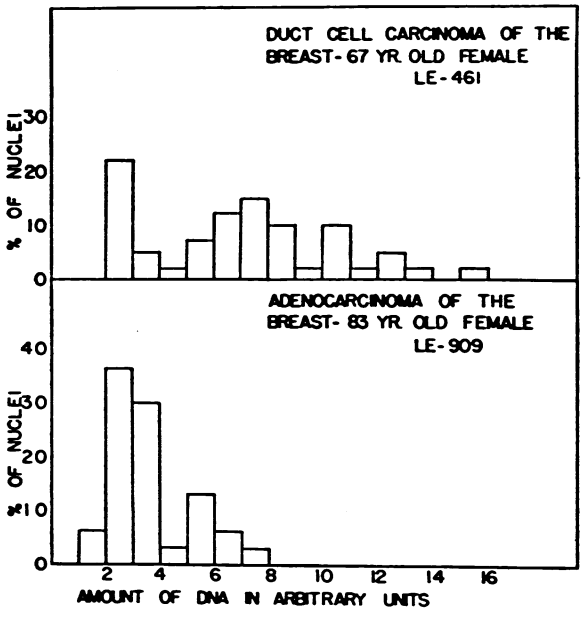


Text-fig. 13. Individual DNA measurements (microspectrophotometry) in nuclei of adenocarcinoma of breast, ages 59 to 62 years.

9 to 12 years. It is evident from the DNA data in Text-figure 15 that interphase cells of a normal liver undergoing mitosis also show a variability and increase in the DNA content as compared with the cells of a liver which is not in the process of mitosis (see Text-fig. 2, case Le 838). The picture is obviously similar to that of malignant cells (perhaps somewhat less striking than in some tumors); but the resemblance is even closer, if one considers that in both instances it is the so-called interphase or resting cell which displays the increase in DNA. Apparently also in human tissues (regardless of whether they are normal or malignant), the build-up of DNA to its double value takes

place at a very early stage of mitosis, that is, *before* structural changes in regard to the chromosomes can be visualized. These findings are in good accord with the ones obtained in dividing animal and plant tissues.[2,12,14,16-18]

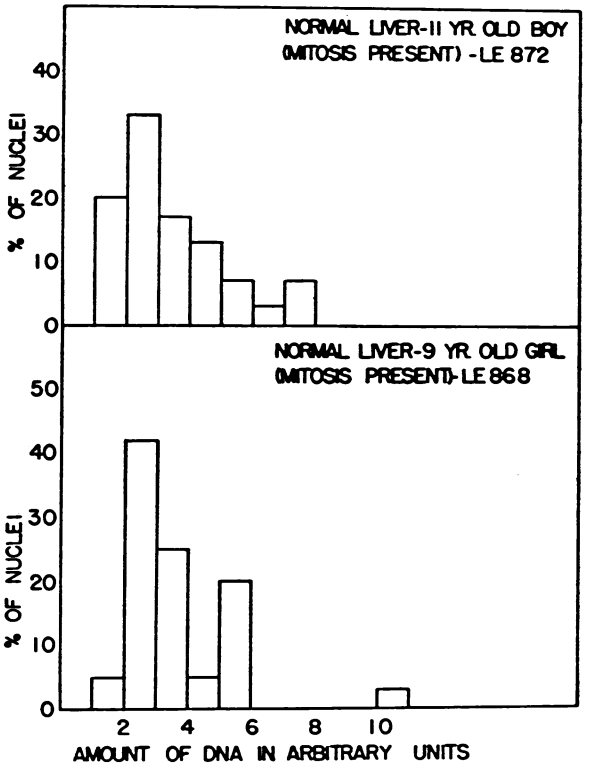
The possibility of interpreting deviating DNA data in tumors only on the basis of mitosis may be a disappointment to the pathologist who has tried again and again to find a diagnostic tool for the recognition of malignant cells. However, it must be kept in mind that normal tis-



Text-fig. 14. Individual DNA measurements (microspectrophotometry) in nuclei of carcinomas of breast, ages 67 to 83 years.

sues in adults usually do not exhibit mitosis, or in other words they usually show the characteristic DNA value with relatively little scattering. Consequently, a DNA histogram of a tissue which reveals scatter and increase of DNA must be looked upon with suspicion in regard to malignancy, unless regeneration is to be expected. One may argue, of course, that microscopic examination of a tissue for mitosis is much simpler and will lead to the same results. However, the absence of mitotic figures in a tissue does not exclude the possibility that a mitotic process is going on. If the time period of the mitotic cycle is a very rapid one, mitotic figures may be missed completely. Since, as pointed out before, DNA synthesis occurs at a very early stage of the mitotic process, namely, already in interphase, and is thus independent of the

time period of the mitotic cycle, DNA increase can be used as a most sensitive indicator for a division process. Studies of this kind may be particularly helpful in the cases which the pathologist designates as borderline cases, such as carcinoma *in situ*, some cases of low-grade malignancy, and, in brief, cases in which mitotic figures are scanty or absent.



Text-fig. 15. Individual DNA measurements (microspectrophotometry) in nuclei of livers of 2 children (mitosis present).

Summary

Microspectrophotometric studies of the DNA content in approximately 2500 individual cells of 49 normal and 27 malignant tissues of humans (ages ranging from 3 weeks to 87 years) gave the following results:

All normal tissues contained cells with a similar basic mean DNA content of 5.6×10^{-9} mg. (or 2.8 arbitrary units). Some tissues (*i.e.*, adult liver) in addition carry cells with nearly exact multiple amounts.

Within normal tissues the DNA content shows a certain but limited degree of variation from cell to cell.

Precancerous and malignant tissues do not exhibit the same uniformity in their mean DNA content as the cells of the homologous normal tissues.

Malignant tissues also reveal a much larger scatter from cell to cell than do the normal cells.

The deviating behavior of DNA in malignant tissues is interpreted on the basis of mitotic processes and is not considered a specific criterion for malignancy.

The possibility of using the DNA measurements as a diagnostic aid in certain questionable tumors is discussed.

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