

A Cytochemical Study of the Stem Cell Concept in Specimens of a Human Ovarian Tumor*

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ABSTRACT

The amounts of DNA in interphase nuclei were compared with the amounts of DNA in metaphase and anaphase figures in Feulgen-stained tissue sections of 5 specimens of the human ovarian papillary serous adenocarcinoma. The relative amounts of DNA per cell were determined by cytophotometric measurements of interphase nuclei at a single wavelength and of mitotic figures by the two wavelength method.

The 5 specimens conformed to the stem cell concept of cell proliferation since anaphase distributions of amounts of DNA were restricted to a narrow range of DNA values indicating the successful mitosis of a single cell type (*stem cell*) out of several cell types whose presence were suggested by the wide spread of interphase and metaphase values. In addition, the data indicated that, in some instances, only the amounts of DNA in anaphase figures can reliably identify the stem cell. Changes in the frequency of dividing cells having doubled amounts of DNA, and/or the presence of cells resulting from endoreduplication can distort the interphase distribution of amounts of DNA and thus give rise to a modal DNA interphase value which is not the same as the DNA value of the stem cell (anaphase figures).

Although measurements of normal tissues have to a large extent confirmed the postulate of constancy of amount of DNA (34), cytophotometric measurements of the interphase nuclei of solid tumors have indicated that they contain cells with differing amounts of chromophores related to DNA (1-3, 17, 32). Thus when the DNA values of tumor cells are examined, it is found that they may fall either into a frequency distribution resembling the pattern of amounts of DNA per cell of a diploid tissue or they may contain the DNA values associated with tissues in which aneuploid and polyploid cells are present. As proliferating tissues, tumors have, in addition, DNA amounts similar to those found in cells synthesizing DNA preparatory to mitosis (34, 37). The DNA frequency distribution of a tumor has, in large part, been attributed to factors related to the mitotic index, premitotic DNA synthesis, and the results of irregular mitosis (2, 3, 35). The pattern of DNA

distribution of a tumor does not appear to be dependent upon the type of tumor tissue, since specimens of the same tumor type may show different distribution patterns (1, 3, 17).

The concept of irregular mitotic mechanisms in dividing tumor cells contributing to a range of cell types distinguishable by different chromosome numbers was first elucidated by Levan and Hauschka (20) from metaphase chromosome counts of ascites tumors. The suggestion was made that from the frequency with which such cell types, differing in chromosome number, appeared in a tumor, the genotype chiefly responsible for proliferation of the tissue could be determined (11, 23). The cell type identified with the chromosome number occurring with the highest frequency was described as that of the *stem cell* from which the main contributions to mitosis were made.

Thus, the stem cell concept of cell proliferation augmented the view that the distribution of DNA in tumor nuclei can partly be accounted for on the basis of aneuploidy and polyploidy concomitant

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with aberrant mitotic mechanisms (2, 3, 35). According to biological and cytochemical evidence presented for this concept, normal mitosis results in the continuation of definitive genotypes which may or may not contain the chromosome number characteristic of the species. The genetic aspect of this concept concerns itself with the selective action of the environment upon the cell population. The concept states that the conditions of growth influence the selection of the compatible chromosome complement or stem cell from the total population of cell types.

Some elements of the principle of stem cell proliferation were foreshadowed by E. B. Wilson (38, p. 173) when in a consideration of multipolar mitoses in tumors, he concluded "that tumors, like normal tissues, grow primarily by typical mitosis and that multipolar figures are of secondary origin." The number of atypical mitoses in tissues may vary from the infrequent occurrence of somatic aneuploidy and polyploidy in some normal (5) and tumor tissues to the more extensive occurrence of such types of aberrancy in mammalian liver, many tumors, plants, invertebrate animals, and mitotically poisoned tissues.

Cytophotometric measurements of DNA have confirmed the existence of the normal proliferative activity of a defined genotype in tumors. Analyses of DNA measurements of mitotic stages in two mouse ascites tumors (32) and in a solid human tumor (1) have supported the stem cell concept. The cell proliferation of tumors has been related to a definitive cell type, which is chiefly responsible for the continuing viability of the tissue.

The present investigation was undertaken to determine whether differences in the pattern of interphase DNA distribution in a group of tumors of the same histological type reflected the identity and character of their stem cell proliferation. Specimens of the ovarian papillary serous adenocarcinoma have shown considerable variations in their pattern of DNA interphase values (3). In the present report, the variability in the DNA interphase distributions of the tumor specimens are compared with the distribution of amounts of DNA of mitotic figures in metaphase and anaphase in the same tumor specimens.

Materials and Methods

Specimens of human ovarian papillary serous adenocarcinoma were prepared for cytophotometric determinations of DNA in Feulgen-stained nuclei as previously described (3). Methanol-fixed tissue sections of

20 μ thickness were hydrolyzed in 1 N HCl for 12 minutes and subjected to the Schiff reagent for 1½ hours. The Feulgen reaction was carried out essentially as described by Stowell (33).

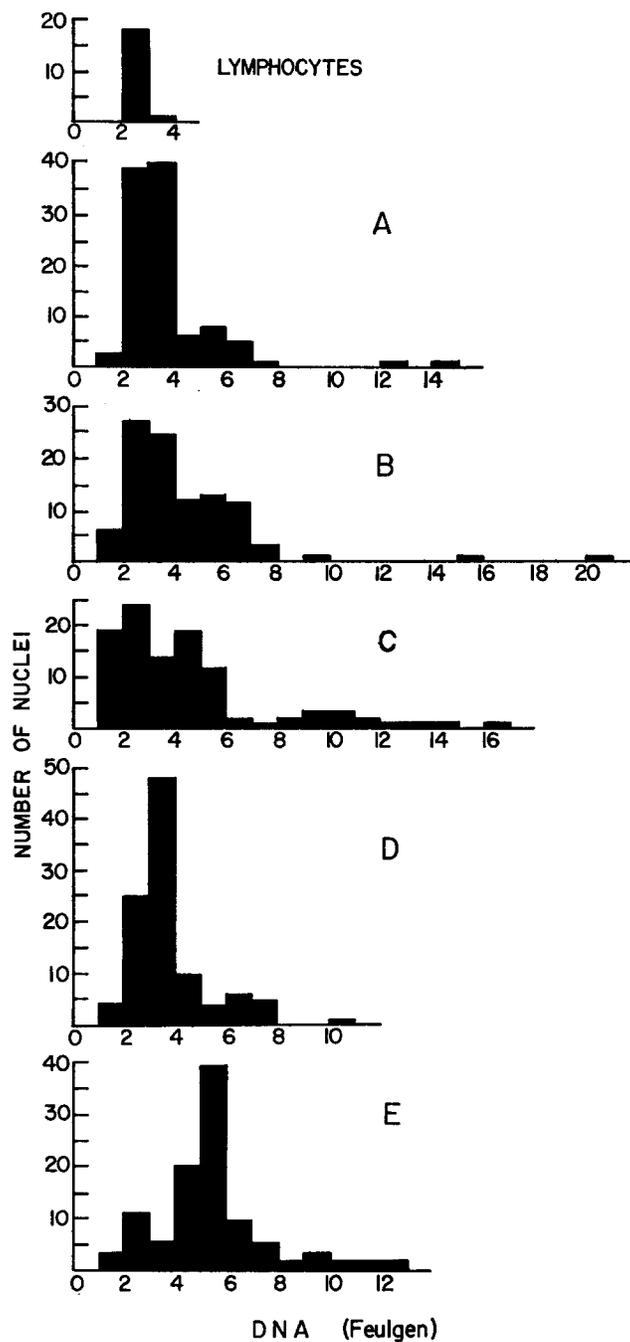
Interphase DNA Determinations at a Single Wavelength:

The DNA determinations of the interphase nuclei in the tumor specimens were determined microspectrophotometrically. The microspectrophotometric apparatus (3) was constructed according to the basic design of Pollister and Moses (31) and was implemented with additions suggested by Pollister (30) and Moses (25). The Feulgen absorption of interphase nuclei was measured at a single wavelength of 560 $m\mu$ isolated from a tungsten filament projection lamp by means of a Bausch and Lomb grating monochromator (3). Individual interphase nuclei in tissue sections were measured by the "plug" method and relative amounts of DNA were calculated from the formula, $E r^2 / F$ (34). The extinction, E , of the nucleus was determined by measuring a plug through the nucleus having a diameter equal to 40 to 50 per cent of the averaged diameters of the nucleus. The radius of the measured area, r , the fraction of the total volume of the sphere measured, F , and the extinction were used in computing the Feulgen-DNA value of a nucleus. In addition, lymphocytes from circulating blood were measured to ascertain the DNA distribution of human diploid cells (29). The relative amounts of DNA in the discoid nuclei of lymphocytes were determined in smears from the formula $E r^2$. Amounts of DNA in the interphase nuclei and in the lymphocytes can be directly compared.

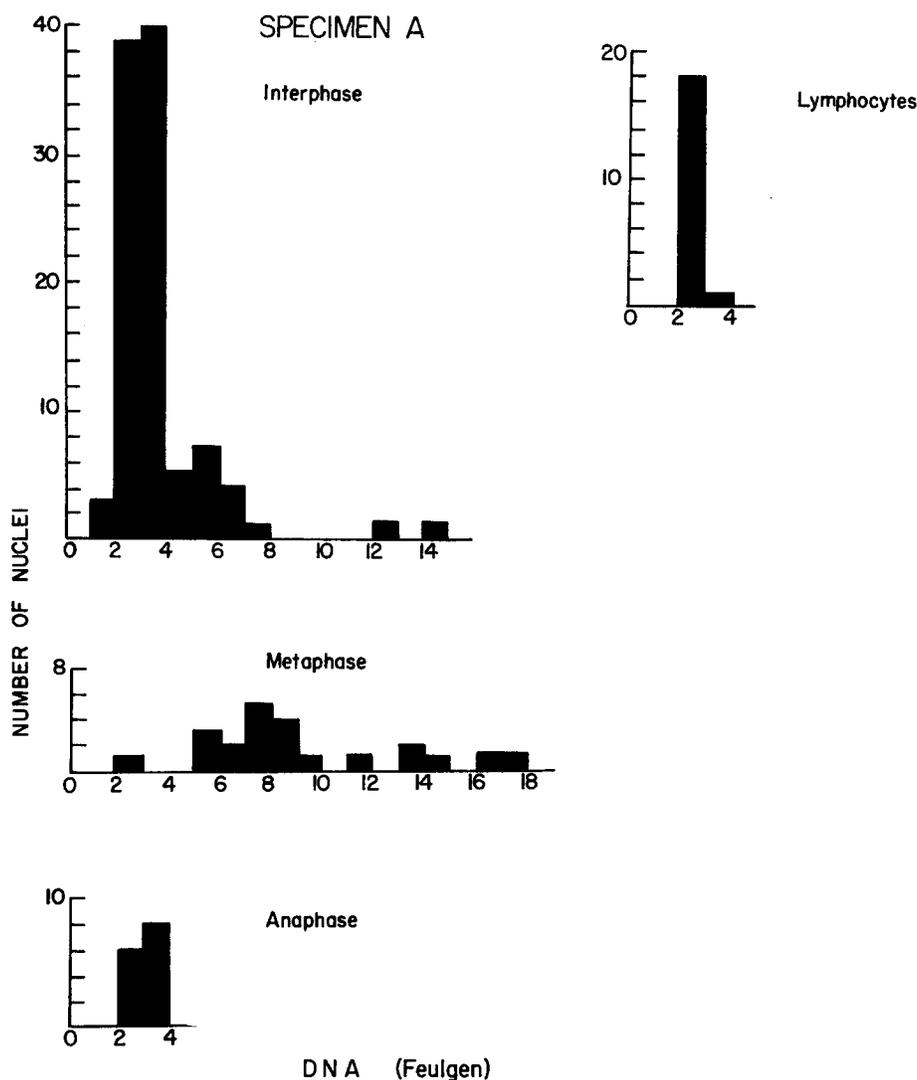
DNA Determinations of Mitotic Figures at Two Wavelengths:

Amounts of DNA in mitotic figures were determined by the two wavelength method. The method was formulated by Ornstein (27) and Patau (28) and has been reviewed by Swift and Rasch (36). The mitotic figures were not measured at a single wavelength because of the irregular geometry of the objects and the inhomogeneous distribution and concentration of absorbing material. Furthermore, the extinction varies throughout a mitotic figure, and difficulty is encountered in measuring its dimensions because of its irregular size and shape. The clumping and aggregation of absorbing molecules in mitotic figures also results in extremely high extinctions when they are measured at a single wavelength.

The two wavelength method, on the other hand, permits cytophotometric evaluation of absorbing substances in objects whose measurement at a single wavelength would not be valid because of errors due to the inhomogeneous distribution and concentration of absorbing material. Such errors would, of course, invalidate the application of the Beer-Lambert laws of



TEXT-FIG. 1. The frequency distributions of the relative amounts of DNA in the interphase nuclei of five tumor specimens (A to E) compared with the distribution of the diploid DNA values of interphase lymphocytes. Each tumor specimen (Specimens A to E) is shown individually with the DNA distributions of its interphase, metaphase, and anaphase nuclei in Text-figs. 2 to 6.



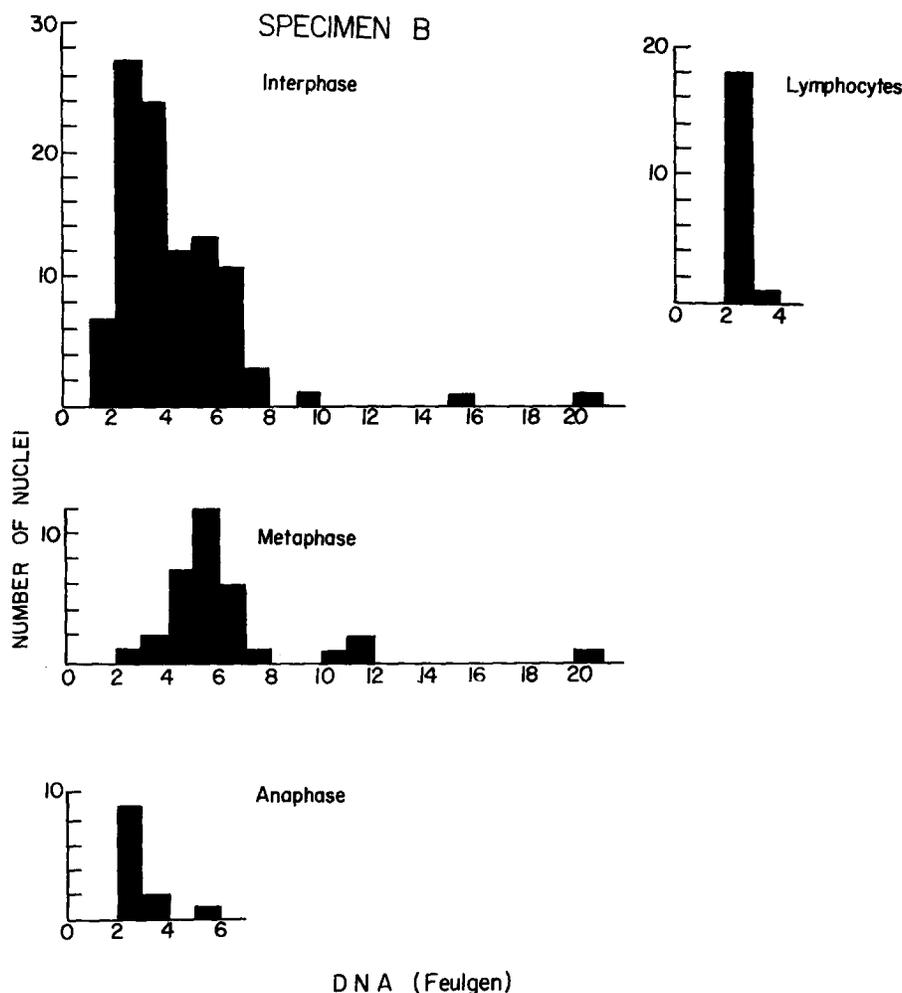
TEXT-FIG. 2. The frequency distributions of the relative amounts of DNA in the interphase, metaphase, and anaphase nuclei of tumor specimen A compared with the distribution of the diploid DNA values of interphase lymphocytes.

photometry upon which measurements at a single wavelength are dependent. Determinations at two wavelengths correct for the distributional error that results from variations in extinction throughout the test object. Similar variations in extinction result if all portions of the absorbing material cannot be brought into focus simultaneously. The use of smears in which the objects are flattened reduces this source of error. The two wavelengths are selected from a region of the spectrum which will not yield high extinctions.

The method requires the entire test object to be included in the measured area. It is, therefore, neces-

sary to have no absorbing material adjacent to the test object. The object plus a small area, clear of absorbing material, is encompassed by the diaphragm in the image plane, to delimit the field to be measured. The clear area is kept as small as possible. Variations in the geometry of the object, such as a mitotic figure, will determine the amount of non-absorbing area that will be encircled by the diaphragm.

The conditions of the method require that $E\lambda_2 = 2E\lambda_1$, in which $E\lambda_1$, and $E\lambda_2$ are the extinctions of a homogeneous part of the object at the two wavelengths employed. Absorption curves run on 5 nuclei of the



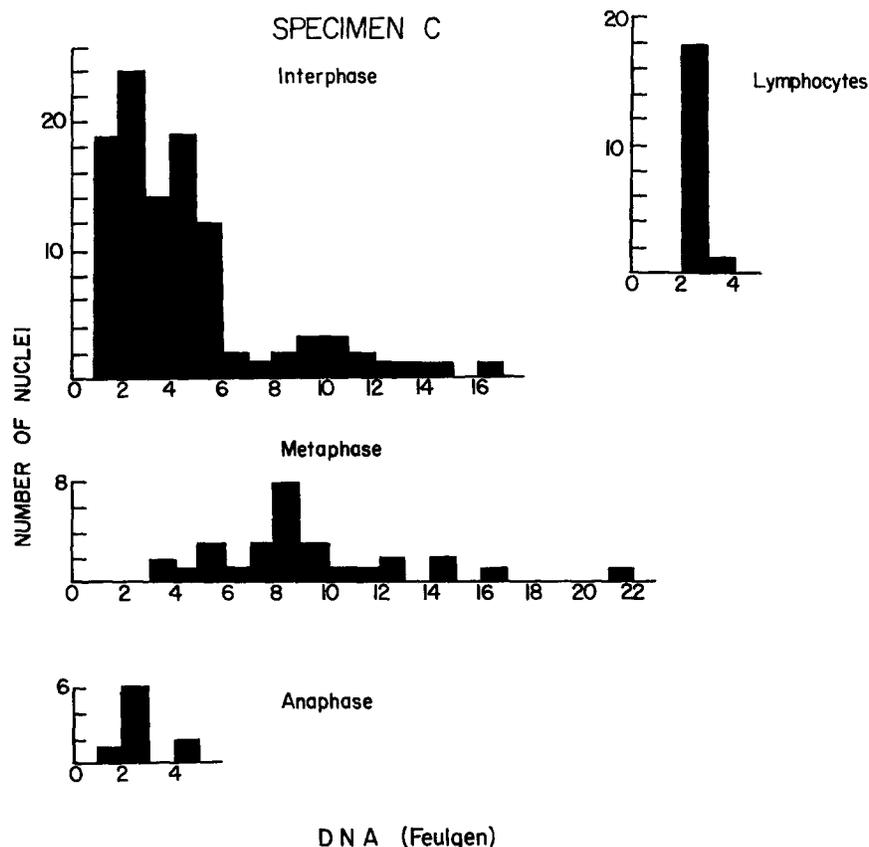
TEXT-FIG. 3. The frequency distributions of the relative amounts of DNA in the interphase, metaphase, and anaphase nuclei of tumor specimen B compared with the distribution of the diploid DNA values of interphase lymphocytes.

tumor material, selected for homogeneous distribution of the absorbing material, indicated that when $\lambda_1 = 490 \text{ m}\mu$ and $\lambda_2 = 514 \text{ m}\mu$, this requirement was satisfied. The DNA content of metaphase and anaphase figures was determined. Light scattering in the material was excluded by mounting the tissues in a medium of closely matched refractive index. Amounts of DNA were calculated from the formula developed by Patau (28).

Greater refinement of both light source intensity and phototube response are needed for the two wavelength measurements than for measurements at a single wavelength. The conventional microspectrophotometric apparatus employed for cytophotometric studies proved not to give sufficient stability and reproducibility for

this purpose. For this reason, an instrument¹ was employed for measurement of mitotic figures in which a storage battery was "floated" from a high current, full wave, line operated rectifier (39). The fluctuation of the light source intensity and phototube response was determined periodically. The variation in response of background readings ranged from a standard error of ± 0.08 per cent to a standard error of ± 0.22 per cent and averaged ± 0.12 per cent. Fourteen tumor nuclei

¹ Indebtedness to Dr. Gabriel C. Godman, Histochemistry Research Laboratory, Columbia University, College of Physicians and Surgeons, for the use of the microspectrophotometric apparatus in his laboratory is gratefully acknowledged.



TEXT-FIG. 4. The frequency distributions of the relative amounts of DNA in the interphase, metaphase, and anaphase nuclei of tumor specimen C compared with the distribution of the diploid DNA values of interphase lymphocytes.

measured successively at one wavelength and at two wavelengths showed a standard error of ± 4.24 per cent for the one wavelength measurements and ± 5.84 per cent for the two wavelength measurements. The ratio of the averaged DNA values of the one wavelength measurements and of the two wavelength measurements provided a factor for the conversion of the two wavelength DNA values into one wavelength units, thus enabling a direct comparison between interphase, metaphase, and anaphase DNA distributions.

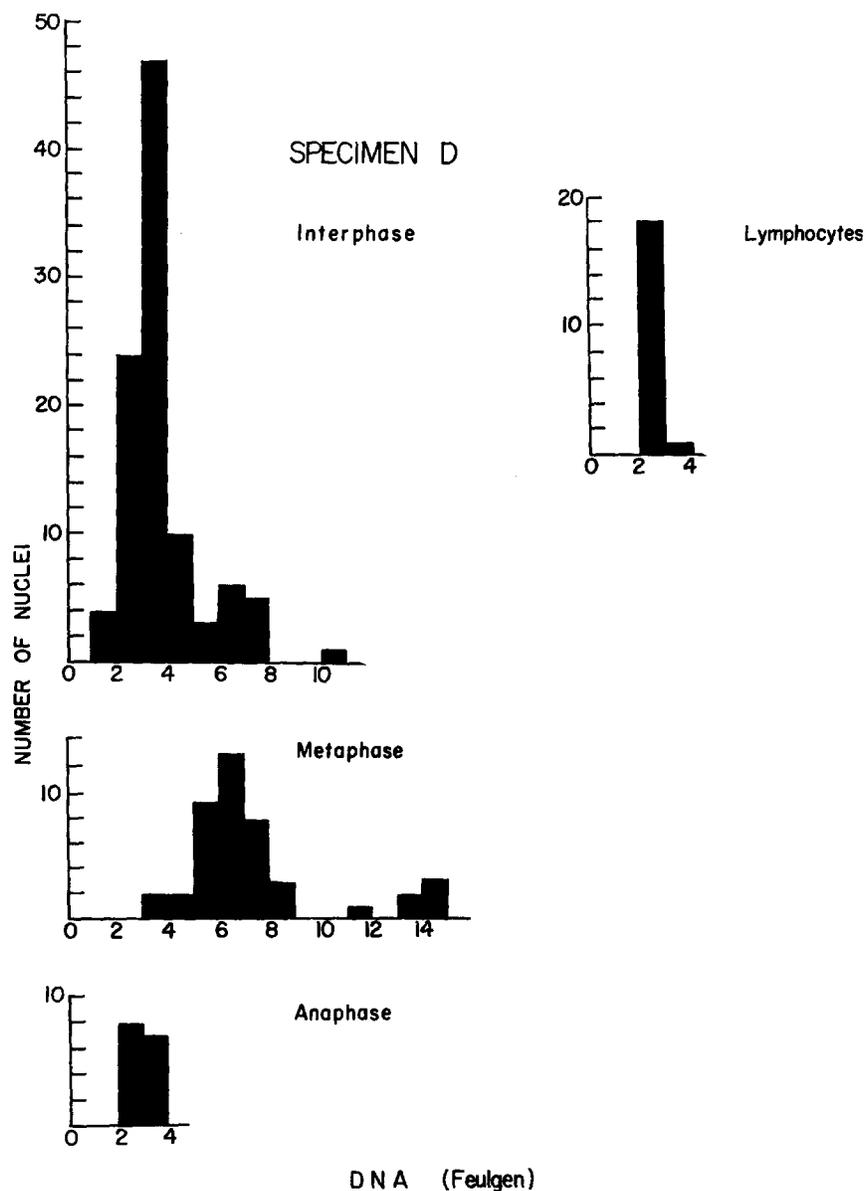
RESULTS

The DNA distribution of metaphase and anaphase figures was compared with the DNA distribution of interphase nuclei in 5 specimens of the human ovarian papillary serous adenocarcinoma. The specimens were chosen on the basis of (1) the presence of numerous mitotic figures and (2) the pattern of the DNA frequency distribution of their interphase nuclei. Many tissue sections of each specimen had to be scanned to find a sufficient

number of mitotic figures that were whole and uncut, and that contained an area around them devoid of absorbing material. Mitotic figures that were overlapped by or contiguous with other nuclei were discarded. Specimens containing dissimilarities in the spread and frequency of the DNA distribution of their interphase nuclei were selected for study.

DNA Interphase Distributions (Text-Fig. 1):

The DNA interphase distributions of the specimens selected for stem cell study are illustrated in Text-fig. 1. The results of DNA measurements of lymphocyte nuclei are shown above the interphase distributions of the tumor specimens. The spread of DNA values in the tumors (Text-fig. 1, Specimens A to E) can be described on the basis of two characteristics. First, in all the tumor specimens most of the DNA values are aggregated in the same region of the distribution as the DNA values



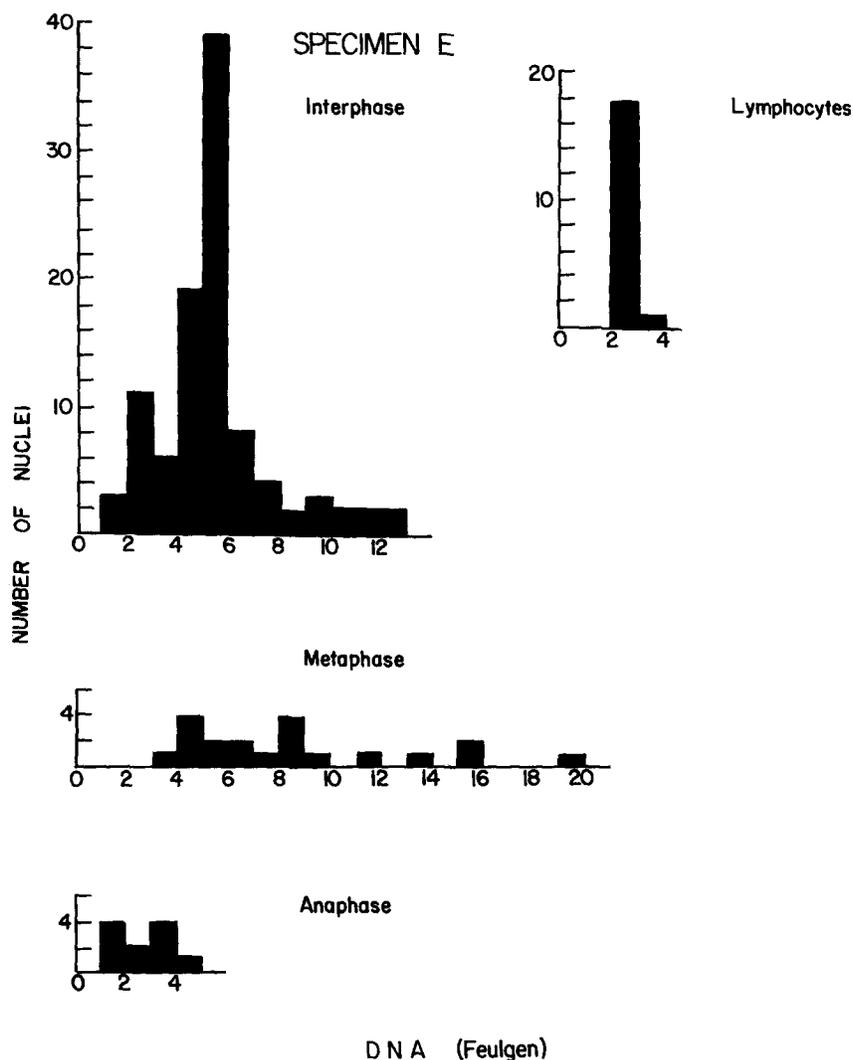
TEXT-FIG. 5. The frequency distributions of the relative amounts of DNA in the interphase, metaphase, and anaphase nuclei of tumor specimen D compared with the distribution of the diploid DNA values of interphase lymphocytes.

found for diploid lymphocyte nuclei and/or in the region adjacent to these values which corresponds to the tetraploid region. The remaining higher DNA values trail away from the main group of values. Secondly, in the main group of DNA values, the specimens differ with respect to the frequency of individual DNA values. The greatest frequency of DNA values in the main group may occur in the diploid region (Text-fig. 1, Specimen

A), or in the tetraploid region (Text-fig. 1, Specimen E), or they may be distributed among the diploid and tetraploid regions (Text-fig. 1, Specimen C).

Stem Cell Proliferation (Text-Figs. 2 to 6):

Metaphase and anaphase figures in the five tumor specimens were measured for DNA content. The measurements of metaphase figures represent



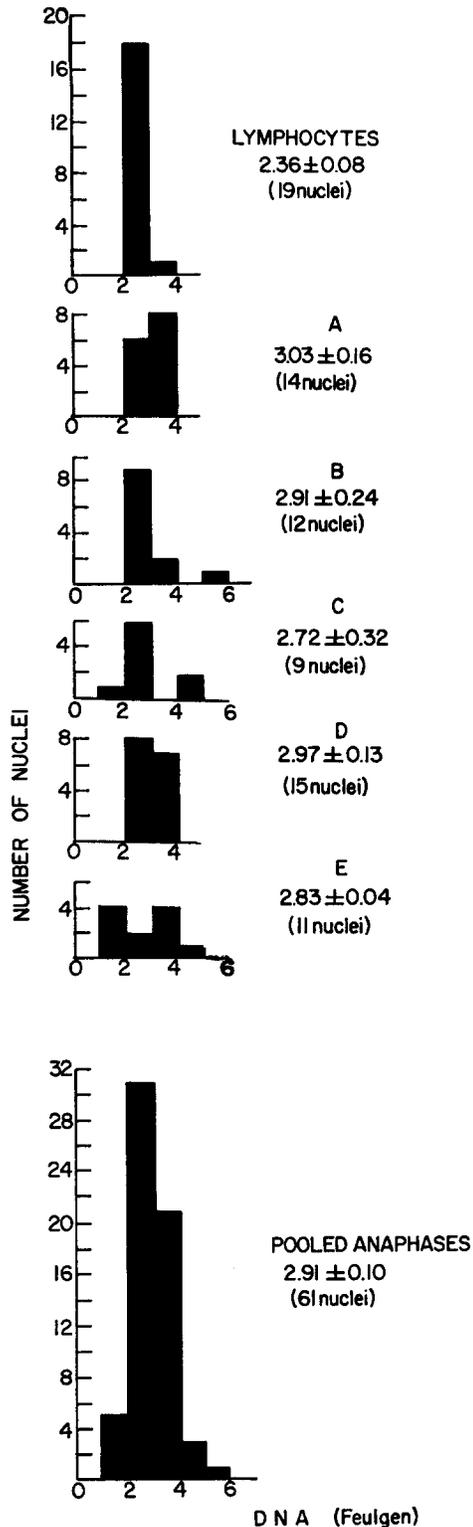
TEXT-FIG. 6. The frequency distributions of the relative amounts of DNA in the interphase, metaphase, and anaphase nuclei of tumor specimen E compared with the distribution of the diploid DNA values of interphase lymphocytes.

the amount of DNA in a doubled set of chromosomes whereas the measurements of anaphase figures represent the amount of DNA in one set of chromosomes resulting from a metaphase separation. A comparison of the interphase, metaphase, and anaphase DNA frequency distributions of each of the specimens (Specimens A to E) is shown in Text-figs. 2 to 6.

It is evident that the metaphase DNA distributions show a wider spread of values than do the DNA distributions of the anaphase figures. In the main, the spread of metaphase DNA amounts is the result of infrequently occurring values dis-

tributed about a dominant class of values. The anaphase amounts of DNA are restricted to a narrow range of DNA values.

The dominant class of DNA metaphase values appears to be distributed in the tetraploid range in each of the five specimens, whereas the DNA anaphase values are distributed in the diploid range. Since DNA synthesis does not occur during division, the amount of DNA in mitotic figures should be proportional to the number of chromosomes (8). Therefore, the interphase cell containing the chromosome number represented by the anaphase DNA values is obviously proceeding



TEXT-FIG. 7.

through metaphase successfully. The interphase cells which vary from the anaphase DNA values and show infrequent metaphase DNA values do not proceed successfully through mitosis.

Characterization of the Stem Cell from the DNA Anaphase Values (Text-Fig. 7):

The anaphase stages represent the cell type or stem cell that is participating in normal mitosis. The DNA distribution of anaphase values was compared with the interphase DNA values of diploid lymphocyte nuclei (Text-fig. 7). The means of the DNA values with their standard errors are shown for the lymphocytes and the anaphase figures of each of the tumor specimens (Specimens A to E).

The lowest anaphase mean DNA value, 2.72 (Text-fig. 7, Specimen C) varies from the highest anaphase mean DNA value, 3.03 (Text-fig. 7, Specimen A) by 10 per cent. This variation falls within the upper limit of experimental error. The apparent discrepancy in the anaphase values of some of the specimens may be due to (1) errors in measurement, or (2) unequal division of metaphase stages. It was observed that in instances in which both anaphases of a metaphase separation were available for measurement, the value of one such figure conformed to the value found for the stem cell population. In no instance did both anaphases fall outside this limit.

It is felt that the chief source of error in some measurements arose from the use of tissue sections in which the absorbing material of some mitotic figures was distributed in such a fashion as to make simultaneous focusing of all the absorbing material impossible. The lymphocyte DNA values which were obtained from smears of flattened interphase nuclei with homogeneously distributed absorbing material show little discrepancy among individual values. Two wavelength measurements of mitotic figures in smears should minimize the focusing errors. If tissue sections are measured, however, discriminate sampling of nuclei is necessary. Deeley *et al.* (7) have stated that errors due to improper focusing of absorbing material in the scanning method may result in a metaphase DNA distribution that resembles the DNA distribution

TEXT-FIG. 7. The frequency distributions of the relative amounts of DNA in the anaphase figures of tumor specimens A to E compared with the diploid DNA values of interphase lymphocytes and with the pooled DNA values of the anaphase figures of the five specimens.

of the interphase nuclei; *i.e.*, in lowered DNA values. The absence of this distortion in the tumor specimens (Text-figs. 2 to 6) indicates that the focusing error is not an important consideration in the evaluation of the present data which was obtained by the two wavelength method.²

The DNA anaphase values of the five specimens (61 nuclei) were pooled (Text-fig. 7, Pooled Anaphases). The standard error of the pooled anaphase DNA values was ± 3.34 per cent which compares favorably with a standard error of ± 3.20 per cent for the lymphocytes (19 nuclei). The variation between the lymphocyte mean DNA amount (2.36) and the mean for the pooled anaphase DNA values (2.91) is 19 per cent. To what extent this 19 per cent variation includes errors in measurement, or biological factors such as unequal division of metaphase figures cannot be determined. It is, however, apparent that the mean anaphase DNA value falls very close to the mean DNA value for the diploid lymphocyte nucleus. The exact relationship between them cannot be determined without chromosome counts. The stem cell of the specimens of the solid ovarian papillary serous adenocarcinoma can be characterized from the data as within the diploid range.

DISCUSSION

The data presented in this report have supported the validity of the concept of stem cell proliferation in five specimens of the same type of solid tumor. Although the specimens varied in the spread and frequency of their DNA interphase values, the variation did not appear to alter the identity of the stem cell which was found to be in the diploid range in each of the specimens. The use of tissue sections presented some disadvantages for cytophotometric determinations of DNA in mitotic figures. However, these disadvantages did not affect the validity of the data obtained by the two wavelength method, since the standard error of the pooled DNA anaphase values obtained from tissue sections and the standard error of the DNA lymphocyte values obtained from the smears were in good agreement.

The diploid character of the stem cell could not be predicted from an examination of the inter-

phase DNA values of Specimen E (Text-fig. 6). The DNA frequency distribution of its interphase nuclei clearly indicated a modal value in the tetraploid range. The metaphase and anaphase DNA frequency distributions which coincide with the chromosome number frequencies for mitotic stages of this tumor do not reflect the division of a stem cell with a tetraploid number of chromosomes. It is evident that in this specimen the interphase DNA frequency distribution does not reveal the identity of its stem cell, and it is therefore apparent that the stem cell cannot be interpreted from a DNA frequency distribution of interphase nuclei in all instances, as has been suggested by some workers (1, 17). This therefore requires reexamination of the identification, by Leuchtenberger *et al.* (17), of "tetraploid" tumors from DNA interphase distributions and of the estimation of the chromosome number of the stem cells of tumors from DNA interphase distributions by Ising and Levan (14). It is suggested that cytophotometric investigation of anaphase figures may best provide the identification of the stem cell in such material.

The discrepancy between the DNA frequency distribution of interphase nuclei and the identity of the stem cell may depend partly on the occurrence of DNA synthesis for mitosis during interphase. Stem cells in the diploid range which are in the process of synthesizing DNA or have completed DNA synthesis would be expected to appear in the tetraploid range of an interphase DNA frequency distribution. This position in the distribution would also be occupied by cells with a tetraploid number of chromosomes. A combination of diploid cells having doubled amounts of DNA and tetraploid cells having the tetraploid amount of DNA would result in a distortion of an interphase frequency distribution relative to the anaphase frequency distribution. The number of such cells would depend upon the mitotic index and the length of the interphase cycle after DNA synthesis. It has been emphasized by Mazia (24) that DNA synthesis and cell division are separate events. He points out that DNA synthesis and "chromosome duplication are necessary conditions but not immediate stimuli to mitosis" (24). Some reports have described the presence of interphase cells in which nuclear DNA synthesis may have been completed without the subsequent occurrence of cell division (8, 16, 26). A delay or failure in cell division following DNA synthesis in the diploid cells would tend to further distort an interphase DNA frequency distribution.

² A paper by Mendelsohn (*J. Biophysic. and Biochem. Cytol.*, 1958, 4, 407) has appeared in which he shows that gross changes in the focus of the objective does not affect the results of repeated two wavelength determinations on the same droplet of dye.

The contrast between the narrow range of DNA values in anaphase and the wide range of DNA values in interphase and metaphase has been, in part, attributed to mitotic irregularities. The spread of interphase DNA values has been described as the result of several types of abnormal mitosis. Two types of mitotic abnormalities that would result in increased DNA values are endoreduplication and *c*-mitosis. Cells in the process of endoreduplication would not be reflected in the prophase or metaphase population. However, endoreduplicated cells would appear in the prophase and metaphase population if they later divided by normal mitosis. A *c*-mitosis type of abnormality would result in the reconstitution of metaphase nuclei and consequently add to the interphase population. Both of these types of mitotic abnormality would contribute to the distortion of an interphase frequency distribution. It has also been suggested that the "genetically unbalanced" genotypes degenerate.

Since endoreduplication involves DNA synthesis and chromosome doubling without visible evidence of mitosis, what characteristics can be employed to distinguish between tetraploid interphase cells described as arising by this mechanism and diploid interphase cells which have doubled their DNA in preparation for visible mitosis? DNA measurements will not reveal any differences. Furthermore, it has been suggested that in interphase cells preparing for mitosis, chromosome doubling and DNA synthesis are closely related events (26). In effect then, cells resulting from endoreduplication are indistinguishable from cells preparing for division in an interphase DNA distribution. Such cells would not be reflected in the metaphase population but would affect the shape of the DNA interphase frequency distribution. Freed and Hungerford (10) reported the presence of polyploid DNA values in sublines of the Ehrlich ascites tumor that could not be accounted for on the basis of metaphase chromosome counts. They suggested endomitotic and endoreduplication mechanisms as mediating this condition.

In the tumor specimens, stem cell proliferation by a cell type in the diploid range was independent of the mitotic mechanisms that resulted in the presence of cells with increased amounts of DNA. Apparently, the selection of the stem cell is not influenced by the conditions of DNA synthesis prior to normal or aberrant mitosis.

General Considerations of Stem Cell Proliferation:

The capacity for survival and mitotic viability in a given environment has been considered to be the main attribute of the stem cell. By single cell isolation experiments, tumor transplantation into genetically varying hosts, by freezing and treating tumors with toxic substances, and by studies on the conversion of solid tumors into the ascites form, the survival and proliferation of tumors have been related to the stem cell (11, 15, 18, 22). That the mosaic composition of the cell population of tumors enables selective forces to favor the proliferation of the cell type, genetically compatible with environmental conditions, has been stressed (11, 15, *inter alia*). Shifts in stem cell proliferation in response to changes in the conditions of growth have been demonstrated by metaphase chromosome counts and DNA studies (10, 15, 19).

The fate of the non-stem cells may be compared to a colchicine-treated population of cells. Bloch (6) has studied the effect of colchicine on DNA synthesis of diploid rat fibroblasts *in vitro*. He concluded that this antimitotic substance had no direct effect on the synthesis of DNA. However, the occurrence of a mitotic block at metaphase and subsequent restitution to interphase of arrested nuclei resulted in an interphase population containing higher DNA classes. The eventual demise of the culture was attributed to toxicity or to production of genetically unbalanced cells. In untreated material, the stem cell population may be a continuous reservoir of cells upon which mitotic irregularities may act to produce a varied interphase distribution.

The stem cell concept can be considered to have wide general application to the proliferation of tissues. Although the infrequent occurrence of somatic aneuploidy and polyploidy encountered in normal mammalian tissues (5) has recently received much attention, the normal mitotic behavior of these tissues has been studied for many years. Normal mitosis by stem cell proliferation has also been noted in tissues in which aberrant mitotic conditions result in several cell types with modified chromosome complements. This condition has been found in animal and human tumors in both the solid and ascites forms (1, 11, 12, 15, 20, 21), and in some normal tissues grown *in vitro* for long periods by successive transplantation (13, 19). Earle's L strain cells (9) which were derived over a decade ago from methylcholanthrene-treated fibroblasts of mouse connective tissue grown *in vitro* were measured cytophotometrically in Feulgen-stained smears. The DNA distribution of interphase and metaphase nuclei showed a wide range of DNA values suggesting the presence of several cell types. The restriction of the DNA values of anaphase and telophase figures to a narrow spread of values in the DNA-tetraploid range indicated that the L strain cells were conforming to the stem cell concept of cell proliferation (4).

Changes in the chromosomal character of the stem

cell which occur when selection favors the proliferation of a specific cell type in a particular environment is not unlike the more permanent changes in somatic chromosome number that occur in the evolution of plants and animals. The stem cell of a tumor or a tissue culture system differs from the stem cell of a normal tissue in its greater capacity for continuous proliferation (18).

The nature of the relationship between the selection of the stem cell by the environment and the ability of the stem cell to complete mitosis successfully is an unresolved question. In a discussion of changes in chromosome number, E. B. Wilson (38, p. 889) concluded that the materials of which chromosomes are composed are of greater importance than the number of chromosomes present. This would suggest that chromosomal components other than DNA may be more directly associated with the selection of the stem cell since the amount of DNA per cell is proportional to the number of chromosomes.

SUMMARY

1. The distribution of amounts of DNA in metaphase and anaphase figures was compared with the DNA distribution of interphase nuclei in 5 specimens of the human ovarian papillary serous adenocarcinoma. The Feulgen absorption of interphase nuclei was measured cytophotometrically at a single wavelength, whereas the Feulgen absorption of the mitotic figures was determined by the two wavelength method.

2. The tumor specimens selected for study varied greatly in the spread and frequency of their DNA interphase values. The variation in the DNA distributions of the interphase cells of the specimens was manifested by differences in the position of the DNA values occurring with the greatest frequency. These modal DNA interphase values occurred either in the diploid region or in the tetraploid region, or they were distributed throughout the diploid and tetraploid regions, as might be expected from dividing tissues in which DNA synthesis for mitosis and mitotic irregularities were occurring.

3. The comparison of the range and frequency of the DNA interphase values with the DNA values for metaphases and anaphases in the same tumors revealed that, whereas the metaphase figures contained a wide spread of DNA values, the amounts of DNA in the anaphase figures were restricted to a range of values approximating that expected of a single genotype. The narrow range of DNA anaphase values revealed that the proliferation of the specimens was dependent on the normal mitosis of a single cell type. The viability of a single

genotype in the presence of several cell types illustrated the conformity of the specimens to the stem cell concept of cell proliferation.

4. Further examination showed that the modal DNA interphase values (the DNA interphase values occurring with the greatest frequency) did not coincide with the position of the DNA anaphase values. Whether the position of the modal DNA interphase values was in the diploid range or in the tetraploid range or distributed throughout the diploid range and the tetraploid range, the position of the stem cell (DNA anaphase distribution) of the 5 tumor specimens remained unaffected and in all instances was found to be in the diploid range.

5. Changes in the frequency of the interphase DNA values which shifted the position of the modal DNA interphase values out of the diploid range were attributed to the presence of interphase cells containing doubled amounts of DNA due to DNA synthesis for cell division but with delayed mitosis, and/or to interphase cells with doubled amounts of DNA due to endoreduplication without visible mitosis. These two cell types cannot be distinguished from each other by their DNA values, but still they occupy the same position in the DNA-tetraploid range.

6. It was concluded that the stem cell cannot in all instances be identified from a DNA interphase distribution and must be verified by the amounts of DNA in anaphase figures.

7. Stem cell proliferation in tumors was discussed in relation to normal mitosis in normal tissues, mitotically poisoned tissues, and more permanent changes in somatic chromosome number such as occur in the evolution of plants and animals.

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