Technical Papers

Critical Evaluation of Feulgen Microspectrophotometry for Estimating Amount of DNA in Cell Nuclei

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In recent years, microspectrophotometry of the Feulgen stain (*1*) has been extensively used as a method for determining amounts of desoxyribosenucleic acid (DNA) in individual cell nuclei (*2–4*).

In spite of certain obvious advantages of this procedure which permits the DNA analysis of a single nucleus *in situ* directly under the microscope and its applicability to the study of the significance of DNA values in a single cell or cell type, the microspectrophotometric method of the Feulgen stain is by no means generally accepted. Based mainly on theoretical considerations of the physical and optical factors involved in the method, the microspectrophotometric method has been repeatedly criticized (*5*) and its validity seriously questioned (*6*).

While the study of such potential sources of error is fully recognized as one of the necessary criterions for the evaluation of Feulgen microscpectrophotometry, we feel that some clarification of the problem might also be derived from a survey of the results obtained by microspectrophotometric analysis and by a comparison of such results with those obtained by other methods.

In this paper, an attempt is made to evaluate the validity of Feulgen microspectrophotometry by using, as a baseline, DNA data of 15,000 nuclei as determined by this method in this laboratory (*7*). Such an approach seems justified because of the considerable number of determinations done under strictly standardized and controlled conditions on a variety of tissues. Furthermore, whenever possible, the Feulgen data were checked by other independent methods. Ultraviolet microspectrophotometry, chromosomal studies, as well as DNA determinations by biochemical analysis, were carried out simultaneously on the same material.

The data presented in Table 1 may be considered mainly from two points of view which seem pertinent in examining the validity of a method: (i) How do the DNA results obtained by microspectrophotometry of the Feulgen stain compare with DNA results by other analytic methods? (ii) How sensitive is this method? In other words, what is the degree of accuracy with which amounts of DNA can be safely determined?

Comparing first the DNA data by Feulgen microspectrophotometry with the results of the biochemical analysis, it can be seen that there is a striking agreement of both methods, giving 1 DNA, 2 DNA, 4 DNA for bull sperm, normal somatic beef, and rodent tumor nuclei, respectively.

It can also be seen that if a tissue, such as rat liver, which contains nuclei with different amounts of DNA (2 DNA, 4 DNA, 8 DNA), is examined, only the microspectrophotometric analysis of *individual nuclei* is capable of reflecting the individual DNA classes, whereas the biochemical analysis that is done on a mass of nuclei can of necessity give only an average value.

The second standard method that can also be used

Table 1. Comparison of the content of DNA in 15,000 individual animal nuclei by microspectrophotometry of the Feulgen stain and other analytical methods. Amounts of DNA are given in basic arbitrary units.

Type of material	Chromosomal counts	DNA by Feulgen* microspectrophotometry	DNA by ultraviolet† microspectrophotometry	DNA by biochemical analysis
Human sperm	1n, haploid	1 DNA		
Bull sperum		1 DNA	1 DNA	1 DNA*†
Insect spermatid (Arvelius)		1 DNA		
Beef liver, beef kidney	2n, diploid	2 DNA	2 DNA	2 DNA*†
Rodents: kidney, spleen, lymphoma		2 DNA	2 DNA	2 DNA†
Rodents: Ehrlich ascites tumor	4n, tetraploid	4 DNA	4 DNA	4 DNA†
Rat liver	2n, diploid	2 DNA	2 DNA	Average value
	4n, tetraploid	4 DNA	4 DNA	of all
	8n, octoploid	8 DNA	8 DNA	DNA classes*†
Hamster liver		2 DNA		
		4 DNA		
		8 DNA		
Human liver	2n, diploid	2 DNA		
	4n, tetraploid	4 DNA		
	8n, octoploid	8 DNA		

* Studies done at the Institute of Pathology, Western Reserve University, Cleveland, Ohio.
† Studies done at the Karolinska Institute, Stockholm, Sweden.

as a tool for the critical examination of Feulgen microspectrophotometry is the counting of chromosomes. It is evident that there is a good accordance between the DNA results of Feulgen microspectrophotometry and chromosome numbers, a comparison that is of special significance in tissues showing multiple DNA values.

The third method used for comparison concerns DNA determinations by *ultraviolet* microspectrophotometry. Although it may be argued that another microspectrophotometric method is not too suitable for comparison, nevertheless, the different principles of the two methods and accordance of the ultraviolet data with the Feulgen data are of sufficient interest to justify its inclusion in Table 1.

Coming now to the second point—namely, the sensitivity and accuracy of the microspectrophotometric DNA determinations—the excellent agreement of the *multiple* DNA values obtained by Feulgen microspectrophotometry with chromosome numbers, as well as with biochemical analysis, illustrates the order of magnitude with which the method can be safely used. Even if the actual DNA values obtained by microspectrophotometry of the Feulgen stain may sometimes show variations up to 15 percent from the theoretical ratios of 1:2:4:8 DNA, such a difference would hardly be capable of obscuring the multiplicity of the DNA values.

On the other hand, the question of the significance of *intermediate* DNA values occurring in nuclei cannot be so readily answered. Although there does not seem to be any doubt about the constant occurrence of such intermediated DNA values in nuclei of proliferating tissues (*3, 8*) it is felt that much more work and probably more exact methods are needed to interpret the degree of accuracy of the intermediate values.

On the basis of the data presented in Table 1, it seems justifiable to say that microspectrophotometry of Feulgen stain can be utilized as a reliable tool for the comparison of relative amounts of DNA in nuclei of different cells, provided that the differences to be detected are sufficiently large.

In support of the validity of the method, it may be added that microspectrophotometry of Feulgen stain also permits an excellent reproducibility of the DNA results, if preparation of nuclei and measurements are done under carefully standardized and controlled conditions. In this laboratory, a group of 12 trained workers, using four different microspectrophotometric setups within a period of more than 4 years, obtained nearly identical DNA results in the same tissues, the largest variation ever encountered being 10 percent. Furthermore, results from other laboratories are essentially in accordance with the data presented in Table 1 (*2–4*).

Taking into consideration the reproducibility of the DNA data obtained by Feulgen microspectrophotometry, their good correlation with chromosomal counts, with ultraviolet microspectrophotometry, and with the biochemical analysis, one can hardly escape the conclusion that the method has its merits and can be used safely for DNA determination at this level of investigation. This, of course, does not exclude the necessity of cautious interpretation, of checking the DNA data by other methods, and of the possible interference of other factors (*5*), if the method is used for the detection of smaller differences in DNA content.

References and Notes

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Hexokinase Reversibility Measured by an Exchange Reaction Using C^{14}-Labeled Glucose

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In the hexokinase reaction, a phosphate group is transferred from adenosine triphosphate (ATP) to glucose to form glucose-6-phosphate:

$$\text{ATP} + \text{glucose} \rightleftharpoons \text{glucose-6-phosphate} + \text{ADP}.$$

The reaction from left to right is associated with a large loss of free energy, and hence equilibrium is far to the right. Net synthesis in the reverse direction has not been demonstrated. In the present study (*1*) evidence of reversibility was obtained by observing an exchange between glucose and glucose-6-phosphate with the aid of C^{14}-labeled glucose. The exchange reaction was found to require catalytic amounts of either ATP or ADP.

The enzyme used in this study was purified from yeast using the method of Berger *et al.* (*2*), the preparation, essentially fraction V, contained 800 units per milligram of protein and, hence, was considered 26-percent pure. Phosphohexose isomerase was present as a significant impurity. During the prolonged reaction times used in this study, this latter enzyme functioned to bring the glucose-6-phosphate $\rightleftharpoons$ fructose-6-phosphate reaction to its equilibrium and, thus, to convert approximately one-third of the glucose phosphate to its fructose isomer. Phosphoglucomutase was also present as a contaminating enzyme. Phosphatase was not detected. The hexokinase proved durable, re-