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DETERMINATION OF NUCLEOTIDE AND ISOPLITH COMPOSITION OF DNA BY THIN-LAYER CHROMATOGRAM SCANNING IN UV LIGHT

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KEY WORDS: DNA derivatives; thin-layer chromatography; UV reflection; nucleotide and isoplith composition of DNA.

The results of previous investigations [3, 7, 9] showed that during scanning of thin-layer chromatograms in reflected light dependence of the areas of the peaks of the densitograms for different substances on the quantity of material in the chromatographic stains is determined both by the quantity and character of the material. Over a certain range of quantity, this dependence may be a virtually linear function [6, 9]. However, despite all the evident advantages of scanning of thin-layer plates compared with the laborious spectrophotometry of eluates for quantitative analysis of DNA derivatives, this approach has not yet been used because of the lack of any suitable coefficients for converting areas of densitographic peaks into corresponding quantities.

The aim of this investigation was the qualitative and quantitative analysis of spectra of UV light reflection by DNA hydrolysis products [2, 10] in thin layers of cellulose after chromatographic separation and determination of the nucleotide and isoplith compositions of DNA.

EXPERIMENTAL METHOD

Nitrogenous bases and pyrimidine isopliths of DNA were fractionated by methods described previously [1, 5]. The quantity of material applied at the start, calculated as DNA, was 5 μg. Scanning was carried out by means of an "Opton" chromatogram spectrophotometer (West Germany) in reflected UV light with a slit measuring 12 × 0.2 mm; the velocity of movement of the beam relative to the plate was 10 mm/min and the tape winding speed of the automatic writer was 30 mm/min. Spectra were recorded in the middle of the zone occupied by a separate component, using the same width of slit as for scanning, relative to a control region having the same R_f value.

EXPERIMENTAL RESULTS

Spectral data for DNA bases separated in an alkaline solvent: N-butanol 60, methanol 20, water 20, 25% NH_4OH 1 (v/v) [8], and an acid solvent: methanol 70, concentrated HCl 20, water 10 [4], are given in Figs. 1 and 2, respectively. The marked differences between the spectral characteristics of the bases in the systems used reflect differences in the degree of protonation of the bases in each system. If dry plates retaining bound water were kept under ordinary conditions, the spectral characteristics of the bases were unchanged. Quantitative analysis showed that coefficients of molar extinction of the substances analyzed on thin-layer chromatograms in reflected light vary depending on the properties of the layer (different batches of cellulose); however, the ratio between the coefficients remained constant in this case. Coefficients which can play the role of coefficients of molar extinction within the

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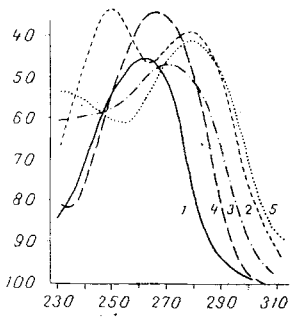

Fig. 1

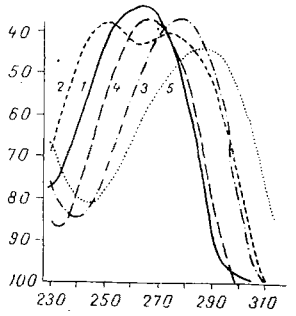

Fig. 2

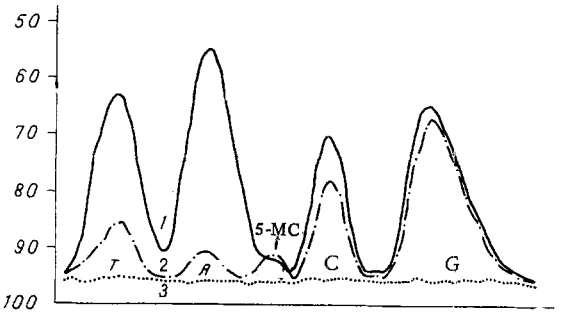

Fig. 3

Fig. 1. Spectra of reflection of UV light by DNA bases after fractionation in an alkaline solvent. Abscissa, wavelength (in nm); ordinate, reflection (in percent); 1) adenine, 2) guanine, 3) cytosine, 4) thymine, 5) methylcytosine.

Fig. 2. Spectra of reflection of UV light by DNA bases after fractionation in an acid solvent. Legend as to Fig. 1.

Fig. 3. Densitogram of chromatographic separation of nitrogenous bases consisting of hydrolysis products of calf thymus DNA. T) Thymine, A) adenine, 5-MC) 5-methylcytosine, C) cytosine, G) guanine. Abscissa, mobility (in relative units). Curve 1) 260 nm, 2) 290 nm, 3) 320 nm. Ordinate, reflection (in percent).

TABLE 1. Spectral Characteristics of Nitrogenous Bases Being DNA Hydrolysis Products in Reflected Light and Their Equimolar Coefficients

Base	Alkaline solvent		Acid solvent	
	maximum, nm	coefficient	maximum, nm	coefficient
Adenine	262	$1{,}00_{260}$	267	$1{,}00_{260}$
Guanine	250/278	$1{,}56_{260}$	253/277	$1{,}67_{260}$
Cytosine	270	$1{,}96_{260}$	280	$2{,}24_{260}$
Thymine	267	$1{,}51_{260}$	268	$1{,}65_{260}$
5-Methylcytosine	279	$1{,}37_{290}$	288	$1{,}16_{290}$

TABLE 2. Composition of Bases (in mole %) of Calf Thymus and *E. coli* DNA

Source of DNA	Method	Adenine	Guanine	Thymine	Cytosine	5-methylcytosine	GC + MC	Error, %
E. coli	Spectrophotometry of eluates	24,6	25,6	24,3	25,5	—	51,1	3–7
Thymus		27,8	22,6	27,4	20,8	1,40	44,8	
E. coli	Scanning of chromatograms	25,2	25,9	25,5	23,4	—	49,3	0,5–2
Thymus		27,0	22,1	28,3	21,3	1,28	44,7	

Legend. Each value represents arithmetic mean of 5 determinations.

TABLE 3. Content of Pyrimidine Sequences (isopliths) (in mole %) in Calf Thymus DNA

Method	Number of pyrimidines in isoplith									
	I	II	III	IV	V	VI	VII	VIII	IX and over	error, %
Spectrophotometry of eluates	10,8	9,6	7,6	6,1	4,1	3,1	2,3	1,5	4,9	
Scanning of chromatograms	10,4	9,5	7,3	6,3	5,3	3,6	2,4	1,1	4,1	0.5–2

range from 0.15 to 1.30 μg of material in the spot are therefore given for different bases such that the coefficient for the base with highest absorption, namely adenine, is conventionally taken as unity. The relative percentage of each base was determined by dividing the product of the area of the peak for that base and its coefficient (Table 1) by the sum of the products of the areas of all peaks (Fig. 3) and the corresponding coefficients, and expressing the result in percent.

Analysis of equimolar quantities of cytidylic and thymidylic oligonucleotides showed that isobestic points were present in their spectra (270 nm). The relative percentages of pyrimidine isopliths can therefore be calculated from the ratio between the areas of the peaks on the densitograms.

The accuracy of the suggested method is shown by data on the base composition of DNA and the content of pyrimidine nucleotides of different lengths (Tables 2 and 3), determined by scanning of thin-layer plates and, for comparison, by spectrophotometry of eluates.

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USE OF BALLOON CATHETERS TO MONITOR MYOCARDIAL RESTING TENSION DURING OPERATIONS WITH CARDIOPLEGIA

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KEY WORDS: cardioplegia; resting tension; contractile function.

Cardioplegia at the present time is the most promising method of protecting the heart against ischemic damage during operations. Meanwhile cardioplegia has not only a protective action, but also a harmful action [1-6]. Possible corrections can be introduced into the course of cardioplegia, the method itself significantly improved allowing for individual differences between hearts, and indications for additional infusions of solution during operation can be objectively determined, however, only if dynamic monitoring of the functional state of the arrested heart is carried out during the operation.

One such technique has been developed in the writers' Institute and has successfully undergone clinical trials [3].

However, this method did not permit a quantitative assessment of changes taking place, and the measuring apparatus required for that purpose was very clumsy and complicated.

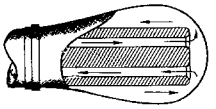

Fig. 1. Scheme of modification of twin-lumen catheter to record myocardial resting tension.

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