SUBSTANCES INTERFERING WITH SPECTROPHOTOMETRIC ESTIMATION OF NUCLEIC ACIDS AND THEIR ELIMINATION BY THE TWO-WAVELENGTH METHOD

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SUMMARY

The uniformity of the optical properties of substances interfering with the spectrophotometric determination of nucleic acids in different tissues has been proved. The absorption spectra of these substances show that the chief conflicting component is most probably tyrosine. The application of the two-wavelength method is therefore very convenient for nucleic acid estimations in animal tissues. The two wavelengths at which the optical density should be measured are 260 mμ and 286 mμ for RNA and 268 mμ and 284 mμ for DNA. A comparison of results obtained by different methods is presented.

INTRODUCTION

It is obvious from investigations made in recent years that in addition to the RNA and DNA fractions, nucleic acid extracts obtained by the Schmidt-Thannhauser procedure[1] contain numerous contaminants interfering with the nucleic acid determination by phosphorus analysis[2–4] and pentose colour reactions[3,5–9], as well as by ultraviolet absorption photometry[9–14]. The Ogur–Rosen method[15], by which purer extracts are obtained, does not, however, ensure the complete absence of interfering substances[6,9,16] and, what is more important, leads to an incomplete separation of RNA from DNA[6,17–21]. In order to avoid the interfering substances, different techniques have been used: reducing the time and lowering the temperature of the alkaline hydrolysis[11]; eliminating the interfering substances by adsorption on Dowex[9], by paper electrophoresis[2] or by paper chromatography[13]; fractionating nucleoproteins to a purer state prior to their determination[22]. The first of these procedures ensures neither the complete elimination of the contaminants nor the complete extraction of RNA[23]. The remaining procedures are too laborious for serial investigations and are not convenient as micromethods.

Taking into consideration the advantages of the spectrophotometric determinations of nucleic acids, a number of authors have tried to apply this method for quantitative nucleic acid determination in tissue fractions[3,15,20,21,24,27], although interfering substances have always been detected. The main contaminants are sup-

posed to be protein breakdown products[9-11,29], which are set free during the hydrolytic separation of nucleic acids. Until now no suitable methods have been proposed for calculating the nucleic acid content by measuring the optical density of the contaminated fractions[14,29,30]. The unknown specific extinction coefficient of the contaminants for suitable wavelengths has made some authors conclude that such a calculation would be impossible[10]. In order to eliminate the cell-protein absorption, a two-wavelength method was introduced twenty years ago for cytochemical estimation of NA[31,32]. Until now such a method has not been used for quantitative determination of RNA and DNA in tissue extracts. In the few investigations in which the difference in the absorbance at two wavelengths is used[21,27,33,34], the appropriate choice of the second wavelength is to some extent arbitrary, since it is not based on the spectral characteristics of the absorbing contaminants.

In this paper we demonstrate the uniformity of the optical properties of the contaminants in different animal tissues. This makes it possible to determine suitable wavelengths at which the absorption of the contaminants would be eliminated by the two-wavelength method.

Prior to the application of the ΔE method it was necessary to prove the additivity of the optical density E_λ of a mixture containing an amount N of nucleic acid with absorptivity k_λ and an amount X of contaminants with absorptivity k'_λ, *i.e.* that

$$E\lambda = k\lambda N + k\lambda X \tag{1}$$

This additivity is admitted generally[34]; but there are data in the literature showing that the absorptivity of a mixture containing adenylic acid and tyrosine is far from being additive[35]. In our experiments, however, we have shown that the optical density of a mixture of pure hydrolysed RNA (or DNA) and protein breakdown products (both in N PCA) is strictly additive[12]. Having proved the validity of (1) in such a way, one can estimate the quantity of nucleic acids by measuring the optical density of the extracts at two wavelengths at which the absorption coefficients of the contaminants are equal. It is convenient to fix the first wavelength at the absorption maximum of nucleic acids. Denoting the second wavelength as λ_0, the amount of nucleic acid according to (1) will be

$$N = \frac{E\lambda_{max} - E\lambda_0}{k\lambda_{max} - k\lambda_0} = K\Delta E \tag{2}$$

The difficulties in determining λ_0 are connected with finding an appropriate absorbing standard. In our investigations it was possible to determine λ_0 from the absorption spectra of the tissue-proteins breakdown products and to show the constancy of this wavelength for most of the normal and malignant tissues investigated.

METHODS

Material

Various normal and malignant animal tissues were obtained in the following way:

1. *Livers* of albino rats, albino mice, frogs (*R. ridibunda*) and tritons (*Triturus cristatus*) were removed immediately after killing the animals by decapitation. 2. *Skin* from the back of albino mice, frogs and tritons was used. The depilation of the mouse skin was performed with a mixture of wax and colophony. 3. *Muscles* from the thigh

of albino mice, frogs and tritons were used. 4. *Epidermis* from the back of albino mice was separated from the dermis according to our method using subcutaneous injection of 2% ice-cold citric acid[36]. 5. *Malignant tumors*: 15-day-old transplantable Guérin carcinoma, Ehrlich ascites carcinoma and benzpyrene-induced skin carcinoma in mice were investigated. Care was taken to eliminate the necrotic parts of the solid tumors. Immediately after removal the ascites fluid was diluted with 100 vol. of ice-cold Krebs–Ringer solution, then centrifuged. 6. *Actinia equina* from the Black Sea was used *in toto* after 30 days of fasting.

Nucleic acids determination

In some of the tissues (rat liver, mouse skin, Guérin carcinoma) the results obtained for the nucleic acid content, determined by phosphorus analysis, pentose reactions and ultraviolet absorption were compared. It was therefore necessary to treat this material according to the classical procedure as follows.

A

(a) *Preliminary treatment.* 1. The weighed material is homogenized in a glass homogenizer with 10% ice-cold TCA. 2. The homogenate is washed twice with 5% ice-cold TCA and once with 96% alcohol. 3. It is then boiled in a water bath for 3 min with alcohol–ether (3:1) and for 30 min with methylalcohol–ether (1:1). 4. It is washed with ether and dried at 37°.

(b) *RNA-fraction.* 1. The dry residue is incubated for 18 h in *N* KOH at 37° (1 ml KOH to each 100 mg fresh tissue). 2. Conc. PCA (0.1 ml to each ml KOH) is added to the ice-cold hydrolysate. 3. The precipitate is centrifuged and washed twice with ice-cold *N* PCA. 4. Supernatant and washings are mixed together and made up to a given volume with *N* PCA.

(c) *DNA-fraction.* The precipitate from (b) is extracted twice according to Ogur–Rosen with *N* PCA, each time for 30 min, at 80° and the two extracts are combined.

When the nucleic acid content is determined only by the ultraviolet absorption the careful extraction of lipids is not necessary. It is also preferable to estimate the amount of nucleic acids on dry weight and to have the possibility of storing the material at some steps of the procedure. In this case the following technique (allowing considerable reduction of the quantity of material needed) was used.

B

(a) *Preliminary treatment.* 1. The material is frozen and cut in 25 μ slices with the freezing microtome. 2. The slices are put in ice-cold absolute alcohol and washed once more in absolute alcohol (if necessary they can be stored in the second alcohol). 3. They are washed twice with ether for 30 min and dried at 37° overnight. The dried material can be stored in a dessicator. 4. A weighed quantity of the material is treated twice with ice-cold 0.2 *N* PCA for 15 min each time (the simplest technique is first to add some drops of 0.2 *N* PCA to the dried residue, stirring it with a glass rod). 5. After washing with 96° alcohol and with ether it is dried at 37°.

The following procedure is like Ab and Ac.

Phosphorus determination was carried out according to KUTTNER AND COHEN[37].

The orcinol reaction was performed according to MEYBAUM[38], with a 45-min heating period[39]. The absorbance was determined by means of a Pulfrich photometer at S_{66}. The data were expressed in phosphorus following the relation $P = \frac{E + 0.045}{0.1250}$.

The deoxyribose was determined by the diphenylamine reaction according to DISCHE[40]. The absorbance was measured with a Pulfrich photometer at S_{61} and the P content calculated by the formula $P = E/0.0195$.

The optical density in the ultraviolet was determined with the Zeiss universal spectrophotometer. The nucleic acid content was expressed in mg of phosphorus on the basis of $\varepsilon(P)_{RNA} = 10130$ and $\varepsilon(P)_{DNA} = 8782$.

Tissue breakdown products

The appropriate absorbing standards were obtained from the tissues in the following way:

1. The material is treated as in Aa or Ba 1–3.

2. The nucleic acids and acid-soluble compounds are extracted simultaneously with *N* PCA at 80° six times, for 15 min each. The complete extraction of absorbing material was controlled spectrophotometrically.

3. The protein residue is washed with distilled water, alcohol, ether, and dried at 37°.

4. The dried material is incubated in *N* KOH for 18 h at 37° or is kept for 1 h in a boiling water-bath, the two procedures giving breakdown products with the same spectra, differing only in the quantity of absorbing substances released.

5. The hydrolysate is cooled and the proteins precipitated with conc. PCA as in Ab 2–4. The mixed supernatant and washings are brought to a given volume and used for spectrophotometry.

In all spectrophotometric measurements of RNA fractions and tissue breakdown products, blanks are used of *N* KOH treated with conc. PCA, centrifuged and diluted with *N* PCA to the same volume as the investigated solution. DNA fractions are measured against *N* PCA.

RESULTS AND DISCUSSION

Optical characteristics of the interfering substances

The absorption spectra of the tissue breakdown products are presented on Fig. 1. In spite of the very different origin of the tissues investigated, these spectra differ mainly in the quantity of absorbing material per 100 g of tissue, having the same spectral characteristics—maximum at 275–278 mμ and minimum at 257–260 mμ. For the ΔE method it is necessary to determine the appropriate wavelength λ_0 at which the absorbance is equal to the absorbance at 260 mμ, for the RNA estimation, and at 268 mμ for the DNA estimation. Under the above conditions of alkaline hydrolysis it was found that all investigated tissues of vertebrates (excluding the frog skin) give breakdown products with a $\lambda_0 = 286$ mμ for RNA and $\lambda_0 = 284$ mμ for DNA. In the frog skin the presence of substances with higher absorption at 260 mμ is detected in the tissue hydrolysates. In this material therefore the λ_0 is shifted to 283 mμ for RNA and to 280 mμ for DNA.

The comparison of the absorption curves of the tissue breakdown products with the spectrum of tyrosine (Fig. 1) makes it seem probable that the main absorbing component of the conflicting substances in the Schmidt–Thannhauser alkaline hydrolysates is this aromatic amino acid. The presence of tyrosine and probably tryptophan in these hydrolysates has already been suggested by MAURITZEN *et al.*[10].

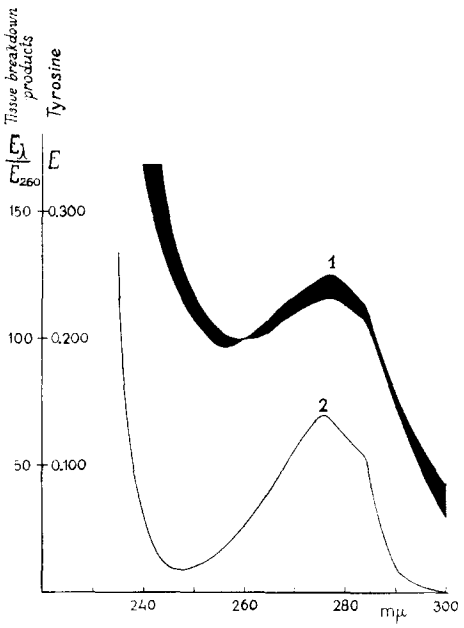

Fig. 1. 1. Absorption spectra of tissue-protein breakdown products from liver (rat, frog, triton), skin (mouse, triton), epidermis (mouse), muscle (mouse, frog, triton) and malignant tumors (Guérin carcinoma, Ehrlich ascites carcinoma and benzpyrene-induced skin carcinoma)—all in normal perchloric acid. Data expressed in percentages of absorbance at 260 mμ. 2. Absorption spectrum of 10^{-4} *M* solution of DL-tyrosine in normal perchloric acid.

The obligatory presence of these amino acids in all animal tissue proteins explains the uniformity of the absorption spectra of the interfering substances released after alkaline hydrolysis of quite different tissues. In this connection some data indicating the similarity of amino acid composition of microsomal nucleoprotein particles should be mentioned[41]. The constancy of λ_0 in different tissues may be explained by taking into account the absorption spectrum of tyrosine as a chief component of the conflicting substances. In *N* PCA the absorptivity of tyrosine at 260 mμ is equal to the absorptivity at 287.5 mμ and the absorptivity at 268 mμ equals the absorptivity at 284.5 mμ. The presence of other substances absorbing to the left of the tyrosine maximum explains the shifting of λ_0 in the different animal tissues to 286 mμ for RNA and to 284 mμ for the DNA.

Elimination of the interfering substances by the two-wavelength method

It is obvious from the above data that the ΔE method is applicable to various animal tissues owing to the identical absorption spectra of their tissue-protein break-

down products. Using equation (2) the quantity of RNA and DNA in mg of phosphorus per 100 g of tissue weight will be:

$$\text{RNA} = K_{\text{RNA}} \frac{(E_{260} - E\lambda_0)\, V}{w.l} \text{ mg per cent P} \tag{3}$$

$$\text{DNA} = K_{\text{DNA}} \frac{(E_{268} - E\lambda_0)\, V}{w.l} \text{ mg per cent P} \tag{4}$$

V being the volume of the extract in ml, w the weight of the investigated material in mg, l the length of the light path in cm. The coefficient K depends on the second wavelength λ_0 as follows

$$K = \frac{M}{\varepsilon(\text{P})} \cdot \frac{r}{(r - 1)} \tag{5}$$

where M is the molecular weight of phosphorus, ε(P) the molecular extinction coefficient of nucleic acid per g-atom of phosphorus, r the ratio of the absorbance of pure nucleic acid at 260 mμ (resp. 268 mμ) to the absorbance at λ_0.

On the basis of the above cited values for λ_0 and ε(P), $r = E_{260}/E_{286}$ being 2.2 for RNA and $r = E_{268}/E_{284}$ being 1.79 for DNA ,the coefficient K_{RNA} is 561 and K_{DNA} is 800. Since λ_0 may be different in some cases, the coefficients should be calculated according to (5). Within certain limits of λ_0 the dependence of K on λ_0 is almost linear and K may be determined with an error smaller than 1% from the equations

$$K_{\text{RNA}} = 9341.2 - 30\,\lambda_0 \text{ (for } \lambda_0 \text{ within the limits 284–289 m}\mu\text{)} \tag{6}$$

$$K_{\text{DNA}} = 15284 - 51\,\lambda_0; \text{ (for } \lambda_0 \text{ within the limits 283–287 m}\mu\text{)} \tag{7}$$

Determination of nucleic acid content by estimation of their various components

In Table I are shown the different values obtained by the determination of the nucleic acids by estimation of their various components. Highest values for RNA are obtained by the orcinol reaction and by ultraviolet photometry when absorption at 260 mμ is used. Although the values obtained in these two ways are in good agreement with most of the data in the literature[6,42,43] they must be considered as being significantly increased on account of the interfering substances. Despite the fact that such substances are known both for the orcinol reaction and for ultraviolet absorption, these two techniques are nevertheless widely employed in nucleic acid determination. The 15 different tissues we have investigated show the ubiquitous presence of u.v.-absorbing material other than nucleic acids, both in the RNA and in the DNA fractions (Fig. 2 and 3). The main difference between the spectra of the pure nucleic acids and the tissue nucleic acids extracts lies in the very strong absorption of the latter at the shorter wavelengths (below 240 mμ) and in their higher absorption at 270–290 mμ. In this respect our results are in good agreement with the recent data of DE DEKEN *et al.*[9].

The estimation of RNA by direct phosphorus analysis gives lower results. Taking into account the established presence of non-nucleotidic phosphorus in the RNA fraction of Schmidt–Thannhauser[2–4], we must consider these values as overrated too.

The RNA content determined by the ΔE method is considerably lower than that estimated by any other procedure. The direct phosphorus analysis gives RNA values 31% (in the rat liver), 22% (in the Guérin carcinoma) and 18% (in the mouse

TABLE I

DETERMINATION OF NUCLEIC ACID CONTENT OBTAINED BY ESTIMATION OF THEIR VARIOUS COMPONENTS

All data are expressed as mg of phosphorus/100 g of fresh tissue.

Tissue	*Number of cases*		*RNA*				*DNA*				*RNA/DNA*			
			ΔE	E_{260}	*Ribose*	*P*	*ΔE*	E_{268}	*Deoxyribose*	*P*	*ΔE*	E_{max}	*Pentose*	*P*
Rat liver	5	M	54.4	98.3	99.1	80.0	29.4	38.1	—	18.5	1.86	2.58	—	4.32
		m	± 1.3	± 2.5	± 4.9	± 3.3	± 1.6	± 1.8		± 0.6				
		per cent	100	181	182	147	100	131		63				
	5	M	51.6	92.2	87.4	74.1	22.0	30.5	20.6	14.0	2.37	3.02	4.24	5.29
		m	± 1.5	± 2.1	± 2.1	± 1.9	± 1.0	± 1.8	± 1.3	± 2.0				
		per cent	100	179	169	143	100	139	94	64				
Mouse skin	5	M	19.2	39.2	38.4	—	27.1	38.5	27.5	—	0.71	1.02	1.40	—
		m	± 1.5	± 1.8	± 2.5		± 2.4	± 3.4	± 1.9					
		per cent	100	204	200		100	142	101					
	5	M	17.1	34.4	33.8	21.8	24.1	31.1	26.0	22.7	0.71	1.10	1.03	0.96
		m	± 1.3	± 2.1	± 2.2	± 1.5	± 1.3	± 1.4	± 1.5	± 1.7				
		per cent	100	201	188	127	100	129	108	94				
Guérin carcinoma	1		51.9	82.7	90.6	63.6	88.2	108.5	91.7	80.3	0.59	0.76	1.14	0.79
		per cent	100	159	175	123	100	123	104	91				

M – mean.
m – standard error of the mean.

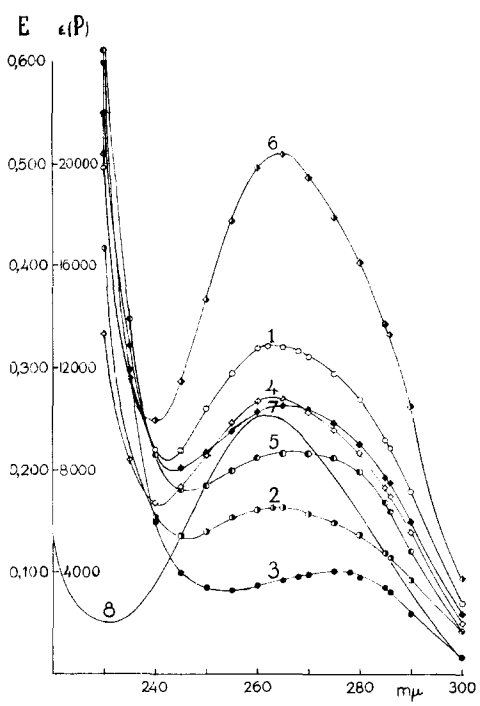

Fig. 2. Absorption spectra of RNA fractions obtained by the Schmidt–Thannhauser procedure and of pure yeast RNA alkaline hydrolysate—all in normal perchloric acid. E = optical density per 100 mg of fresh tissue (1, rat liver; 2, mouse skin; 3, mouse muscle; 4, Guérin carcinoma) or per 20 mg of dry de-lipidated tissue (5, epidermis of mouse skin; 6, Ehrlich ascites carcinoma; 7, benzpyrene-induced skin carcinoma). 8, ε(P) = molar extinction coefficient per g-atom of phosphorus of yeast RNA.

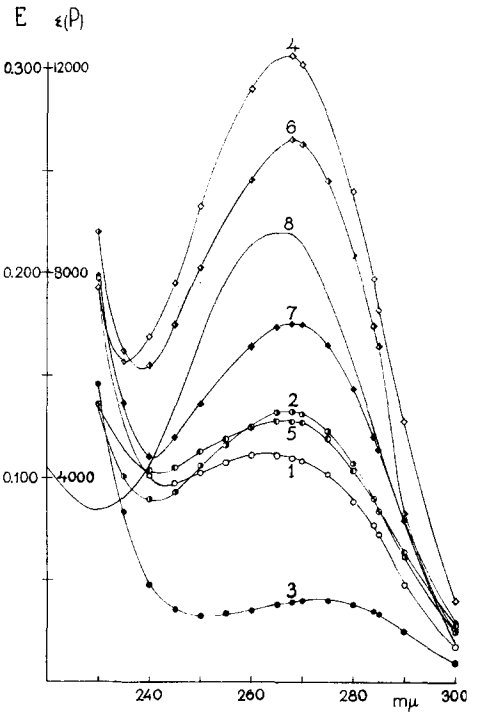

Fig. 3. Absorption spectra of DNA fractions obtained by hot perchloric acid extraction, compared with pure DNA treated in the same way. 1–7, as in Fig. 2; 8, DNA.

skin) higher than those obtained by the ΔE method. That is in good agreement with the data of DAVIDSON AND SMELLIE[2], who reported 25% non-nucleotidic phosphorus in the RNA fraction.

As far as DNA is concerned, the ΔE values are almost identical with the values obtained by the diphenylamine reaction. Taking into account the low sensitivity of the diphenylamine test, it is possible that the difference of 5–10% between the two methods is due to errors in the deoxyribose estimation. In this connection it is necessary to mention the note of DE DEKEN *et al.*[9] pointing out that lower results are obtained with the diphenylamine reaction when the DNA is extracted at a higher temperature and that a reduction of the deoxyribose content of DNA hydrolysate takes place when stored at room temperature.

It is worth noting that the direct phosphorus analysis of the DNA fraction always gives lower results for the DNA content than the values obtained by the ΔE method or by the diphenylamine reaction. The difference is especially great in rat liver where the direct analysis of the phosphorus leads to values 36%, and 32% lower than the ΔE and the diphenylamine results, respectively. This difference between the P analysis and the diphenylamine test is in agreement with the data

TABLE II

RIBONUCLEIC ACID CONTENT OF THE LIVER ESTIMATED BY VARIOUS METHODS

Method	*RNA*	*RNA-P mg per 100 g of fresh tissue**	*Animal*	*Author*
Classical procedures of Schmidt–Thannhauser and Schneider	—	77–100 93	Rat Mouse	LESLIE[42], TSHEPINOGA[43] and BADOLATO *et al.*[6]
Ogur–Rosen	— —	57 74	Rat Rat	BADOLATO *et al.*[6] HADJIOLOV[44]
Milder alkaline hydrolysis	556**	50	Rat	SCOTT *et al.*[11]
Preliminary precipitation	—	58	Rat	MANOÏLOV AND ORLOV[22]
Paper chromatography	2.98***	64	Mouse	KLEINSCHMIDT AND MANTHEY[13]
Adsorption on Dowex-2	2.63***	57	Rat	DE DEKEN-GRENSON AND DE DEKEN[9]
ΔE method		53 67	Rat Mouse	TSANEV AND MARKOV (this paper)

* These data when not given by the authors are calculated on the basis of 9% of phosphorus in the RNA and 24% of dry de-lipidated weight of the liver.
** mg of RNA/100 g of fresh tissue.
*** mg of RNA/100 mg of dry de-lipidated tissue.

of BADOLATO *et al.*[6], who also report that the values for DNA obtained by the phosphorus determination are 17–31% lower than those obtained by the diphenylamine reaction. It has already been pointed out that the Ogur–Rosen method, avoiding the alkaline hydrolysis, gives significantly higher results for DNA than the Schmidt–Thannhauser procedure[6,44,45]. It would seem that this discrepancy is mainly due to incomplete extraction of RNA by the cold *N* PCA. It should be noted, however, that some authors admit an incomplete precipitation of DNA in the Schmidt–Thannhauser method[9,10,46,47]. KLEINSCHMIDT AND MANTHEY[13], using a chromatographic separation of nucleic acid compounds prior to their spectrophotometric determination, report, however, that a single hydrolysis of the tissue with *N* KOH does not result in any loss of DNA. BADOLATO *et al.*[6] report also that they never found diphenylamine-positive material in the RNA fraction. Our results, as well as the results of BADOLATO *et al.*[6] (higher values by the u.v. absorption and the diphenylamine test than by phosphorus analysis) suggest a partial dephosphorylation of DNA during the alkaline hydrolysis in which part of the DNA phosphorus is transferred to the RNA fraction. It is necessary to point out that the excess of phosphorus in the RNA fraction is always greater or equal to the missing phosphorus in the DNA fraction.

It would be of use to compare the values we have obtained for the nucleic acid content in rat liver with the numerous literature data concerning the nucleic acids in this organ. Most authors who have investigated the nucleic acids in rat and mouse liver by the Schmidt–Thannhauser procedure report data within 77–100 mg of P for RNA and within 16–26 mg of P for DNA per 100 g of fresh tissue[6,42,43]. It is obvious that these values can be considered as higher for RNA and slightly lower for DNA than the real ones. Therefore, according to these data, the accepted

RNA/DNA ratio of 4–5 is in fact greatly overrated. The values obtained by the ΔE method are in fairly good agreement with some recent data of authors who have used procedures involving purification of the RNA fraction (Table II). As can be seen, five different procedures, eliminating the interfering substances in quite different ways, give very similar results, which are considerably lower than those obtained by the classical methods.

The DNA content of the rat liver estimated by the ΔE method is 25.7 mg of P per 100 g of fresh tissue, coinciding with the highest values cited in the literature, whereas the direct phosphorus analysis is in agreement with the lowest data reported in the literature.

A comparison between the estimation of the nucleic acid content in different tissues by ultraviolet photometry at the absorption maximum and by the two wavelength-method is given in Table III; the differences are especially striking in the muscles. In every case only a certain part of the optical density at the absorption maximum is due to nucleic acids. Excluding the muscles, the part due to RNA in different tissues is about 50–60%, whereas in the DNA extracts the absorbance due to DNA is about 75%.

All results of the above-cited authors as well as our own indicate that the values given in the literature for the RNA content in various tissues determined by the

TABLE III

NUCLEIC ACID ANALYSIS IN VARIOUS TISSUES BY ULTRAVIOLET PHOTOMETRY AT THE ABSORPTION MAXIMUM AND BY THE ΔE METHOD

All data are expressed as mg of phosphorus/100 g of fresh tissue or of dry de-lipidated tissue*.

Material	*Number of cases*	*RNA*			*DNA*			*RNA/DNA*	
		E_{260}	ΔE	$\Delta E/E_{260}$	E_{258}	ΔE	$\Delta E/E_{258}$	E_{max}	ΔE
Liver									
1. Rat	10	95.3	53.3	0.56	34.3	25.7	0.75	2.80	2.12
2. Frog	1	69.2	41.5	0.60	84.8	63.5	0.75	0.82	0.65
3. Triton	2	42.1	23.6	0.56	80.9	58.4	0.72	0.52	0.41
Skin									
4. Mouse	10	169.0*	84.0*	0.50	198.0*	142.0*	0.72	0.85	0.59
	10	36.8	18.1	0.49	34.8	25.6	0.74	1.06	0.71
5. Frog	2	31.0	18.3	0.59	24.6	13.0	0.53	1.26	1.41
6. Triton	2	51.3	30.5	0.60	59.1	42.2	0.72	0.87	0.74
Epidermis									
7. Mouse	5	351.0*	179.0*	0.51	241.8*	169.4*	0.70	1.45	1.05
Muscle									
8. Mouse	4	130.9*	29.2*	0.22	56.3*	20.8*	0.37	2.47	1.40
9. Frog	2	17.8	3.4	0.20	11.4	1.6	0.15	1.55	2.17
10. Triton	3	19.7	2.9	0.15	21.3	10.1	0.47	0.92	0.29
Malignant tumors									
11. Guérin carcinoma	1	82.7	51.9	0.63	108.5	88.2	0.81	0.76	0.59
12. Ehrlich ascites carcinoma	5	781.6*	491.3*	0.63	473.2*	344.9*	0.73	1.65	1.42
13. Benzpyrene-induced skin carcinoma	7	401.0*	211.0*	0.53	299.2*	213.9*	0.72	1.37	0.99
Actinia equina									
14. Total body	2	341.0*	190.0*	0.56	154.4*	91.6*	0.59	2.21	2.07

usually accepted Schmidt–Thannhauser technique are considerably higher than the real ones. It should be considered as obligatory in nucleic acid determinations to utilize only procedures that eliminate the interfering substances. In this respect the two-wavelengths method has all the advantages of the spectrophotometric methods, while avoiding at the same time the laborious purifying procedures.

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