MOUSE LIVER NUCLEIC ACIDS

II. ULTRA-VIOLET ABSORPTION STUDIES*

by

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The utilization of ultra-violet absorption methods for the study of nucleic acids in relation to biological problems has become especially important in view of the ingenious applications described by CASPERSSON[1]. The characteristic absorption of ultra-violet light by nucleic acids has been known for many years and has been attributed to their purine and pyrimidine constituents[2]. Although many investigators have studied the absorption characteristics of a number of the nitrogenous constituents of the nucleic acids[3, 4, 5], less literature appears to be available concerning quantitative relationships between nucleic acids and their absorption of ultra-violet light.

Previous investigations utilizing acid, alkaline, and enzymatic hydrolysis on samples of yeast ribose and calf thymus desoxyribose nucleic acids, disclosed increases in absorption of ultra-violet light by both nucleic acids following each of the hydrolytic procedures[6]. It was concluded from these studies that the hydrolytic state as well as the specific purine and pyrimidine content must be considered in the interpretation of ultra-violet absorption characteristics of nucleic acids.

The ultra-violet absorption characteristics of mouse liver nucleic acids following various conditions of hydrolysis, and the utilization of spectrophotometric techniques for the quantitative estimation of these substances were investigated.

EXPERIMENTAL

Highly purified preparations of mouse liver pentose and desoxypentose nucleic acids were utilized for the spectrophotometric studies[7]. All ultra-violet absorption measurements were made with the model DU Beckman spectrophotometer using standard 1 cm light path silica cells.

1. *The effect of hydrolysis on the ultra-violet absorption characteristics of mouse liver desoxypentose nucleic acid*

The liver desoxypentose nucleic acid samples were prepared by gentle extraction and purification procedures[7]. Solutions of the sodium salt were found to be viscous at concentrations of less than 1 mg per ml, suggesting a high degree of polymerization[8].

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From previous studies, molecular size appeared to affect the ultra-violet absorption characteristic of nucleic acids. By employing LEVENE's extraction and purification procedures[9], known to yield degraded desoxyribose nucleic acid preparations from calf thymus glands, higher extinction values, $E^{1\%}_{1\,cm}$, were noted in these samples as compared to other samples of desoxyribose nucleic acid isolated from the same source by gentle procedures[7]. The effect of acid, alkaline, and enzymatic hydrolysis on the ultra-violet absorption characteristics of mouse liver desoxypentose nucleic acid was studied by the following methods: From a stock solution containing 1.72 mg sodium desoxypentose nucleate per ml, four 1.00 ml aliquots were removed and placed into 50 ml volumetric flasks. To one flask, $M/10$ phosphate buffer (pH 7.0) was added to volume. This served as the non-hydrolyzed control. To the second flask, 1 ml 10% trichloroacetic acid was added. The flask and contents were heated at 90° for 20 minutes in a water bath, neutralized with sodium hydroxide, and made to volume with $M/10$ phosphate buffer, pH 7.0. To the third flask was added 1 ml 1 N sodium hydroxide. The flask and contents were heated at 95° for 30 minutes in a water bath, neutralized with hydrochloric acid, and made to volume with $M/10$ phosphate buffer, pH 7.0. To the last flask was added 1 ml of a solution containing 0.1 mg desoxyribonuclease (Worthington Biochemical Co.) and 1 ml of 0.01 M magnesium chloride. The flask and contents were incubated at 37.0° for 15 hours. Following the incubation, the flasks were made to volume with $M/10$ phosphate buffer, pH 7.0. Appropriate blank solutions were prepared by following identical procedures, but substituting distilled water for the sodium nucleate in each case.

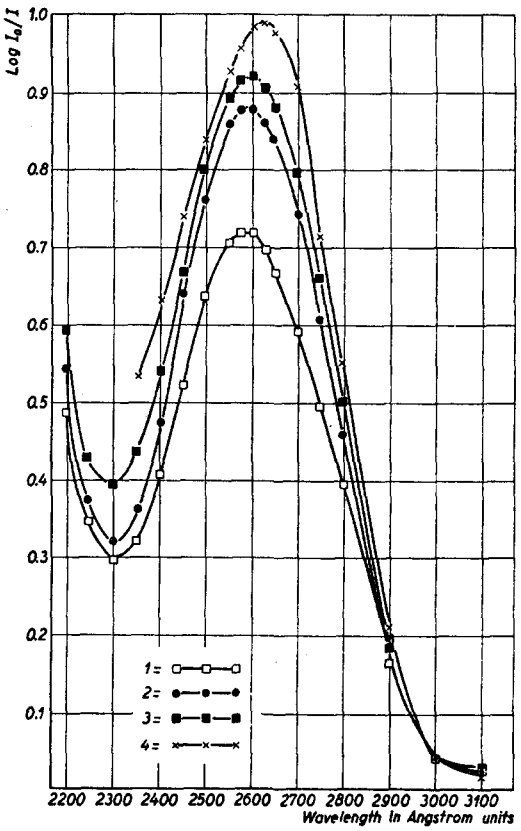

Fig. 1. A comparison of the ultra-violet absorption curves of non-hydrolyzed (curve 1: □—□—□), alkaline hydrolyzed (curve 2: •—•—•), enzymatically hydrolyzed (curve 3: ■—■—■), and acid hydrolyzed (curve 4: ×—×—×) samples of sodium desoxypentose nucleate. Each sample contained 34.4 μg sodium desoxypentose nucleate per ml. All samples were neutralized and buffered at pH 7.0 prior to the absorption measurements.

The prepared solutions were read against their respective blanks in the spectrophotometer. The results of this study are presented in Fig. 1. By each method of hydrolysis employed, an increase in absorption of ultra-violet light over the non-hydrolyzed control sample was noted.

The trichloroacetic acid hydrolyzed sample showed a 34% increase in absorption of ultra-violet light as compared to the non-hydrolyzed control. Also, a slight shift in the absorption maximum toward the longer wave-lengths was observed. Dilute acid hydrolysis in general on all desoxypentose nucleic acid samples investigated resulted in an increased absorp-

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tion and a shift in the absorption maximum toward the longer wave-lengths. The trichloroacetic acid hydrolysis procedure was found to liberate completely the purines to their free forms from the nucleic acid macromolecule. Previous experiments using commercial preparations of guanylic and adenylic acids disclosed a reduction in absorption of ultra-violet light by these compounds following hydrolysis with dilute acids[6].

The desoxypentose nucleate showed a significant increase in absorption following alkaline hydrolysis. Lower molecular weights have been reported for samples of desoxypentose nucleic acid subjected to alkaline hydrolysis[10]. The lability of desoxypentose nucleic acid towards alkali therefore appears to be well substantiated.

The increase in absorption of ultra-violet light following enzymatic hydrolysis of the nucleate was less than the increase following acid hydrolysis. Only partial degradation of the nucleate by desoxyribonuclease was found. About 45% of the total nucleate phosphorus was rendered dialyzable through prepared collodion membranes by the action of enzyme. The apparent inability of desoxyribonuclease to hydrolyze desoxyribose nucleic acid completely has been previously reported by other investigators[11].

2. The effect of hydrolysis on the ultra-violet absorption characteristic of mouse liver pentose nucleic acid

Experiments comparable to those previously described for the desoxypentose nucleate were devised to study the effect of hydrolysis on the ultra-violet absorption characteristics of mouse liver pentose nucleate.

From a prepared stock solution of sodium pentose nucleate containing 1.32 mg nucleate per ml, four 1.00 ml aliquots were removed and placed into separately labelled 50 ml volumetric flasks. To one flask, $M/10$ phosphate buffer (pH 7.0) was added to volume and served as the non-hydrolyzed control sample. To the second flask was added 1 ml 10% trichloroacetic acid. The flask and contents were heated for 20 minutes at 90°. The solution was neutralized with sodium hydroxide and made to volume with $M/10$ phosphate buffer, pH 7.0. To the third flask, 1 ml 1 N sodium hydroxide was added. The flask and contents were incubated at 37° for 15 hours. Following which the solution was neutralized with hydrochloric acid and made to volume with $M/10$ phosphate buffer, pH 7.0. To the last flask was added 1 ml of a solution containing 0.05 mg crystalline ribonucleinase (Armour Co.). The flask and contents were incubated at 37° for 15 hours, then diluted to volume with $M/10$ phosphate buffer, pH 7.0. Appropriate blank solutions were prepared by following identical procedures, but substituting distilled water for the sodium nucleate in each case.

Absorption curves were determined for each sample (Fig. 2). Alkaline hydrolysis of the pentose nucleate was found to result in the greatest increase in absorption. Uranyl-trichloroacetic acid reagent when added to such hydrolyzed preparations failed to yield a precipitate, suggesting that hydrolysis was complete[12]. A shift in the absorption maximum toward the shorter wave-lengths was noted. At 2600 Å wave-length, a 32% increase in absorption of ultra-violet light by the sample hydrolyzed by alkali was found over the non-hydrolyzed control sample.

The aliquot of sodium pentose nucleate hydrolyzed with trichloroacetic acid showed a significant increase in absorption. Only 25% of the total purines of the pentose nucleate were hydrolyzed to their free form. Rather than a shift toward the longer wave-lengths in the absorption maximum as found for the desoxypentose nucleate, a slight shift

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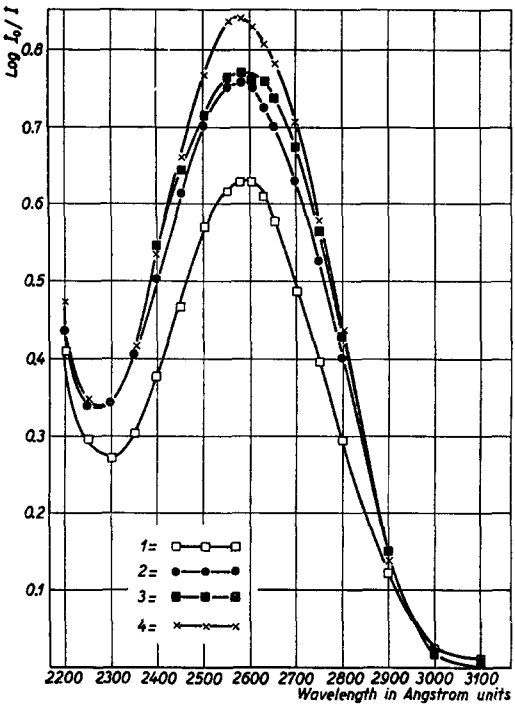

Fig. 2. A comparison of the ultra-violet absorption curves of non-hydrolyzed (curve 1: □—□—□), enzymatically (ribonucleinase) hydrolyzed (curve 2: •—•—•), acid hydrolyzed (curve 3: ■—■—■), and alkaline hydrolyzed (curve 4: ×—×—×) samples of sodium pentose nucleate. Each sample contained 26.4 μg sodium pentose nucleate per ml. All samples were neutralized and buffered at pH 7.0 prior to the absorption measurements.

toward the shorter wave-length was noted. The significance of these shifts remains to be determined.

Enzymatic hydrolysis of the pentose nucleate, resulted in an increased absorption, found to be less than that observed following alkaline hydrolysis. This finding might be correlated with the inability of the enzyme ribonuclease, to hydrolyze this and other pentose nucleic acids completely[7, 13]. A degree of correlation appears to exist between the absorption increases noted and the extent of nucleate degradation. A slight shift in the absorption maximum toward the shorter wave-lengths was noted following the enzymatic hydrolysis. A similar shift was described previously by KUNITZ on samples of yeast ribose nucleic acid treated with ribonucleinase[14]; however, increases in overall absorption of ultra-violet light to the extent observed for the present sample of mouse liver pentose nucleate apparently were not observed.

3. *Quantitative aspects of ultra-violet spectrophotometric methods for the estimation of nucleic acids*

From the experiments previously described, it is apparent that the absorption of ultra-violet light by nucleic acids is dependent not only upon the purine and pyrimidine content, but also is influenced markedly by hydrolyzable chemical linkages present within the nucleic acid macrostructure. To study further the relationship between the state of degradation of nucleic acids and their extinction coefficient, additional experiments were performed on several preparations of yeast ribose nucleic acid.

Four separate commercial samples of yeast ribose nucleic acid were utilized for this study. Each sample was dissolved in water in the presence of an excess of sodium acetate, neutralized to pH 7.0 with HCl, then shaken a number of times with chloroform to remove possible protein contaminants[15]. The solutions were finally dialyzed in cellophane tubing against frequent changes of distilled water for a period of 72 hours in a cold box at 2–5°. Aliquots were removed from the prepared solutions for phosphorus determinations[16] and for the ultra-violet absorption studies. Extinction values were determined for each of the samples at 2575 Å wavelength prior to and following trichloroacetic acid and alkaline hydrolysis. The hydrolytic procedures were identical to those previously described for the liver pentose nucleate. The wave-length selected was found to be near the point of maximum absorption in each case.

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The results are summarized in Table I. Comparable increases in extinction values were found for the four samples of yeast ribose nucleic acids following each hydrolytic procedure. The extinction values for the non-hydrolyzed control samples were similar, suggesting the influence of the dialysis in grading the various samples to nearly comparable molecular sizes.

TABLE I

A COMPARISON OF EXTINCTION VALUES FOR YEAST RIBOSE NUCLEIC ACIDS PRIOR TO AND FOLLOWING HYDROLYSIS

Commercial source of nucleic acid sample	μg Nucleic acid phosph. per ml	Before Hydrolysis		Following Trichloroacetic acid hydrolysis		Following Sodium Hydroxide Hydrolysis	
		Density (Log I_o/I) 2575 Å	$E^{1\%\,P*}_{1\,cm}$ 2575 Å	Density (Log I_o/I) 2575 Å	$E^{1\%\,P*}_{1\,cm}$ 2575 Å	Density (Log I^o/I) 2575 Å	$E^{1\%\,P*}_{1\,cm}$ 2575 Å
1. Pfanstiehl Co.	1.91	0.564	2960	0.654	3430	0.680	3560
2. Schwartz Co.	1.94	0.572	2960	0.644	3330	0.696	3580
3. Fischer Co. Lot W 452334	1.88	0.544	2900	0.614	3270	0.652	3480
4. Fischer Co. Lot W 481974	2.20	0.662	3010	0.728	3300	0.788	3580

* $E^{1\%\,P}_{1\,cm}$ represents the calculated extinction for a 1% concentration of nucleic acid phosphorus and a 1 cm thick absorbing layer.

Subsequent investigations were directed toward a study of suitable quantitative relationships for the accurate estimation of mouse liver nucleic acids by ultra-violet absorption methods. Procedures have been described in the literature for the quantitative extraction of nucleic acids from small tissue samples using hot trichloroacetic acid[17]. It was considered desirable to establish relevant extinction values for the quantitative estimation of nucleic acids extracted in this manner by ultra-violet absorption measurements. Three separate samples of mouse liver pentose nucleic acid and two separate samples of mouse liver desoxypentose nucleic acid were prepared in the form of their soluble sodium salts by previously described procedures[7]. Measured aliquots were removed from the prepared stock nucleic acid solutions for phosphorus analysis[16] and for the spectrophotometric study. Nucleic acid concentrations were computed from the phosphorus analysis. Previous findings[7] disclosed the phosphorus content of mouse liver pentose and desoxypentose nucleic acids to be 9.48 and 10.0% respectively. Extinction values were determined for each of the samples at 2600 Å wave-length following their hydrolysis with 5% trichloroacetic acid at 90° for 20 minutes. Extinction values were also determined on non-hydrolyzed and alkaline hydrolyzed aliquots of each of the nucleic acid preparations for purposes of comparison. The alkaline hydrolysis procedures were identical to those previously described. Blank solutions were treated in precisely the same manner as the samples. The results of this study are summarized in Table II. Good agreement was found in extinction values between the three isolated pentose nucleic acids, as well as between the two desoxypentose nucleic acid samples. The desoxypentose nucleic acid samples were found to show much lower extinction values than the pentose nucleic acid prior to their hydrolysis.

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TABLE II

A COMPARISON OF EXTINCTION VALUES DETERMINED AT 2600 Å WAVE-LENGTH FOR NUCLEIC ACIDS ISOLATED FROM MOUSE LIVER PRIOR TO AND FOLLOWING ACID AND ALKALINE HYDROLYSIS

Nucleic Acid Sample	Non-Hydrolyzed Control		Following Trichloroacetic Acid Hydrolysis		Following Sodium Hydroxide Hydrolysis	
	$E^{1\%P}_{1cm}$*	$E^{1\%}_{1cm}$**	$E^{1\%P}_{1cm}$	$E^{1\%}_{1cm}$	$E^{1\%P}_{1cm}$	$E^{1\%}_{1cm}$
PNA (1)	2650	254	3210	309	3500	337
(2)	2640	255	3240	313	3440	332
(3)	2640	255	3200	309	3480	336
DNA (1)	2230	224	3050	307	2720	274
(2)	2220	223	3080	310	2720	274

* The calculated extinction value for a 1% concentration of nucleic acid phosphorus and a 1 cm light path at 2600 Å wave-length.

** The calculated extinction value for a 1% concentration of nucleic acid and a 1 cm light path at 2600 Å wave-length.

4. *Quantitative estimation of mouse liver pentose and desoxypentose nucleic acids*

Experiments were performed to test the applicability of ultra-violet absorption methods for the quantitative estimation of nucleic acids in liver tissue.

Ten male mice of 3 months age after 18 hours fasting were anaesthetized and their livers removed. Each liver immediately following excision was sponged free from excess blood and accurately weighed to the nearest mg. The weighed liver was ground in an all-glass homogenizer (Scientific Glass Co.) in a precisely measured volume of cold distilled water such that 9 ml of water was added for each gram of tissue.

Equivalent aliquots were removed from each prepared homogenate for extraction and estimation of nucleic acids. Following the removal of acid soluble constituents with cold 5% trichloroacetic acid, the tissues were extracted twice with cold 95% ethyl alcohol and three times with hot alcohol-ether (3 : 1) to remove lipids. The tissues were next extracted with 5% trichloroacetic acid for a period of 20 minutes at 90° to remove the nucleic acids. The extracted nucleic acids were made to known volumes and aliquots were removed for analysis.

The analyses consisted of estimations for pentose, desoxypentose, phosphorus, and also quantitative measurements for total nucleic acids by ultra-violet absorption methods. From the estimations for pentose and desoxypentose, the respective amounts of nucleic acid present were computed on the basis of previous chemical analyses on highly purified preparations of both types of nucleic acids[7].

The pentose was estimated by the orcinol reaction with suitable corrections[18]. The desoxypentose was estimated by the diphenylamine reaction[19]. Total nucleic acid phosphorus was determined by the method of FISKE AND SUBBAROW[16]. Total nucleic acid was estimated by ultra-violet absorption measurements using the constant, $E^{1\%}_{1cm}$ equal to 310 at 2600 Å wave-length (see Table II).

The results of the chemical and spectrophotometric analyses for nucleic acids of normal mouse liver are presented in Table III. The results of these analyses indicated a fairly good general agreement between the various measurements. Individual variations in the nucleic acid content of mouse liver appears to be most pronounced with respect to the desoxypentose nucleic acids. Considering ratios of pentose to desoxypentose

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nucleic acids for the samples of mouse liver investigated, an extreme variation in this ratio among the animals was found to exist.

TABLE III

THE QUANTITATIVE ESTIMATION OF NUCLEIC ACIDS IN NORMAL MOUSE LIVER BY CHEMICAL AND ULTRA-VIOLET ABSORPTION TECHNIQUES

Mouse Number	Milligrams per gram of wet weight liver					
	(1) Pentose Nucleic Acid (PNA)	(2) Desoxy-pentose Nucleic Acid (DNA)	(3) Pentose plus Desoxy-pentose Nucleic Acid	(4) Total Nucleic Acid by U.V. Absorption	(5) Total Nucleic Acid Phosph. Found	(6) Calculated PNA Phosph. + DNA Phosph.
1	9.11	3.12	12.2	12.0	1.25	1.19
2	7.68	3.17	10.9	11.0	1.10	1.06
3	9.37	3.30	12.7	11.9	1.22	1.23
4	7.74	4.08	11.8	12.0	1.15	1.15
5	7.88	3.84	11.7	12.1	1.12	1.15
6	8.64	3.25	11.9	12.3	1.13	1.16
7	8.09	2.67	10.8	11.2	1.03	1.05
8	7.34	2.65	9.99	10.3	0.950	1.02
9	8.80	2.80	11.6	12.1	1.16	1.19
10	8.84	3.30	11.7	11.2	1.11	1.15

All calculations were based on chemical compositions previously found for highly purified preparations of mouse liver pentose and desoxypentose nucleic acids.

(1) The pentose nucleic acid was estimated from pentose determinations.
(2) The desoxypentose nucleic acid was estimated from desoxypentose determinations.
(3) Column 1 plus column 2.
(4) Total nucleic acid estimated by ultra-violet absorption techniques.
(5) Total nucleic acid phosphorus, experimentally determined.
(6) Total nucleic acid phosphorus, calculated from nucleic acid values reported in columns 1 and 2.

DISCUSSION

A reliable quantitative measure of nucleic acids by ultra-violet absorption techniques can be assured only when appropriate extinction coefficients are realized for the particular sample being investigated. Samples of nucleic acid isolated by gentle extraction and purification methods showed extremely low extinction values as compared to similar samples isolated by procedures known to cause considerable degradation. From such observations, one wonders what the extinction values for these same nucleic acids might be prior to their extraction from the tissue.

The absorption of ultra-violet light by nucleic acids has been attributed to their purine and pyrimidine constituents. The nature of the chemical alterations following acid, alkaline, and enzymatic hydrolysis of nucleic acids leading to a marked increase in the capacity of the nitrogenous ring structures to absorb ultra-violet light remains to be determined. Of possible significance in this respect is the variety of hydrolytic agents capable of inducing these alterations. Also, increases in the capacity of the purine and pyrimidine components to absorb ultra-violet light appeared to parallel the extent of degradation of the nucleic acid sample. From these observations it must be concluded that nucleic acids contain labile linkages which on rupture (presumably by chemical

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and enzymatic hydrolysis) increase the absorption capacity of one or more of the nitrogenous ring structures.

SUMMARY

1. Alterations in the ultra-violet absorption characteristics of mouse liver pentose and desoxypentose nucleic acids following treatment with acid, alkali, and specific enzyme were demonstrated.

2. Four separate yeast ribose nucleic acid samples showed a consistent increase in absorption by each preparation following acid and alkaline hydrolysis.

3. Extinction values were determined for mouse liver pentose and desoxypentose nucleic acids following specific hydrolytic procedures for purposes of establishing constants for the estimation of these substances by ultra-violet spectrophotometric methods.

4. Investigations dealing with the estimation of nucleic acids in normal mouse liver were presented using spectrophotometric procedures in conjunction with chemical analyses for phosphorus, pentose, and desoxypentose.

RÉSUMÉ

1. Nous avons démontré des changements de l'absorption charactéristique dans l'ultraviolet des acides pentose- et désoxypentose-nucléiques de foie de souris, après traitement par l'acide, par l'alcali ou par un enzyme spécifique.

2. Quatre échantillons différents d'acide ribonucléique de levure ont montré un même accroissement de l'absorption après hydrolyse acide ou alcaline.

3. Nous avons déterminé les valeurs de l'extinction pour les acides pentose- et désoxypentose-nucléiques du foie de souris après hydrolyse par des procédés spécifiques, afin d'établir des constantes en vue de l'évalution de ces substances par des méthodes spectrophotométriques.

4. Nous avons cherché à évaluer la quantité d'acide nucléique dans le foie normal de souris en combinant des procédés spectrophotométriques avec les déterminations chimiques du phosphore, des pentoses et des désoxypentoses.

ZUSAMMENFASSUNG

1. Veränderungen der charakteristischen Ultraviolet-Absorption der Pentose- und Desoxypentose-Nukleinsäuren aus Mäuseleber, nach Behandlung mit Säure, Alkali und spezifischem Enzym, wurden nachgewiesen.

2. Vier verschiedene Proben von Hefe-Ribonukleinsäure zeigten nach saurer und alkalischer Hydrolyse eine regelmässige Zunahme der Absorption.

3. Die Werte der Extinktion wurden für Pentose- und Desoxypentose-Nukleinsäuren aus Mäuseleber nach spezifischer Hydrolyse bestimmt um die Konstanten für eine ultravioletspektrophotometrische Bestimmungsmethode dieser Substanzen zu ermitteln.

4. Die Bestimmung der Nukleinsäuren in normaler Mäuseleber durch eine Kombination von spektrophotometrischen Arbeitsweisen mit chemischen Phosphor-, Pentose- und Desoxypentoseanalysen wurde untersucht.

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