MOUSE LIVER NUCLEIC ACIDS

I. ISOLATION AND CHEMICAL CHARACTERIZATION*

by

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Prior to an investigation of nucleic acids of mouse liver in relation to normal and malignant growth, it was considered desirable to first isolate and characterize these substances. Detailed chemical analyses of mouse liver nucleic acids appear to be lacking in the literature. LEVENE's[1] concept of the presence of equimolar proportions of nucleotides within ribose nucleic acid isolated from yeast and desoxyribose nucleic acid isolated from calf thymus has been seriously questioned[2, 3, 4, 5], and is found to be an incorrect representation of the structure of mouse liver nucleic acids.

EXPERIMENTAL

Isolation of Mouse Liver Nucleic Acids

Although numerous methods have been described in the literature for the isolation of nucleic acids[6, 7, 8], each appears to have its specific disadvantages for a satisfactory isolation of the two types of nucleic acids from the same tissue source. Our method consisted in a preliminary separation by centrifugation into nuclear and cytoplasmic fractions, followed by extraction with neutral sodium chloride. All steps throughout the entire isolation and purification procedure were accomplished at or near neutral p_H with extraction times being maintained at a minimum. In this manner it was hoped to avoid as far as possible chemical alterations due to adverse p_H or enzymatic degradation.

Two hundred male mice 4 months of age were fasted for 18 hours to reduce liver glycogen. The excised bled livers were pooled in convenient numbers, usually 20, and ground in an all glass homogenizer (Scientific Glass Co.) with 4 volumes of cold 0.85% sodium chloride. The desoxypentose nucleoproteins have been considered to be relatively insoluble in this range of salt concentration[9]. The homogenized tissue containing free nuclear and cytoplasmic constituents was spun in an angle centrifuge at 1,500 × g for 4 minutes. This procedure results in a separation of the homogenate into nuclear and cytoplasmic fractions[10, 11]. The sedimented nuclear fraction is usually contaminated with some cytoplasmic constituents (chiefly agglutinated mitochondria), a relatively few intact liver cells, and some red blood cells. However, by microscopic examination, the supernatant fluid containing the cytoplasmic constituents is practically free of nuclei. All manipulations to this point were carried out as close to 0° as possible, in order to minimize enzymatic degradation of the nucleic acids.

Isolation of Pentose Nucleic Acid: To isolate the pentose nucleic acid from the cytoplasmic fraction, solid sodium chloride was added to a final concentration of 10%. The resulting suspension was boiled for 30 minutes to extract the pentose nucleic acid[12]. The hot mixture was filtered with

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the aid of a Buechner funnel. The insoluble residue was washed with small amounts of 10% sodium chloride. The resulting clear, yellow filtrate was poured into two volumes of cold 95% ethyl alcohol from which the pentose nucleic acid settled readily as a white flocculent mass. After centrifugation and repeated washings with large volumes of 95% ethyl alcohol, the nucleic acid was dissolved in a minimum volume of distilled water yielding a clear colorless solution. The solution was shaken a number of times with chloroform to remove traces of remaining protein contaminants[13]. The pentose nucleic acid was further purified by dialyzing at 2–5° in cellophane tubing against a large volume of distilled water, which was frequently changed over a period of 72 hours. The final yield of pentose nucleic acid (as the sodium salt) was 640 mg or about 40% of the original estimated total.

Isolation of Desoxypentose Nucleic Acid: The sedimented nuclear fraction was treated at once with enough saturated sodium chloride solution[7, 14], to restore the original volume and was agitated for several minutes in a Waring blender to insure thorough extraction. Centrifugation in 250 ml cups at 0° resulted in the separation of an insoluble residue of protein at the bottom and a flocculent insoluble residue floating at the top of the cup. The intermediate clear, reddish, liquid phase containing the extracted desoxypentose nucleic acid and some contaminating hemoglobin was removed. The insoluble residue was re-extracted with saturated sodium chloride in the Waring blender and centrifuged. Again an insoluble residue formed at the top and the bottom of the cups. The intermediate clear, reddish, liquid phase was again removed. The insoluble residues were discarded. The two soluble extracts were combined and poured into two volumes of 95% ethyl alcohol. The desoxypentose nucleic acid separated in a gelatinous mass which could be removed from the alcoholic solution by winding around a stirring rod. The desoxypentose nucleic acid, still grossly contaminated with protein material but relatively free of hemoglobin was dissolved in a minimum volume of saturated sodium chloride solution. An insoluble residue of protein remained which was removed with the aid of a Buechner funnel. The resulting highly viscous solution was poured into two volumes of 95% ethyl alcohol. A gelatinous, colorless precipitate formed which became fibrous with winding on a stirring rod. The precipitate was dissolved in a minimum volume of distilled water and shaken vigorously a number of times with chloroform to remove the last traces of protein contaminants[13]. The solution was finally dialyzed in cellophane tubing against a large volume of frequently changed distilled water for 72 hours at 2–5°. The final yield of desoxypentose nucleic acid (as the sodium salt) was 290 mg or about 45% of the total originally estimated to be present.

CHEMICAL CHARACTERIZATION OF THE ISOLATED NUCLEIC ACIDS

All analytical data are reported on a per cent of weight basis. The solids were determined by drying aliquots of the stock solutions at 65° until visible water was removed, followed by drying at 110° as used by GULLAND[2], until constant weight was attained. Because of the hygroscopic nature of the sodium nucleates, it was necessary to take special precautions during the final weighings.

1. *Isolation and Characterization of the Purines Present in Mouse Liver Pentose and Desoxypentose Nucleic Acids*

Analyses for nucleic acid purines consisted of chemical determinations for total purine nitrogen and guanine. Values for adenine were calculated by difference. Spectrophotometric procedures were also used for the estimation of both adenine and guanine to serve as a check on the chemical methods.

Purine Nitrogen: In order to evaluate the effectiveness of copper[15, 16, 17, 18, 19] for purposes of quantitatively separating purines from nucleic acid hydrolysates, model experiments were devised. Equimolar mixtures of adenine, guanine, cytidylic acid, and uridylic acid were prepared in an attempt to approximate a dilute acid hydrolysate of nucleic acid. The mixtures were buffered at the desired pH and the purines precipitated by the addition of copper sulfate and sodium bisulfite[18]. Nitrogen determinations were performed on the copper precipitated fractions[20, 21]. The results are reproduced in Table I. Solutions buffered at pH 4.5 prior to precipitation with copper were found to give closest to theoretical values.

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TABLE I

THE EFFECT OF pH ON THE SEPARATION OF PURINES BY THE COPPER-BISULFITE PROCEDURE FROM APPROXIMATELY EQUIMOLAR MIXTURES OF ADENINE, GUANINE, CYTIDYLIC AND URIDYLIC ACIDS

Tube #	pH of Solution	mg Purine N Added	mg Pyrimidine Nucleotide N Added	mg N Recovered	Per Cent Error
1	4.0	0.400	0.209	0.415	+3.75
2	(Acetate	0.400	0.209	0.413	+3.25
3	Buffer)	0.400	0.209	0.418	+4.50
4	4.5	0.400	0.209	0.407	+1.75
5	(Acetate	0.400	0.209	0.410	+2.50
6	Buffer)	0.400	0.209	0.412	+3.00
7	5.0	0.400	0.209	0.427	+6.75
8	(Acetate	0.400	0.209	0.425	+6.25
9	Buffer)	0.400	0.209	0.420	+5.00
10	5.5	0.400	0.209	0.424	+6.00
11	(Acetate	0.400	0.209	0.420	+5.00
12	Buffer)	0.400	0.209	0.420	+5.00
13	7.0	0.400	0.209	0.286	−28.5
14	(Phosphate	0.400	0.209	0.288	−28.0
15	Buffer)	0.400	0.209	0.278	−30.5

The rate of purine hydrolysis from the isolated liver nucleic acids was determined by precipitating the liberated purine with copper at pH 4.5. Table II shows that maximum purine liberation, therefore presumably total purine liberation, was accomplished following 15 minute hydrolysis of the pentose nucleic acid and 10 minute hydrolysis of the desoxypentose nucleic acid at 100° in 1 *N* HCl.

TABLE II

RATE OF PURINE HYDROLYSIS FROM MOUSE LIVER SODIUM NUCLEATE PREPARATIONS IN 1 *N* HCl AT 100° C

Hydrolysis Time Minutes	Sodium Pentose Nucleate		Sodium Desoxypentose Nucleate	
	Nucleate Hydrolyzed (mg)	Purine N Released (mg)	Nucleate Hydrolyzed (mg)	Purine N Released (mg)
5	6.68	0.433	4.25	0.427
10	6.68	0.645	4.25	0.440
15	6.68	0.676	4.25	0.445
30	6.68	0.675	4.25	0.441
60	6.68	0.678	4.25	0.442

The procedure adopted for the routine isolation of purines from nucleic acids was as follows: Triplicate samples of nucleic acid containing approximately 0.5 mg purine nitrogen were placed into 15 ml centrifuge tubes. The samples were hydrolyzed in 1 *N* HCl for 20 minutes in a boiling water bath, then neutralized with sodium hydroxide and buffered at pH 4.5 with 3 ml molar acetate buffer. The tubes were heated in a boiling

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water bath and while hot, 0.2 ml 10% copper sulfate and 0.2 ml saturated sodium bisulfite were added to each tube. A brownish-white flocculent precipitate formed in a few minutes. The precipitate was centrifuged from the hot solution and washed three times with hot water. The precipitated purines were dissolved in dilute HCl and transferred to micro-Kjeldahl digestion flasks for the determination of total purine nitrogen. A digestion mixture containing potassium sulfate, copper sulfate, and selenium in sulfuric acid was used[20] for the digestions.

Guanine Estimations: The hydrolyzed nucleic acids were analysed for guanine by the colorimetric method of HITCHINGS[22]. It was found to be unnecessary to isolate the guanine prior to estimation. Identical values for guanine were found when determined directly on nucleic acid hydrolysates as compared to determinations following isolation of the purines from these hydrolysates.

The results of the analyses for purine nitrogen and for guanine on the liver nucleic acids are presented in Table III. Calculated values for adenine are also included. In order to substantiate these values, guanine and adenine were also determined spectrophotometrically.

TABLE III

THE PURINE COMPOSITION OF THE SODIUM SALTS OF MOUSE LIVER DESOXYPENTOSE NUCLEIC ACID (Na DNA) AND PENTOSE NUCLEIC ACID (Na PNA)

	Na DNA		Na PNA	
	Found	Calculated*	Found	Calculated*
Total Purine N	10.4% 10.4 10.6	10.6%	10.0% 10.2 10.1	10.2%
Guanine	10.1% 10.1 10.3	11.4%	15.2% 15.2 15.4	11.0%
(Adenine)**	11.2%	10.3%	5.83%	9.84%
Molar Ratio Adenine/Guanine	1.22	1	0.428	1

* Calculations based on assumed large polynucleotide structures consisting of tetrasodium salts of statistical tetranucleotides, *i.e.* $(C_{39}H_{45}O_{24}N_{15}P_4Na_4)_x$ for Na DNA and $(C_{38}H_{43}O_{28}N_{15}P_4Na_4)_x$ for the Na PNA.

** Adenine values represent calculated figures.

Estimation of nucleic acid purines by ultra-violet absorption: The procedure used was similar to that proposed by KERR and associates[23]. The purines were separated as insoluble complexes with copper as described above. The copper was removed with hydrogen sulfide by the procedure recommended by HITCHINGS AND FISKE[18]. There has been some question regarding the loss of purine by adsorption onto particles of copper sulfide by this procedure[16]. To test this possibility, nitrogen was determined on some purine fractions before and after removal of copper; no loss of purine nitrogen was found.

The solution containing the purines was aerated to remove hydrogen sulfide and made 0.1 *N* with respect to HCl. The ultra-violet absorption curves for the liver nucleic

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acid purines are shown in Fig. 1. All absorption values were computed in terms of molecular extinctions (ε) on the basis of purine nitrogen determinations. From the absorption curves it becomes readily evident that the purines isolated from both nucleic acids are guanine and adenine. A curve for an equimolar mixture of adenine and guanine is included for comparison. This standard purine solution was prepared from purified samples of adenine and guanine dried at 110° to constant weight. Nitrogen analysis by the micro-Kjeldahl procedure as recommended by PREGL[21] showed for the adenine sample 51.2% N (calculated 51.8%) and for the guanine sample 46.0% N (calculated 46.3%).

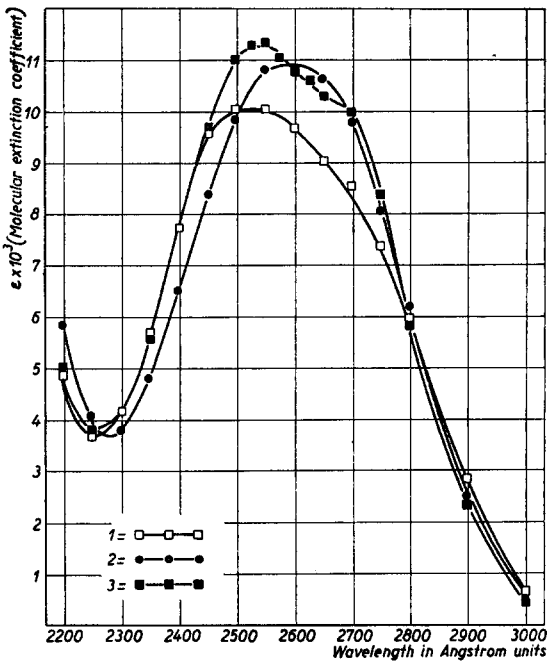

Fig. 1. Ultra-violet absorption curves of purines isolated from mouse liver pentose nucleic acid (curve 1: □—□—□), from mouse liver desoxypentose nucleic acid (curve 2: •—•—•), and an equimolar mixture of adenine and guanine (curve 3: ■—■—■). All absorption curves were determined in 0.1 *N* HCl in 1 cm silica absorption cells with a model DU Beckman spectrophotometer.

The quantities of adenine and guanine present in the purine fractions isolated from the liver nucleic acids were calculated from the known molecular extinction coefficients of adenine and guanine at 2650 Å (Table IV). The reliability of the method is demonstrated by the good agreement found between three completely separate analyses performed on each of the nucleic acid samples.

TABLE IV

SPECTROPHOTOMETRIC ANALYSIS OF THE PURINES ISOLATED FROM THE SODIUM SALTS OF MOUSE LIVER DESOXYPENTOSE NUCLEIC ACID (Na DNA) AND PENTOSE NUCLEIC ACID (Na PNA)

Sample	Log I_0/I 2650 Å	Purine Concentration (moles/liter, $\cdot 10^{-5}$)	Molecular Extinction Coefficient (2650 Å)	Molar Proportions		Average Molar Ratio Adenine/ Guanine
				Adenine	Guanine	
Guanine	0.548	7.26	7,550	0	1	
Adenine	0.958	7.37	13,000	1	0	
Na DNA (1)	0.564	5.34	10,600	0.557	0.443	
(2)	0.565	5.33	10,600	0.563	0.437	1.25
(3)	0.575	5.48	10,500	0.546	0.454	
Na PNA (1)	0.901	9.93	9,080	0.290	0.710	
(2)	0.895	9.79	9,140	0.294	0.706	0.401
(3)	0.831	9.21	9,030	0.275	0.725	

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The results of the chemical and spectrophotometric analyses on the purines of the liver nucleic acids are found to substantiate each other (compare Table III and IV). From these results, it is concluded that neither mouse liver pentose nor desoxypentose nucleic acids contain equimolar quantities of adenine and guanine nucleotides. The pentose nucleic acid was found to contain a considerable excess of guanine, while the desoxypentose nucleic acid was found to contain somewhat less guanine than adenine. Analyses for purine nitrogen disclosed near theoretical values, assuming a statistical tetranucleotide composition, for both nucleic acids (Table III). Variations in guanine and adenine ratios within a given nucleic acid sample would be expected to contribute practically insignificant differences to the computed nucleic acid purine nitrogen values. Although pyrimidine analyses were not performed*, the near theoretical values for total purine nitrogen suggest the presence of equimolar proportions of total purine and total pyrimidine nucleotides in each of the nucleic acids. Subsequent analyses for carbohydrate and phosphorus were found to substantiate this relationship.

2. *Additional Chemical Analyses on Nucleic Acids of Mouse Liver*

Additional analyses consisted of total nitrogen, total phosphorus, pentose, and desoxypentose. Total nucleic acid nitrogen was determined by the micro-Kjeldahl method as applied by PREGL[21]. Phosphorus was determined colorimetrically by the method of FISKE AND SUBBAROW[24]. The carbohydrate component of the pentose nucleic acid was estimated by the orcinol reaction as applied by MEJBAUM[25]. The procedure measures only the purine-bound pentose. Two separate series of quadruplicate determinations were performed to arrive at an average value. Since occasional variation; in analyses for pentose were found with the orcinol method, the duplicate series were considered essential. The desoxypentose was estimated by the method of DISCHE[26]. A highly purified desoxyribose nucleic acid isolated from calf thymus by LEVENE's procedure[1] (p. 298) was used as a standard. Chemical analyses for purine nitrogen, total nitrogen and phosphorus indicated near theoretical values by assuming a statistical tetranucleotide composition.

The results of this series of analyses are summarized in Table V. Experimentally determined values for phosphorus and specific carbohydrate groups were found to correlate well with calculated values for these constituents when assuming statistical tetranucleotide structures. Thus, the presence of equimolar proportions of total purine to total pyrimidine nucleotides appears to be substantiated by these analyses. The high values for total nitrogen for both nucleic acids remain to be explained. The excess nitrogen was found to be present with all preparations of mouse liver nucleic acids thus far investigated. Whether the excess nitrogen can be explained by contaminating protein or an excess of cytidylic acid remains to be determined.

3. *The Liberation of Inorganic Phosphorus From Mouse Liver Pentose Nucleic Acid by Dilute Sulfuric Acid*

Further experiments were performed on the pentose nucleic acid to characterize it more fully. Hydrolysis of ribose nucleic acid isolated from yeast with dilute sulfuric

* Pyrimidine analyses were not performed on these nucleic acid samples because of the lack of suitable quantitative techniques at the time. However, KERR and associates[23] in a recent publication have described simple and appropriate spectrophotometric procedures for their estimation in pentose nucleic acids.

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TABLE V

NITROGEN, PHOSPHORUS AND CARBOHYDRATE CONTENT OF THE SODIUM SALTS OF MOUSE LIVER DESOXYPENTOSE NUCLEIC ACID (Na DNA) AND PENTOSE NUCLEIC ACID (Na PNA)

	Na DNA		Na PNA	
	Per Cent Found	Per Cent Calculated*	Per Cent Found	Per Cent Calculated*
Total N	16.4 16.4 16.5	15.9	15.8 16.0 15.8	15.3
Phosphorus	9.4 9.4 9.4	9.4	8.8 8.8 9.0	9.0
Pentose (Purine only)	—	—	21.5	21.8
Desoxypentose (Purine only)	19.4 19.4 19.0	20.3	0.1	0.0

* Calculations based on assumed large polynucleotide structures consisting of tetrasodium salts of statistical tetranucleotides, *i.e.* $(C_{39}H_{45}O_{24}N_{15}P_4Na_4)_x$ for Na DNA and $(C_{38}H_{43}O_{28}N_{15}P_4Na_4)_x$ for the Na PNA.

acid was reported by JORPES[27] to cleave only part of the phosphoric acid groups free; the parts resisting the treatment were the pyrimidine nucleotides. JORPES found that only 53% of the total phosphorus of a yeast ribose nucleic acid sample was hydrolyzed by this procedure; similar findings for this type of nucleic acid were reported by SCHMIDT and associates[28]. DAVIDSON AND WAYMOUTH[29], working with isolated sheep liver pentose nucleic acid, report 54.2–54.7% of the total phosphorus hydrolyzed with 2% sulfuric acid after 2½ hours at 100°.

Dilute sulfuric acid hydrolysis of phosphorus from mouse liver pentose nucleic acid was studied by heating in 3% sulfuric acid at 100° for varying lengths of time. This was followed by inorganic phosphorus analysis[24]. The results of this study indicated that 54.5% of the total nucleic acid phosphorus was hydrolyzed to the free state after 3 hours. Hydrolysis for 4 hours showed essentially the same result. Mouse liver, yeast, and sheep liver pentose nucleic acids appear to be similar with respect to phosphate liberation by dilute acid. An extensive study dealing with the kinetics of phosphate liberation from ribose nucleic acids and various nucleotides has been recently reported by BACHER AND ALLEN[30].

4. *The Action of Crystalline Ribonucleinase on Mouse Liver Pentose Nucleic Acid*

An enzymatic characterization of the liver pentose nucleic acid was performed utilizing crystalline ribonucleinase (Worthington Co.). A sample of purified yeast ribose nucleic acid (Schwartz Co.) was treated enzymatically in the same manner for purposes of comparison.

To separate centrifuge tubes were added 1 ml volumes of mouse liver pentose and yeast ribose nucleic acids containing approximately 2 mg nucleic acid per ml. To each

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tube was added 1 ml of a solution containing 0.10 mg ribonucleinase per ml. The tubes were incubated at 37.0° for 24 hours. Following the incubation, 2 ml of 10% trichloroacetic acid solution containing 0.31% uranium acetate was added to each tube. This reagent has been utilized to separate enzymatically hydrolyzed from non-hydrolyzed constituents[31]. The tubes were centrifuged and both separated fractions retained. The uranium was removed from both fractions by alkalinization[32]. The enzymatically hydrolyzed fractions (uranyl-trichloroacetic acid soluble fractions) and the non-hydrolyzed fractions (uranyl-trichloroacetic acid insoluble fractions) were analyzed for phosphorus[24] and pentose[25]. Analyses for these constituents were also performed on the intact nucleic acids for purposes of comparison. Control tubes containing nucleic acid alone when precipitated with the uranyl-trichloroacetic acid reagent showed less than 1% soluble material for the liver pentose nucleic acid, and slightly larger quantities for the yeast ribose nucleic acid.

The results of the study of ribonucleinase hydrolysis on the liver pentose and yeast ribose nucleic acids are presented in Table VI. The enzyme was found to hydrolyze both nucleic acids incompletely. Similar findings have been reported for sheep liver ribose nucleic acid[29] and yeast ribose nucleic acid[33]. LORING and associates[33] found a greater proportion of pyrimidine nucleotides hydrolyzed from yeast ribose nucleic acid by the action of ribonucleinase. Similar results were found for both the nucleic acids investigated in this study. This conclusion is based on the ratios of phosphorus to pentose found in the two fractions (*i.e.* hydrolyzed and non-hydrolyzed) as compared to the intact nucleic acid. The orcinol reaction as applied[25] measures only the purine-bound pentose. Thus, an increase in the ratio of phosphorus to pentose in the hydrolyzed fractions, as compared to the intact nucleic acid, can only mean that a greater proportion of pyrimidine nucleotides has been liberated from the nucleic acids. The yeast ribose and liver pentose nucleic acids appear to be similar with respect to enzymatically hydrolyzable linkages.

TABLE VI

ENZYMATIC DEGRADATION OF MOUSE LIVER PENTOSE AND YEAST RIBOSE NUCLEIC ACIDS BY CRYSTALLINE RIBONUCLEINASE

Nucleic Acid Fraction	Liver Pentose Nucleic Acid			Yeast Ribose Nucleic Acid		
	Phosphorus (moles $\cdot 10^{-6}$)	Pentose (moles $\cdot 10^{-6}$)	Phosphorus/ Pentose Ratio (molar)	Phosphorus (moles $\cdot 10^{-6}$)	Pentose (moles $\cdot 10^{-6}$)	Phosphorus/ Pentose Ratio (molar)
Intact Nucleic Acid	6.52	3.22	2.02	6.61	3.37	1.96
Hydrolyzed Fraction	4.90	1.97	2.49	4.32	1.75	2.47
Non-Hydrolyzed Fraction	1.86	1.16	1.60	2.48	1.61	1.54

DISCUSSION

Recent investigators utilizing chromatographic techniques for the separation of

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nucleic acid constituents have reported on the chemical composition of several pentose and desoxypentose nucleic acids isolated from different sources[3, 4, 5]. Their results do not indicate equimolar ratios of the respective nucleotides in each of the nucleic acid samples investigated. Wide variations in chemical composition were found for pentose nucleic acids isolated from several different sources[5]. Analyses of desoxypentose nucleic acids isolated from thymus and spleen did not show statistical tetranucleotide relationships; however, very similar chemical compositions were recorded for both nucleic acids[4].

It is apparent however, that more detailed analyses on nucleic acids isolated by gentle extraction procedures are yet necessary in order to formulate any definite chemical concepts regarding the true chemical nature of these substances.

SUMMARY

1. Methods for the isolation of pentose and desoxypentose nucleic acids from mouse liver tissue, applying gentle extraction and purification procedures were presented. Differential centrifugation techniques were incorporated to effect a preliminary separation of the tissue into nuclear and cytoplasmic fractions.

2. Chemical analyses indicated that neither nucleic acid conformed to a statistical tetranucleotide in composition. However, near equal molar proportions of purine nucleotides to pyrimidine nucleotides appeared to be present. Purine analyses disclosed a considerable excess of guanine in relation to adenine for the pentose nucleic acid, and an excess of adenine over guanine in the desoxypentose nucleic acid. A spectrophotometric procedure was described for purine analyses which served to check the chemical methods.

3. Mouse liver pentose nucleic acid was found to be similar to yeast and sheep liver ribose nucleic acids with respect to containing equivalent quantities of dilute acid labile phosphate.

4. Crystalline ribonucleinase was found to hydrolyze the liver pentose nucleic acid incompletely. A preferential hydrolysis of pyrimidine over purine nucleotides by the enzyme was found for the liver nucleic acid as well as for yeast ribose nucleic acid.

RÉSUMÉ

1. Nous présentions des procédés d'extraction et de purification doux pour l'isolation des acides pentose- et désoxypentose-nucléiques de foie de souris. Nous avons effectué une séparation préliminaire des fractions nucléaire et cytoplasmique du tissu de foie de souris à l'aide de techniques de centrifugation différentielle.

2. Des analyses chimiques ont montré que la composition des acides nucléiques isolés ne correspond pas à un tétra-nucléotide statistique. Cependant les purine- et pyrimidine-nucléotides se trouvent en proportions molaires à peu près égales. Des déterminations de la quantité de purine ont montré que les acides pentose-nucléiques contiennent beaucoup plus de guanine que d'adénine; c'est l'inverse pour les acides désoxypentose-nucléiques. Nous avons décrit un procédé spectrophotométrique pour la détermination quantitative de la purine, procédé qui a servi au contôle des méthodes chimiques.

3. L'acide pentose-nucléique du foie de souris ressemble à l'acide ribonucléique de la levure et du foie de mouton, en ce qu'il contient des quantités équivalentes de phosphate hydrolysable par les aides dilués.

4. Nous avons trouvé que la ribonucléinase cristalline hydrolyse de façon incomplète l'acide pentose-nucléique du foie et que les pyrimidine-nucléotides sont hydrolysés avant les purine-nucléotides dans les acides nucléiques du foie aussi bien que dans l'acide ribonucléique de la levure.

ZUSAMMENFASSUNG

1. Milde Extraktions- und Reinigungsmethoden zur Isolierung von Pentose- und Desoxypentose-Nukleinsäuren aus Mäuselebergewebe werden beschrieben. Eine erste Trennung der Kern- und Protoplasmafraktionen wurde durch Zentrifugieren bewerkstelligt.

2. Durch chemische Analysen wurde gezeigt, dass keine der Nukleinsäuren in ihrer Zusammensetzung einem statistischen Tetranukleotid entsprach. Dagegen waren Purin- und Pyrimidinnu-

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kleotide in nahezu molaren Proportionen vorhanden. Purinbestimmungen ergaben, dass die Pentose-Nukleinsäure einen grossen Überschuss an Guanin im Vergleich zum Adenin enthält, während das Verhältnis bei der Desoxy-Nukleinsäure gerade umgekehrt liegt. Eine spektrophotometrische Bestimmungsmethode für Purin wurde beschrieben, die zur Kontrolle der chemischen Methoden diente.

3. Es wurde festgestellt, dass die Pentose-Nukleinsäure aus Mäuseleber der Ribonukleinsäure aus Hefe und Schafsleber insofern ähnlich ist, als sie entsprechende Mengen Phosphat enthält, die unter dem Einfluss von verdünnter Säure hydrolysiert werden.

4. Die Pentose-Nukleinsäure aus Leber wurde durch kristalline Ribonukleinase unvollständig hydrolysiert. Sowohl in der Nukleinsäure aus Leber, wie in der Ribonukleinsäure aus Hefe hydrolysierte das Enzym die Pyrimidinnukleotide vor den Purinnukleotiden.

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