PHOSPHORUS COMPOUNDS IN ANIMAL TISSUES

III. A COMPARISON OF METHODS FOR THE ESTIMATION OF NUCLEIC ACIDS

By WALTER C. SCHNEIDER*

(From the McArdle Memorial Laboratory, Medical School, University of Wisconsin, Madison)

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Recently two methods for the estimation of nucleic acids in animal tissues appeared almost simultaneously (3, 4). Although the techniques employed in these independently conducted experiments differed considerably, the results agreed remarkably well. Some discrepancies in the results obtained by the two methods were, however, evident. For this reason and because of the increasing interest in the rôle of nucleic acids in cellular physiology, it seemed of interest to compare the results obtained by the two methods on the same sample of tissue.

The present report describes the results of the analysis of six rat tissues for nucleic acids by the two methods and also describes an improved method for nucleic acid analysis which incorporates the principles of both methods.

Methods and Materials

Preparation of Tissues—Adult rats of the Sprague-Dawley strain were used for analysis. The animals were killed by decapitation and the tissues were removed immediately and chilled on cracked ice. Portions of the tissue were weighed and homogenized in distilled water at 0° in the apparatus of Potter and Elvehjem (2).[1]

Removal of Acid-Soluble Phosphorus Compounds and Phospholipides—Equal aliquots of the tissue homogenates were pipetted into each of two 15 ml. centrifuge tubes and acid-soluble compounds were removed as described previously (4). Phospholipides were then removed from the tissue residues by the method reported previously (4) with the exception that the extractions with boiling alcohol-ether were omitted, since it was found that these extractions did not appreciably increase the yield of phosphorus extracted.

Nucleic Acid Removal, Method of Schneider (4)—The residue contained in one of the tubes after removal of acid-soluble and fat-soluble compounds

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[1] Now obtainable from the Central Scientific Company.

was heated with 5 per cent trichloroacetic acid to extract the nucleic acids (see (4)). This extract will be referred to as Fraction I. The protein residue which remained after heating with trichloroacetic acid was dissolved in 2 per cent NaOH to form Fraction II (see (4)).

Nucleic Acid Removal, Modification of Method of Schmidt and Thannhauser (*3*)—The method of Schmidt and Thannhauser was modified slightly to include heating with trichloroacetic acid to obtain the desoxyribose nucleic acid (DNA) in solution so that it might be determined colorimetrically. The procedure was as follows: The tissue residue which remained in the second centrifuge tube, after removal of acid- and alcohol-soluble materials, was treated for 20 hours at 37° with 1 N KOH (see (3)). This treatment resulted in solution of the tissue (Fraction III). DNA and protein were precipitated with acid as described (3) and the filtrate containing the pentose nucleic acid (PNA) and protein phosphorus was removed and termed Fraction IV. The precipitate containing the DNA was heated with trichloroacetic acid (see (4)) to dissolve the DNA. The trichloroacetic acid extract was designated Fraction V.

Analyses—Total phosphorus was determined by the method of LePage and Umbreit (1). DNA was measured by the reaction with diphenylamine (4) in Fractions I and V. PNA was measured in Fractions I and IV by the orcinol reaction (4). Nucleic acids served as standards for both reactions (see (4)). Inorganic phosphorus was precipitated from Fraction IV as the calcium salt and determined colorimetrically (1).

Results

The analytical results are reported in Table I. In confirmation and extension of the previous results (4, 5), it was found that all of the phosphorus present in the extracts of tissues heated with trichloroacetic acid could be accounted for by nucleic acids as measured by pentose determinations (see Table I, compare Fraction I-A plus I-B with I-C).

The DNA values found by the method of Schmidt and Thannhauser (see Fraction IV-D, Table I) were higher than the results reported earlier by these authors (3). The most striking case was that of spleen in which the DNA content was found to be almost 3 times as high as they had reported. A possible explanation of the higher results we obtained may lie in the fact that our analyses include four animals, while the results of Schmidt and Thannhauser apparently were based on single analyses. With the exception of liver and brain, the DNA found by the method of Schmidt and Thannhauser (phosphorus analysis) agreed remarkably well with the DNA found by desoxyribose analyses (compare Fractions IV-D and V-A). The DNA found by desoxyribose determinations in the modified method is in excellent agreement with the DNA found by the method of Schneider

TABLE I

Analysis of Rat Tissues for Nucleic Acids

The results are expressed in terms of mg. of phosphorus per 100 gm. of fresh tissue. Four samples of each tissue were analyzed. The mean values are given and the variation from the mean is expressed as the standard deviation, $\sqrt{\Sigma d^2/n}$. DNA = desoxypentose nucleic acid; PNA = pentose nucleic acid.

Method	Schneider (4)						Modified Schmidt and Thannhauser (3)							
Compound	DNA	PNA	Nucleic acids, DNA + PNA	Nucleic acids	"Phospho-protein"	Original	Original	PNA + inorganic P	Inorganic P	PNA	PNA	DNA	DNA	DNA
From measurement as	Desoxy-pentose	Pentose	Pentoses	Total P	Total P	Total P	Total P	Total P	Inorganic P	Total P minus inorganic P	Pentose	Total P	Desoxy-pentose	Total P
Fraction No.	I-A	I-B	I-A + I-B	I-C	II	I-C + II	III	IV	IV-A	IV-B (IV − IV-A)	IV-C	IV-D (III − IV)	V-A	V-B
Pancreas	47.9	198	246	254	17.3	271	255	204	4.2	199.8	188	51	49.1	41.0
	±3.2	±8.9	±12.1	±5.1	±0.2	±5.3	±13.6	±13.7	±0.3	±13.6	±7.7	±6.8	±1.6	±0.5
Thymus	276	53.1	329	311	25.9	337	320	59.1	8.5	50.6	53.3	260.9	274	253
	±16.0	±2.5	±18.5	±10.0	±2.3	±12.3	±18.1	±3.2	±1.5	±4.8	±1.5	±14.7	±10.4	±13.0
Liver	26.4	73.5	99.9	95.2	22.1	117.3	122	91.1	3.7	87.4	77.7	30.9	26.0	25.5
	±1.8	±4.5	±6.3	±4.1	±5.1	±9.2	±5.8	±8.6	±1.8	±10.0	±4.1	±3.1	±1.5	±3.0
Kidney	41.8	40.7	82.5	76.8	23.3	100.1	92.6	53.6	4.3	49.3	42.3	39.0	40.4	36.5
	±2.7	±2.2	±4.9	±3.1	±5.6	±8.7	±5.3	±7.4	±2.5	±8.9	±0.4	±5.9	±1.1	±1.4
Spleen	140	49.9	190	179	26.1	205.1	214	67.0	6.1	60.9	58.4	147	144	132
	±22.3	±4.0	±26.3	±25.2	±12.5	±37.7	±33.0	±14.0	±4.7	±14.6	±5.7	±20.8	±15.8	±17.6
Brain	20.1	18.8	38.9	37.6	23.3	60.9	59.8	35.0	7.0	28.0	20.5	24.8	19.1	15.0
	±1.4	±0.2	±1.6	±4.0	±0.3	±4.3	±2.5	±3.7	±3.9	±6.5	±0.1	±4.5	±1.4	±0.3

(compare Fractions V-A and I-A), indicating that the DNA has been effectively separated in the modified method.

The PNA values found by the method of Schmidt and Thannhauser were somewhat lower than the results reported by these authors (see Fraction IV-B, Table I, and (3)). The PNA found by pentose determinations was in fair agreement with the PNA calculated as described by Schmidt and Thannhauser (compare Fractions IV-C and IV-B) and in good agreement with the PNA found in the trichloroacetic acid extraction method (compare Fractions IV-C and I-B).

DISCUSSION

The observation that the amounts of nucleic acids found by pentose determinations in the modified method of Schmidt and Thannhauser and in the method of Schneider are the same indicates that DNA and PNA have been effectively separated in the former method. This finding indicates that the choice of the method to be used in the measurement of nucleic acids will depend upon whether a separation of the nucleic acids is necessary. If a separation of DNA and PNA is required, as would probably be the case in work with isotopes, the method of Schmidt and Thannhauser as modified in this paper is the method of choice. On the other hand, if such a separation is unnecessary, the trichloroacetic acid method can be expected to afford a simple, rapid, and accurate means of extraction and estimation of nucleic acids. In connection with the choice of methods, it should be recalled that in the trichloroacetic acid method of Schneider (4) a correction must be applied for the reaction of DNA in the orcinol reaction. Such a correction is unnecessary in the modified method, since the nucleic acids have been effectively separated.

The use of pentose determinations for the measurement of nucleic acids has been criticized on the ground that not all of the pentose in the nucleic acids reacts in the colorimetric methods (3). Our own results (Table I, and (4, 5)), in which nucleic acids were used as standards in the colorimetric reactions, show that the use of pentose is a valid procedure for the determination of nucleic acids, since in all of the tissues studied the nucleic acid found by pentose determinations agreed well with the nucleic acids as measured by phosphorus determinations. Obviously, it is a sound practice not to rely upon either pentose or phosphorus analyses alone but to employ both. A still better procedure would be to determine the phosphorus, the pentose, and the purine and pyrimidine bases simultaneously in order to arrive at a correct estimate of the nucleic acid content of the tissue. The nitrogenous bases could probably be measured by the use of ultraviolet spectrophotometry.

SUMMARY

1. The method of Schmidt and Thannhauser for the determination of nucleic acids was modified by extracting the DNA with trichloroacetic acid so that the DNA could be determined colorimetrically.

2. The following rat tissues were analyzed by the modified method and by the method of Schneider: pancreas, thymus, liver, kidney, spleen, and brain.

3. Both methods yielded essentially the same results when the nucleic acid estimations were based on pentose determinations. Results based upon phosphorus determinations were less consistent and reliable. The need for making both types of measurements was emphasized.

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