Technical Papers

A Criticism of the Indiscriminate Use of the Schmidt-Thannhauser Method for the Fractionation of Nucleic Acids in Biological Material

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Interest in the nucleic acids from a variety of tissues is being displayed by an increasing number of biologists. Many who would not consider themselves well versed in biochemical procedures are attracted to the Schmidt-Thannhauser (*1*) method of separation and determination because of its relative simplicity when compared with other methods. The chief attraction of this method is that it can be followed by only one type of determination (i.e., phosphorus) rather than by the two or more methods necessary when the sugar moieties of the nucleic acids are used as the basis for analysis. It has become customary among many biologists to accept without question the general applicability of this method. It is the purpose of this communication to point out and emphasize to biologists some of the hazards inherent in this method.

The Schmidt-Thannhauser method of separation of ribonucleic acid (RNA) from deoxyribonucleic acid (DNA) rests upon the claim of Steudal and Peiser (*2*) that RNA is quantitatively hydrolyzed to acid-soluble nucleotides by treatment with 0.75 *N* NaOH at room temperature in 24 hr, whereas DNA resists this treatment and remains completely precipitable by mineral acid. According to the theories of Fonó (*3*), Brown and Todd (*4*), and Markham and Smith (*5*), the difference between these two nucleic acids in their susceptibility to alkaline hydrolysis is due to the formation by RNA of highly alkali-labile "cyclic" nucleotides by esterification of the free —OH group on the 2′or 3′ carbon atom of ribose. Deoxyribose has no corresponding —OH group, and so DNA is incapable of forming such "cyclic" nucleotides and therefore can not be degraded in this manner. In the usual Schmidt-Thannhauser methods, the material to be analyzed is incubated at 37° C for a period of from 18 hr to three days with 1 *N* KOH, 1 *N* NaOH, or 0.3 *N* NaOH. At the end of this incubation period, DNA is precipitated by the addition of suitable amounts of mineral acid. It is then assumed that all the DNA is precipitated and that all the RNA remains in the supernatant fluid. Phosphorus determinations on the supernatant fluid (after adjustment for any phosphoproteins) are considered an estimate of RNA and the phosphorus content of the precipitate after hydrolysis is considered an estimate of DNA.

Assuming for the moment that the separation of these acids by such a procedure is adequate, the use of phosphorus determinations as a means of estimating these nucleic acids is itself fraught with many dangers, the most obvious of which are: (1) the danger of contamination of one fraction with phosphorus-containing compounds from another fraction, (2) the possibility of differential extraction and/or precipitation of phosphorus and the sugar moieties, and (3) the inadequacy of the general assumption of a constant percentage of phosphorus in the nucleic acid derivatives. Schneider (*6*) made a detailed comparison of the results yielded by the Schmidt-Thannhauser method and his own method (*7*) which depends upon the simultaneous extraction of both types of nucleic acids with hot trichloroacetic acid (TCA). RNA and DNA are determined by estimation of ribose and deoxyribose in portions of this extract. In the particular tissues which Schneider (*6*) used for comparison good agreement between the two methods was obtained.

Evidence is accumulating in the literature that there may be serious discrepancies in results obtained by the two methods. These discrepancies obviously are not due to errors produced by the use of phosphorus determinations alone (contaminations, differences in phosphorus composition, etc.) but are more likely due to the inadequacy of the actual separation in the Schmidt-Thannhauser method. Davidson (*8*) noted that the use of the Schmidt-Thannhauser technique gave, for several tissues, higher ratios of ribonucleic acid phosphorus to deoxyribonucleic acid phosphorus than other methods. Davidson, Frazer, and Hutchinson (*9*) showed that the total phosphorus of a Schneider extract from a rabbit liver homogenate contained onyl 75% of the phosphorus found in the fractions corresponding to the DNA and RNA of a Schmidt-Thannhauser hydrolyzate. They suggested that the Schneider procedure did not split off all the nucleic acid phosphorus and they showed that about 25% of this Schneider residue, if subjected to Schmidt-Thannhauser procedure, is alkali-soluble. Of this soluble material, about 85% is acid-soluble organic phosphorus, but it was not shown that this fraction contained ribose. More recently, Mauritzen, Roy, and Stedman (*10*) have shown specifically that, in a Schmidt-Thannhauser fractionation of thymus nuclei, a significant amount of degraded DNA appears in the acid supernatant fluid ("RNA fraction"). The general validity of the basic assumption of Steudel and Peiser (*2*) is thereby called in question. Either the material taken for analysis contains some DNA already in a sufficiently degraded state to be soluble in alkali or, contrary to Steudel and Peiser, some DNA can be hydrolyzed by treatment with alkali. Most recently,

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TABLE 1

COMPARISON OF SCHMIDT-THANNHAUSER AND SCHNEIDER METHODS ON MAMMARY TUMOR TISSUE
Results expressed as μg/mg dry tissue

Tissue sample*	Total phosphorus†		Ribonucleic acid (RNA)‡			Deoxyribonucleic acid (DNA)§			Residual phosphorus
	Schmidt-Thann. alk. hydrol.	Schneider TCA extract	Phos. on Schmidt-Thann. supern.	Orcine on Schmidt-Thann. supern.‖	Orcine on Schneider extract	Phos. on Schmidt-Thann. ppt.	Diphenyl-amine on Schmidt-Thann. ppt.	Diphenyl-amine on Schneider extract	Schneider residue
1									
A	13.3	10.6	7.9	49.6	44.1	5.0#	45.8#	76.6	1.1
B	12.3	11.1	7.6	47.6	41.5	5.2**	48.1**	66.4	0.5
2									
A	7.4	7.3	4.3	29.2	36.6	2.5#	26.5#	52.8	0.0
B	8.1	8.1	4.2	30.0	37.0	4.1**	40.9**	52.4	0.0
3									
A	12.7	11.5	10.4	33.8	37.6	2.1#	25.7#	103.0	1.2
B	11.7	12.1	10.0	31.6	38.8	1.9**	32.0**	110.8	1.3

* Samples 1, 2 and 3 were from mammary tumors of different stages of development.
** Schmidt-Thannhauser precipitate hydrolyzed with NaOH.
† Phosphorus determined by the method of Fiske and SubbaRow (modified) (*17*).
‡ RNA determined by the Mejbaum (*13*) orcinol method.
§ DNA determined by the Dische (*15*) diphenylamine method.
‖ Average of two determinations.
Schmidt-Thannhauser precipitate extracted with hot trichloroacetic acid (in sample 2A, extraction was short and did not extract all of the DNA from the precipitate).

McIndoe and Davidson (*11*), working with free nuclei, have found that by dissolving and reprecipitating several times the original Schmidt-Thannhauser precipitate, the DNA which was originally precipitatable tended to become degraded and alkali-soluble. Such a finding makes some of the longer incubation periods used by some workers highly suspect. Furthermore, it has been shown by Davidson and Smellie (*12*) that the ribonucleotide fraction derived by a modified Schmidt-Thannhauser separation from rat liver homogenate contains at least five other organic phosphate components. Ribonucleotides contribute only 75% of the total phosphorus of this fraction. Only one of the contaminating components (*A*) which could be separated ionophoretically from the nucleotides has a detectable absorption in the ultraviolet. The principal contaminants in this case are not deoxyribonucleotides. These observations emphasize the great caution that should be exercised before it is assumed that the "RNA fraction" from any unknown material contains only ribonucleotides.

Data collected in the course of another investigation support the finding of Mauritzen, Roy, and Stedman (*10*) that in certain tissues DNA, either because it is less polymerized or because it is less resistant to alkaline hydrolysis, is not completely precipitated by acidification after initial incubation with 1 *N* NaOH. Mammary tumor tissue from C3H mice was analyzed by both Schmidt-Thannhauser and Schneider methods (Table 1). It is clear that an appreciable portion of the DNA is *not* precipitated upon acidification of the Schmidt-Thannhauser alkaline hydrolyzate. Short-

TABLE 2

APPARENT DIFFERENCES IN PHOSPHORUS AND SUGAR CONTENTS OF NUCLEIC ACIDS OF MAMMARY TUMOR TISSUE WHEN ANALYZED BY THE SCHMIDT-THANNHAUSER AND SCHNEIDER METHODS
Results expressed as μg/mg dry tissue

Tissue sample*	Method	Ribonucleic acid fraction (RNA)				Deoxyribonucleic acid fraction (DNA)			Total phosphorus		
		Time of alkaline hydrol.	Super-nat. phos.	RNA in super-nat. orcine†	RNA in TCA extr. orcine	Phos. on ppt.	DNA in ppt. diphenyl-amine†	DNA in TCA extr. diphenyl-amine	Phos. in alk. hydrol.	Phos. in TCA ext.	Calc. from orcine and diphenyl-amine‡
A	Schmidt-Thann.	4 hr	8.2	42.6	.....	3.6	47.7	.....	11.2	.....	8.7
B	Schmidt-Thann.	24 hr	8.8	38.2	.....	2.9	36.4	.....	11.3	.....	7.2
C	Schneider	.....	.....	.....	49.3	.....	.....	101.3	.....	12.2	14.8
D	Schneider	.....	.....	.....	53.5	.....	.....	104.9	.....	10.7	15.4

* All tissue samples were from the same initial homogenate.
† Average of two determinations.
‡ Calculated on the basis of 9.8% phosphorus in DNA and 9.5% phosphorus in RNA.

ening the period of hydrolysis has no effect on the amount of DNA precipitated (Table 2). This could be taken to support the interpretation that the degraded part of the DNA fraction is already in the alkali-soluble form before the material is treated with alkali. This finding does not necessarily contradict the finding of McIndoe and Davidson (*11*) that the repeated reprecipitation of DNA from isolated nuclei results in increasing degradation of the DNA which was at first precipitable.

It can be seen from Table 2 that there are some discrepancies between the total extractable phosphorus values given by direct phosphorus analysis and the amounts calculated on the basis of theoretical phosphorus contents of 9.8% and 9.5% for DNA and RNA respectively (Schneider, *7*). These can probably be accounted for on the basis of (a) a slight interference in the Mejbaum (*13*) orcinol reaction by DNA (von Euler and Hahn, *14*) and (b) high values on the Dische (*15*) diphenylamine reaction in the presence of protein (Dounce, *16*). In the experiments reported here, the standard solutions of nucleic acids used for calibration were made up in solvents of exactly the same composition as those used in the unknowns and were varied to correspond to the method of extraction or fractionation.

Data similar to those reported here have been obtained on occasion from mouse liver and from old mouse uterine tissue. It is suggested that, before the Schmidt-Thannhauser method is used as a method for the determination of DNA and RNA, a check should be made with another method to find out whether effective separation is achieved in the particular tissue being studied. Acid precipitation after alkaline hydrolysis of suitably prepared nucleic acid-containing tissue homogenates does not always result in complete separation of the deoxyribonucleic acids. For this reason, and because the acid-soluble fraction may also contain non-nucleotide organic phosphorus, it is unsafe to use phosphorus estimations on the acid-soluble and acid-insoluble fractions as the sole means of distinguishing the DNA and RNA.

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The Occurrence of Acquired Hemolytic Anemia in Subjects of Blood Group O

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In a previous study of the blood groups of 26 patients suffering from hemolytic anemia (*1*), there was noted a slight increase in the incidence of blood group O. To investigate this trend in a larger sample, the blood groups of 58 subjects of hemolytic anemia were analyzed in relation to the type of hemolytic anemia involved, i.e., congenital or acquired.

Of the 58 cases of hemolytic anemia studied, 31 were diagnosed as congenital hemolytic anemia, and 27 as acquired hemolytic anemia. The diagnoses were made by competent hematologists and were verified by frequent references to the progress of the individual cases. All the patients who had acquired hemolytic anemia were unrelated; of those with congenital hemolytic anemia, 7 were members of 3 families (two instances of a father and daughter, and one of a father, daughter, and granddaughter), and the rest were unrelated.

In Table 1, the ABO blood group distribution among subjects of hemolytic anemia is presented, and the preponderance of group O among those with acquired hemolytic anemia is evident. The distribution of ABO blood groups in the 58 subjects of hemolytic anemia was compared with the normal distribution of the ABO blood groups in an unselected, white, population.

There was found to be a statistically significant increase in the incidence of blood group O among patients with acquired hemolytic anemia, whereas no significant deviation from the expected distribution of ABO blood groups occurred among patients with congenital hemolytic anemia.

TABLE 1

The Distribution of ABO Blood Groups in 58 Patients with Hemolytic Anemia

Type of hemolytic anemia	Total No.	%	ABO blood groups							
			O (N = 45%) (*2*)		A (N = 41%)		B (N = 10%)		AB (N = 4%)	
			No.	%	No.	%	No.	%	No.	%
All types total	58	100	36	62.1	13	22.4	9	15.5	0	0
Congenital	31	100	15	48.4	11	35.5	5	16.1	0	0
Acquired	27	100	21	77.8	2	7.4	4	14.8	0	0