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Determination of Pentose Nucleic Acid in Trichloroacetic Acid Extracts of Human Placental Tissue

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The sugar component of pentose nucleic acid (P.N.A.) is the basis of a variety of procedures for determination of this substance [1,2,3]. The specificity of these reactions, however, is poor, and when applying them to the quantitative assay of P.N.A. in biological material great care has to be taken.

A method for quantitative extraction of nucleic acids by hot trichloroacetic acid (T.C.A.) has been devised by Schneider [4]. By applying colorimetric methods to the estimation of pentose and desoxypentose sugars, the nucleic acids can be determined without having to separate the two acids.

In an investigation of the nucleic acids of human placenta the T. C. A. procedure has been used [5,6]. However, a large variety of sugars are present in this tissue. Corey [7] has demonstrated the presence of large amounts of glycogen in the placenta. On T. C. A. hydrolysis this substance may yield glucose. Jones, Gey and Gey [8] have presented ample evidence of the production of chorionic gonadotrophin by placental tissue. This hormone is a mucoprotein containing galactose [9], and the sugar may be split off during the acid hydrolysis. Embryonic tissue has been found to possess considerable fructolytic power [10], and it may be presumed that fructose plays an important role in embryonic tissue [11]. Huggett, Warren and Warren [12] have presented conclusive evidence of the production of fructose by sheep placenta. It is thus necessary to take into consideration the possible presence of all these sugars, the corresponding amino sugars and uronic acids, in the T. C. A. extract of human placenta.

For determination of P. N. A. in the hot T. C. A. extract Schneider [4] uses the orcinol reaction. Some criticism may, however, be raised against this method. As has been shown by Mejbaum [1], the reaction obeys Beer's law only for concentrations between 1 μg and 18 μg pentose per ml. When the

concentration is increased from 18 μg to 24 μg pentose per ml, the extinction coefficient decreases by about 8 %. Frequent dilution of the sample is thus necessary, which introduces some error in the determination. Moreover, the influence of several carbohydrates on the pentose-orcinol reaction has been observed. Schneider[4] emphasizes the necessity of correcting for any desoxypentose nucleic acid (D. N. A.) present, which involves determination of this substance in the sample. Mejbaum[1] points out the influence of glucose. In most determinations of P. N. A. by the orcinol reaction, including that done by Schneider[4,13], no attention seems to have been paid to this source of error. Brown[14] has studied the pentose-orcinol reaction in the presence of glucose, and he has also pointed out the influence of fructose on the reaction.

These considerations and the supposed presence in human placental tissue of a great variety of sugars, which are known to interfere with the pentose-orcinol reaction, made it desirable to find another method for determination of P. N. A. This paper is concerned with the application of Dische's[3] basic cysteine reaction (BCyR) to the determination of P. N. A. in hot T. C. A. extracts of human placental tissue.

The present investigation was carried out along two lines. The influence on the pentose-cysteine reaction of sugars that might be present in the placenta was first studied. This part is a confirmation and extension of some of the studies by Dische[3]. The possible presence of disturbing substances in the hot T. C. A. extract was then investigated.

EXPERIMENTAL

After extraction of cold T.C.A.- and lipid-soluble substances the nucleic acids were extracted by Schneider's[4] method. The P.N.A. content was estimated by a slight modification of Dische's[3] BCyR. One volume, containing the P.N.A. to be estimated, is chilled in an ice-water bath. Four volumes of concentrated chilled (+ 5° C) H_2SO_4 are added. After vigorous shaking for half a minute the tube is placed in a water bath at 60° C for 20 minutes. It is then immersed in a water bath at 25° C, where it is stood for ten minutes. 0.1 ml of 3 % cysteine-HCl is added and the contents are mixed. The tube is again placed in the water bath at 25° C and the sample is read in a Beckman quartz spectrophotometer after exactly ten minutes, using a 1 cm cell. A reagent blank is run. When performing the reaction on T.C.A. extracts from biologic material, corrections are made for the absorption caused by the reaction products with H_2SO_4.

As a standard of reference for the P.N.A. determinations on the T.C.A. extracts, yeast nucleic acid from Hoffmann-La Roche was used. It was purified according to Hammarsten's[15] method and then hydrolyzed in 5 % T.C.A. at 90° C for 20 minutes.

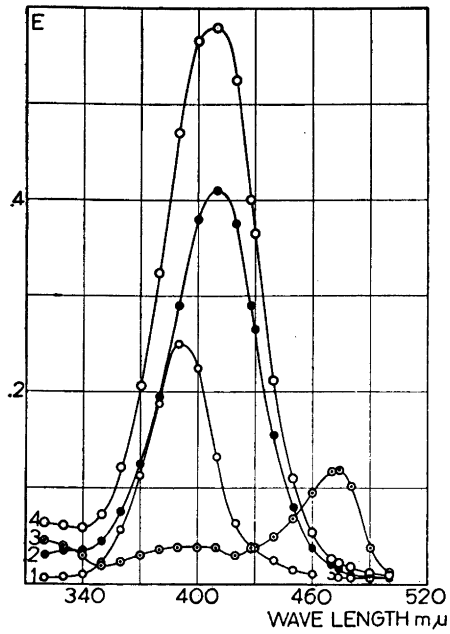

Fig. 1. Basic cysteine reaction. 1. Yeast nucleic acid (3.39 μg P). 2. 32.4 μg dextrose. 3. Desoxypentose nucleic acid (12.05 μg P). 4. 22 μg fructose. All curves read 10 to 20 minutes after adding cysteine HCl.

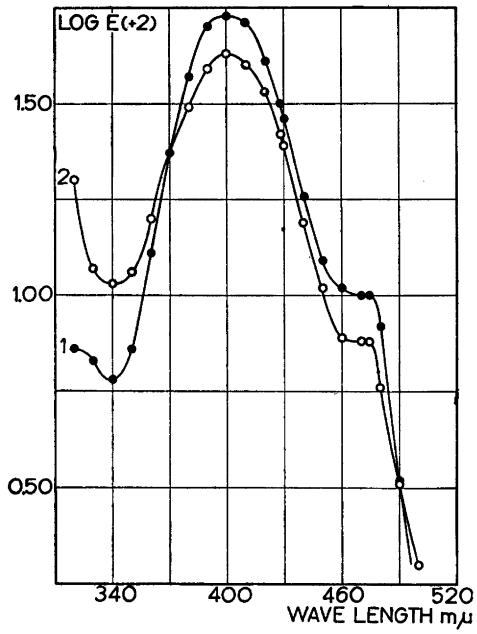

Fig. 2. Basic cysteine reaction. 1. A mixture of yeast nucleic acid (3.39 μg P), 32.4 μg dextrose and desoxypentose nucleic acid (9.6 μg P). 2. T. C. A. extract from human placenta. Curves read 10 to 20 minutes after adding cysteine HCl. Log extinction plotted against wave length.

RESULTS

As pointed out by Dische [3], every class of sugar yields spectrophotometrically measurable reaction products, when the reaction is done as described above. Fig. 1 shows the absorption curves for yeast nucleic acid, D. N. A., dextrose and fructose. Fig. 2 shows the absorption curve for a hot T. C. A. extract from human placenta. On comparing this curve with that obtained from a mixture of yeast nucleic acid, D. N. A. and dextrose, it is clear that one or more hexoses are present.

It will be seen in Fig. 1 that dextrose and D. N. A. show the same extinction at 390 mμ and 428 mμ. The difference in extinctions of P. N. A. at these wave lengths follows Beer's law. Thus, sugars behaving similarly to dextrose and desoxypentose do not interfere with the quantitative determination of P. N. A. Table 1 gives the figures for differences between extinctions at these

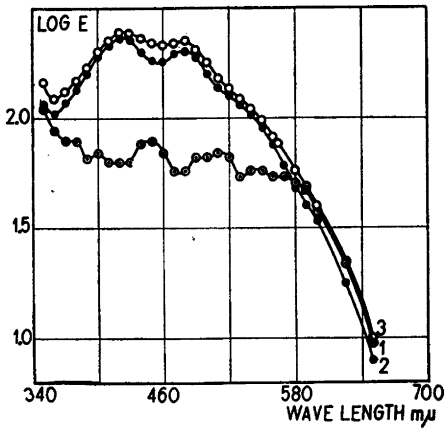

Fig. 3. Resorcin reaction. 1. T. C. A. extract. 2. 441 μg fructose. 3. 441 μg fructose + T. C. A. extract. Log extinction plotted against wave length.

wave lengths for various sugars. It will be seen that quantitative estimation of P. N. A. is possible in the presence of desoxypentose, dextrose, galactose, mannose, glucosamine, and galactosamine, but not in the presence of large quantities of fructose, fucose or uronic acids, especially not galacturonic acid.

The resorcin reaction was carried out to investigate whether fructose is present in the hot T. C. A. extract. Concentrated hydrochloric acid to give a final concentration of 12 %, and 0.1 ml of a 50 % resorcin solution were used. The mixture was placed in a water bath at 100° C for five minutes and the

Table 1. Differences of densities at 390 mμ and 428 mμ for various sugars in Dische's BCyR. Readings 10 minutes after addition of cysteine HCl.

Substance Amount: 10 μg	△ 390.428 × 1 000
Nucleic acid bound pentose *	107
Nucleic acid bound desoxypentose *	zero
Glucose	zero
Galactose	zero
Mannose	zero
Fructose	34
Fucose	16
Glucosamine	zero
Galactosamine	zero
Glucuronic acid	3
Galacturonic acid	32

* Calculated by the percentage of pentose of a structural polytetranucleotide.

Table 2. Recovery tests. Yeast nucleic acid added to T.C.A. extracts of human placenta.

μg P.N.A. phosphorus		Difference in per cent
Added	Found	
3.78	3.85	1.19
3.78	3.88	2.58
3.78	3.90	3.08
9.44	9.70	2.69
9.44	9.56	1.26
9.44	9.56	1.26
12.88	13.38	3.74
12.88	13.43	4.10
12.88	13.33	3.38
	Average	2.59

coloured product was extracted with amyl alcohol. Fig. 3 shows the absorption curves for different solutions. On comparing these curves it will be found that the presence of fructose in the T. C. A. extract is not probable. The fructose test was also carried out according to Roe's [16] method. The hot T. C. A. extract gave no measurable absorption at 490 mμ.

Dische's [3] test for fucose was done to find out if this substance could be detected in the hot T. C. A. extract. The difference between extinctions at 400 mμ and 380 mμ decreased slightly after two hours, as compared with the difference ten minutes after addition of 0.6 ml of water to the mixture. The result obtained speaks in favour of the absence of fucose in the extract. The possibility that the T. C. A. would depress the reaction was ruled out.

Galacturonic acid has never been demonstrated with certainty in animal tissues. Glucuronic acid, on the other hand, may be presumed to be present in placental tissue. It will be seen in Table 1 that the error associated with the estimation of nucleic acid-bound pentose in the presence of an amount of glucuronic acid equal to that of the nucleic acid sugar is fairly small, being about 2.8 %. It was considered desirable, however, to rule out the presence of glucuronic acid in the T.C.A. extract by direct evidence. To this end the glucuronic acid reaction devised by Dische [17] was used. The result of the test was such as to rule out the presence of any significant amount of glucuronic acid in the hot T.C.A. extract. The T.C.A. had no effect on this reaction.

These tests seem to show that the P.N.A. content in T.C.A. extracts of human placenta can be determined by this method.

The relation between concentration and absorption was investigated by the method of least squares [18,19]. When using a 1 cm cell, the method can be employed for amounts of P.N.A. phosphorus between 0.75 μg and 16 μg. The standard deviation ($n = 20$) expressed as a percentage of the average is 2.28.

The extinction per μg phosphorus shows some variation for different batches of yeast nucleic acid. Three batches were investigated. All the preparations were purified by the method of Hammarsten. The extinction values were a maximum of 7.9 % apart, the average extinction being 0.062 per μg phosphorus.

Table 2 summarizes the data from a series of recovery tests. Known amounts of purified yeast nucleic acid were added to T.C.A. extracts of human placenta and estimated by the method outlined above.

SUMMARY

The applicability of a modification of the pentose-cysteine reaction to the estimation of P.N.A. in hot T.C.A. extracts of human placental tissue has been investigated. The influence on the reaction of fructose, fucose and glucuronic acid, carbohydrates which are probably present in this kind of tissue, has been demonstrated. The presence of these substances in the hot T.C.A. extracts has been ruled out. The accuracy of the method has been evaluated by statistical analysis, the standard deviation being 2.28 % of the average.

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REFERENCES

1. Mejbaum, W. *Z. Hoppe-Seylers Physiol. Chem.* **258** (1939) 117.
2. v. Euler, H., and Hahn, L. *Svensk Kem. Tidskr.* **58** (1946) 251.
3. Dische, Z. *J. Biol. Chem.* **181** (1949) 379.
4. Schneider, W. C. *J. Biol. Chem.* **161** (1945) 293.
5. Brody, S. *Exptl. Cell Research* **3** (1952) 702.
6. Brody, S. *Acta Chem. Scand.* **7** (1953) 502 and 507.
7. Corey, E. L. *Am. J. Physiol.* **112** (1935) 263.
8. Jones, G. E. S., Gey, G. O., and Gey, M. K. *Bull. Johns Hopkins Hosp.* **72** (1943) 26.
9. Gurin, S. *Proc. Soc. Exptl. Biol. Med.* **49** (1942) 81.
10. Dickens, F., and Greville, G. D. *Biochem. J. (London)* **27** (1933) 832.

11. Needham, J. *Biochemistry and Morphogenesis*, Cambridge 1942.
12. Huggett, A. St. G., Warren, F. L., and Warren, N. V. *J. Physiol.* **113** (1951) 258.
13. Schneider, W. C. *J. Biol. Chem.* **164** (1946) 747.
14. Brown, A. H. *Arch. Biochem.* **11** (1946) 269.
15. Hammarsten, E. *Acta Med. Scand.* **196** (1947) 634.
16. Roe, J. H. *J. Biol. Chem.* **107** (1934) 15.
17. Dische, Z. *J. Biol. Chem.* **171** (1947) 725.
18. Snedecor, G. W. *Statistical Methods*, Iowa State College Press, Ames 1938.
19. Bonnier, G., and Tedin, O. *Biologisk Variationsanalys*, Bonnier, Stockholm 1940.

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