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A Spectrophotometric Study on the Desoxypentose Nucleic Acid — Cysteine Reaction

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In 1944 Dische [1] described the reaction between desoxypentose nucleic acid (D.N.A.) and cysteine in sulphuric acid. Stumpf [2] presented a paper dealing with the quantitative colorimetric application of Dische's reaction to the specific estimation of D.N.A. This reaction has been used by Hedén [3], Kurnick [4] and others.

In an investigation [5,6] of the amount of nucleic acids in various organs the present writer used, among other methods, the extraction procedure described by Schneider.[7] D.N.A. was estimated according to Stumpf's method, but the values showed a great scatter. In my experience the error inherent in the method is fairly great. Stumpf gives no information about the standard error, nor do others. According to Stumpf the extinction curve follows Beer's law for amounts between 25 and 550 μg D.N.A. Amounts below 100 μg, however, give an extinction less than 0.1, when read in a Beckman spectrophotometer using a 1 cm cell.

In most of my preparations the total amount of D.N.A. was less than 100 μg. In an attempt to use the D.N.A.-cysteine reaction under these conditions and in order to investigate the various factors affecting the reaction, a systematic investigation of the reaction has been made.

EXPERIMENTAL

Concentration of sulphuric acid. It was observed that the sensitivity of the method was considerably increased when concentrated acid was used. The colour produced is yellow and there is a very sharp absorption peak at 474 mμ (Fig. 1). The optimal final concentration of the acid is 86.5 per cent, which means 5 ml of concentrated acid in a total volume of 5.55 ml (Fig. 2).

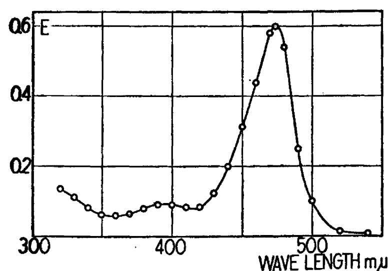

Fig. 1. Absorption spectrum for the reaction product of D.N.A. and cysteine in concentrated sulphuric acid.

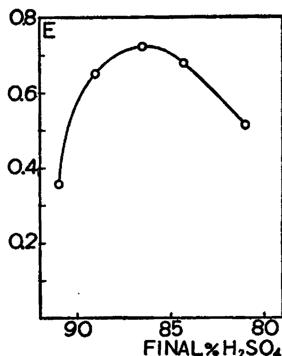

Fig. 2. Influence of concentration of sulphuric acid on the intensity of the D.N.A.-cysteine reaction.

Concentration of cysteine hydrochloride. Maximal absorption, other factors being unchanged, was obtained with 0.05 ml of a 1 % cysteine hydrochloride solution on a total volume of 5.55 ml (Fig. 3). A preparation from Merck (U.S.A.) gave the most consistent results.

Influence of temperature. It was observed that the temperature had a pronounced effect on the reaction. To get reproducible results it is necessary to avoid overheating at the moment of mixing the D.N.A.-solution and the sulphuric acid. The test tubes are therefore thoroughly chilled and the sulphuric acid is cooled to at least −10° C before mixing is done. The cold acid is then pipetted into the test tubes along their sides, so that two layers are formed. This is done with the test tubes immersed in an ice-water bath. The contents are then mixed.

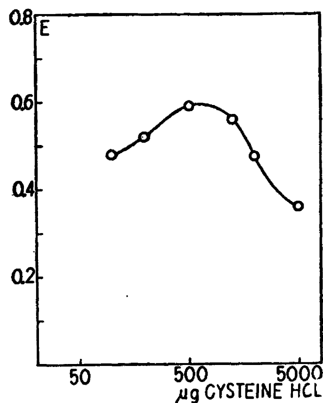

Fig. 3. Influence of concentration of cysteine HCl on the intensity of the D.N.A.-cysteine reaction.

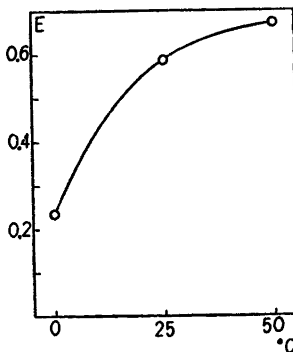

Fig. 4. Influence of environmental temperature during colour development.

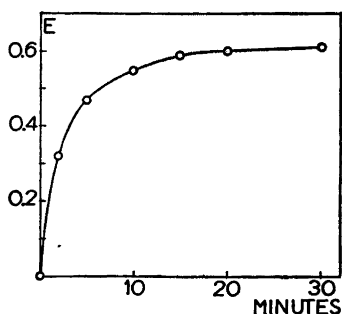

Fig. 5. Rate of colour development.

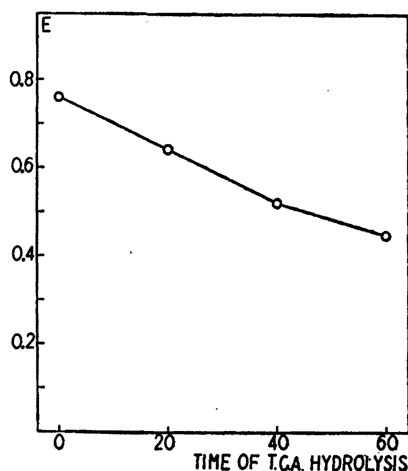

Fig. 6. Influence of trichloroacetic acid hydrolysis of D.N.A. on the intensity of the D.N.A.-cysteine reaction.

When the tubes are allowed to stand at room temperature until reading in the spectrophotometer, there is a considerable scatter in the results. To study the influence of the environmental temperature during colour development the tubes were immersed in a water bath at 0°, 25° and 50° C immediately after mixing. The results are shown in Fig. 4.

Colour development. The increase in colour intensity is very marked during the first few minutes after mixing. After fifteen minutes the curve flattens out (Fig. 5).

Influence of trichloroacetic acid (T.C.A.) hydrolysis of D.N.A. This part of the investigation was carried out with a view to apply this method of determination to nucleic acid extracts according to the technique devised by Schneider [7]. The D.N.A. preparation used

Table 1. Recovery tests.

Mixture	μg D.N.A.P.		Difference in per cent
	Added	Found	
D.N.A. + P.N.A.	1.30	1.34	3.0
—»—	8.90	8.78	1.4
—»—	5.08	4.97	2.2
D.N.A. + Schneider-extract	3.62	3.67	1.3
—»—	2.42	2.47	2.3
—»—	4.83	4.75	1.7
—»—	4.20	4.24	0.9

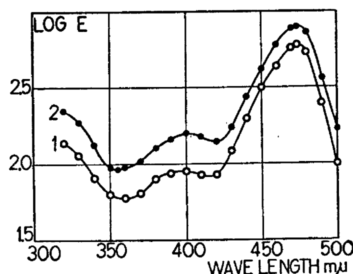

Fig. 7. D.N.A.-cysteine reaction. 1. D.N.A. solution. 2. T.C.A. extract of human placenta (time of T.C.A. hydrolysis 20 minutes). Log extinction plotted against wave length.

as a standard was prepared by the method of Hammarsten [8]. When an aqueous solution of D.N.A. was diluted with the same volume of 10 % T.C.A., a cloudy precipitate was obtained. This precipitate was dissolved by warming the solution in the water bath at 90° C for a few minutes. The influence of T.C.A. hydrolysis for varying length of time at this temperature on the intensity of the D.N.A.-cysteine reaction is shown in Fig. 6. As 0 minutes hydrolysis is taken the value obtained by determination of an aqueous solution of D.N.A.

Specificity of the reaction. 1 mg of yeast nucleic acid and 0.2 mg of either dextrose, galactose, mannose, galactosamine, glucosamine or glucuronic acid gave no extinction at 474 mμ.

Proposed method of determination of D.N.A. The above mentioned observations illustrate the sensitivity of the method. The marked influence of a variety of factors stresses the necessity of working under strictly defined conditions.

The D.N.A. solution to be determined should amount to 0.5 ml. To this is added with an 'Agla' micrometer syringe 0.05 ml of a 1 % l(+)cysteine hydrochloride solution. The test tubes are then placed in an ice-water bath for about ten minutes. 5 ml of properly chilled concentrated sulphuric acid is then added as described above. After mixing the contents of the tubes, these are placed in a water bath at 25° C. The optical densities

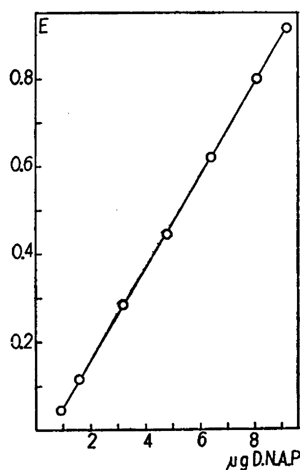

Fig. 8. Standard absorption curve for D.N.A. hydrolysed in 5 % T.C.A. for 20 minutes.

Regression line $y = -0.056 + 0.1054\,x$

$\bar{x} = 60.54 \pm 6.548.$

$\bar{y} = 0.454 \pm 0.0553.$

Regression coefficient 0.1054 ± 0.000625

Correlation coefficient 0.9996 ± 0.000179

$\sigma(y-Y) = [0.006395] = 1.54$ *per cent.*

y = *extinction* (E)

x = *μg D.N.A. phosphorus*

are read after exactly (stop watch) 20 minutes in a Beckman quartz spectrophotometer at 474 mμ wave length using a 1 cm cuvette. A reagent blank is run.

Recovery tests. A known amount of D.N.A. was added to pentose nucleic acid solutions and to hot T.C.A. extracts of human placenta. The D.N.A. was determined according to the method described here. The results are summarized in Table 1.

To demonstrate further the applicability of this reaction to the estimation of desoxypentose nucleic acid in a hot T.C.A. extract the absorption curves for a pure D.N.A. solution and a hot T.C.A. extract according to the reaction described here were determined. (Fig. 7.)

Statistical analysis. The relation between concentration and absorption has been investigated. The data fit a straight line for amounts of D.N.A. phosphorus between 0.96 μg and 9.2 μg (Fig. 8). The rectilinear line has been calculated by the method of least squares [9,10]. The standard deviation is calculated as the square root of the mean square. As this figure is a logarithm, the standard deviation as a percentage of the average has been calculated from the antilog of the standard deviation [11]. It amounts to 1.54 per cent.

SUMMARY

The colorimetric determination of D.N.A. by the cysteine reaction has been investigated and a standard procedure is proposed. The concentration range falls between 0.96 μg and 9.2 μg D.N.A. phosphorus. The error of the method is 1.54 per cent. The influence of T.C.A. hydrolysis of D.N.A. on the cysteine reaction has been studied with a view to apply the method to T.C.A. extracts of nucleic acids.

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