The application of the CERIOTTI reaction to determination of deoxyribonucleotides

CERIOTTI has described the colorimetric determination of deoxyribonucleic acid by a reaction with indole in 3 *N* HCl[1,2]. The earlier method of DISCHE using a lower concentration of HCl is less sensitive[3]. However, of the monodeoxyribonucleotides (or deoxyribosides), only thymidylic acid[4] has been reported to yield a color in the CERIOTTI procedure. A colorimetric response of several additional deoxyribonucleotides is described herein.

The procedure used is similar to that described by CERIOTTI[1]. To 2 ml of sample containing 0.100 μmole of deoxyribosidic compound in a 16 × 125 mm culture tube is added 1 ml 0.04 % indole and 1 ml conc. HCl (sp.gr., 1.18) with mixing after each addition. The tube is closed with a Teflon-lined screw-cap and placed into a boiling water bath within 5 min after acid addition. After heating for a given time, the tubes are cooled in a water bath at 20°. Absorbancies are determined at 490 mμ in curvettes with a 1-cm light path using a Beckman model DU spectrophotometer. Unless otherwise stated, absorbancies are without chloroform extraction. Chloroform extraction, when necessary, is carried out prior to rereading absorbancies, by twice shaking with 4 ml of reagent-grade chloroform (Mallincrodt Chemical Works, St. Louis, Missouri) and aspirating off the chloroform. Readings are against a reagent blank treated like the sample.

Fig. 1 shows the absorbancy at 490 mμ, and the effect of heating time for 5 deoxyribosidic compounds after being subjected to the indole–HCl treatment. The color development from each compound indicates the color response is general for deoxyribonucleotides. This is in contradiction to the inference of CERIOTTI that probably only the purine deoxyribonucleotides should respond[1]. However, the absorbancy arising from deoxycytidylic acid and deoxyuridine is less for a given concentration, than for the two purine deoxyribonucleotides and thymidylic acid. The increase in colorimetric response of the pyrimidine deoxyribotide compounds on heating beyond 10 min is in distinct contrast to the decrease seen with the purine

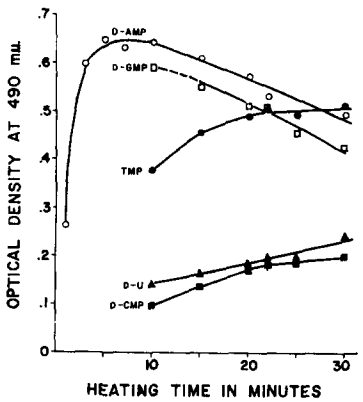

Fig. 1. Color formed by deoxyribosidic compounds on heating with indole and HCl for various times. 0.10 μmole in 4 ml final volume. O—O Deoxyadenylic acid (D-AMP); □—□ Deoxyguanylic acid (D-GMP); ●—● Thymidylic acid (TMP); ▲—▲ Deoxyuridine (D-U); ■—■ Deoxycytidylic acid (D-CMP).

deoxyribonucleotides. Heating beyond 30 min gives poor results because of the appearance of turbidity. The absorbancy at 490 mμ resulting from heating deoxyribosidic compounds with indole and HCl for 22 min is presented in Table I. The results show that the method is not completely specific for deoxyribosidic compounds, but that absorbancy arising from a ribosidic compound is readily extracted by chloroform, as contrasted to the poor extraction from a deoxyribosidic compound. Hence, specificity may be checked by an absorbancy reading after chloroform extraction. Duplicate determinations of deoxyribosidic solutions varied by an average of 2 % from the mean and determinations of different days varied in a range of 10 %. The reagent blank for 22-min heating has an absorbancy of around 0.042 against water. The determination may not be carried out in the presence of ammonium formate since upon heating 0.5 *M* ammonium formate containing indole and HCl, a dense orange color appears which is removed only by repeated extractions into chloroform, in which it assumes a pink color.

TABLE I

RESULT OF HEATING VARIOUS COMPOUNDS WITH INDOLE AND HCL FOR 22 MIN

Compound (0.10 μmole in 4 ml final sol.)	*Absorbancy at 490 mμ*	*after two extractions with $CHCl_3$*
Deoxyadenylic acid	0.528	0.468
Deoxyguanylic acid	0.511	0.450
Thymidylic acid	0.503	0.444
Deoxycytidylic acid	0.164	0.166
Deoxyuridine	0.196	0.148
Adenylic acid	0.085	0.014
Guanylic acid	0.108	0.028
Cytidylic acid	0.005	
Uridylic acid	0.010	

A method previously described for purine and pyrimidine deoxyribonucleotides involves reaction with cysteine and conc. H_2SO_4[5,6]. The sensitivity of this method for deoxycytidylic acid approximates that of the indole–HCl method in terms of concentration in the final reaction volume; however, the indole–HCl method is three or more times as sensitive on the basis of concentration prior to reagent addition. The indole–HCl determination of deoxyribosidic compounds described here is useful because it avoids the use of conc. H_2SO_4, and because of its directness and ease. It may be applied to determine if a purified nucleotide is a deoxyribonucleotide. The method is not generally suitable for determining total deoxyribosidic content of a mixture of deoxyribonucleotides monophosphates because of their differing molar extinction coefficients in this procedure.

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Received June 19th, 1959