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Brief Notes

The Chemical Determination of Deoxyribonucleic Acid in Tissue Cultures.*

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The protein content and dry weight of tissue fragments may vary considerably without any real change in cell number. In view of the rather constant amount of deoxyribonucleic acid per cell found for any given species (1) it has been suggested that it should be used for the measurement of cell number (2). In tissue culture studies where it has been used in this way micro modifications of the procedure of Schmidt and Thannhauser (3, 4) have been employed for the determination of deoxyribonucleic acid phosphorus. With very small amounts of tissue this requires considerable technical skill and the fractionation is tedious. For this reason the method has not been generally applied to the measurement of growth in tissue cultures although its theoretical desirability has been recognized. This communication describes a rapid and simple method for deoxyribonucleic acid determinations on amounts of tissue of the order of 1 mg. dry weight.

The determination is essentially a direct application of the method of Ceriotti (5). The original method depended on the application of a modification of Dische's indole reaction (6, 7) for deoxyribonucleic acid to a tissue fraction obtained by the method of Ogur and Rosen (8). It was found that the fractionation procedure could be replaced by simple extraction of the tissue with hot perchloric acid, deoxyribonucleic acid being estimated by applying Ceriotti's method to the extract.

The final method adopted was as follows:[1]

Method

The tissue is washed quickly with BSS to remove excess medium. (This is usually done by mixing the cells with BSS and centrifuging to separate them from the washing, which is discarded.) Two ml. 1.0 N perchloric acid are added to the tissue in a centrifuge tube and the mixture incubated in a water bath at 70°C. for 20 minutes, stirring occa-

[1] *Reagents:*

1. Hanks's balanced salt solution (BSS).
2. 1.0 N perchloric acid.
3. 0.04 per cent indole solution (dissolved by warming).
4. Concentrated hydrochloric acid (36 per cent *w/w*).
5. Chloroform.
6. DNA standard solution. A few mg. of a pure specimen of DNA are dissolved in 1.0 N perchloric acid by heating at 70°C. Ultraviolet absorption and/or phosphorus content are determined on it. For use this stock standard solution is diluted with 1.0 N perchloric acid to give a phosphorus content of about 1 μg. per ml. which corresponds to an optical density at 260 mμ of about 0.275, a 1 cm. cell being used.

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sionally with a platinum wire. After centrifugation the supernatant fluid is decanted off and retained. One ml. 1.0 N perchloric acid is added to the tissue and the incubation and extraction repeated, the second supernatant fraction being added to the first.

TABLE I

Determination of Deoxyribonucleic Acid by the Indole Method

Comparison of estimates made by performing the reaction on simple hot perchloric acid extracts and on completely fractionated tissues (as described by Ceriotti). Figures represent the means and standard deviations of estimates on six equal samples in each case.

Tissue	Complete fractionation DNA found	Perchloric acid extraction DNA found
	μg	μg
Embryonic chick liver	27.3 ±1.49	27.3 ±2.82
Embryonic chick heart	17.0 ±0.94	17.1 ±1.4
Strain L cell	24.52 ±1.75	24.52 ±0.073

Two ml. of the combined supernatant fractions are pipetted into a boiling tube, followed by 1 ml. indole solution and 1 ml. concentrated hydrochloric acid. The tubes are covered with caps to prevent contamination and placed in a boiling water bath for 10 minutes. After removal and cooling the samples are extracted three times with 4 ml. chloroform each time, the chloroform (denser) layers being discarded. The tubes are centrifuged for a few minutes at a low speed to clear emulsions and the optical density of the yellow color is determined at 490 mμ. Blanks, using 1.0 N perchloric acid in place of the test solution, and standards, containing 2 ml. of the diluted DNA standard, should be included in each set of determinations. As with all new methods the applicability of the technique to new materials should be checked by the simultaneous use of established methods in preliminary experiments.

Results

A comparison of estimates of DNA obtained by this simplified procedure with estimates on completely fractionated tissues is shown in Table I. The materials chosen represent typical tissue culture materials.

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