A SIMPLIFIED QUANTITATIVE ASSAY FOR TRITIATED THYMIDINE INCORPORATED INTO DEOXYRIBONUCLEIC ACID*

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The wide use in metabolic studies of H^3-TdR** as a precursor, which specifically labels DNA, prompted the development of this method. It incorporates the advantages of liquid scintillation counting, i.e., sensitivity to the weak beta emission of tritium, simplicity of sample preparation, and freedom from limitations of self-absorption. It does not require specific isolation of DNA from other acid insoluble cell constituents. Reproducibility has been tested and is reported here.

The method, as routinely employed, was applied to tritiated DNA prepared enzymatically (Bollum and Potter, 1958). The tritiated DNA solution (0.5 ml in a 10x75 mm incubation-hydrolysis tube) was freed of acid soluble compounds by the usual methods; acid precipitation (cold TCA, final concentration 7 percent in 0.03N HCl), ethanol wash, and two-fold alkali (0.1N NaOH) dissolution, and acid precipitation. Soluble tritiated thymine residues from the DNA, provided by formic acid hydrolysis (Wyatt and Cohen, 1958), satisfied the solubility requirements of the toluene liquid scintillation solution. This was accomplished by sealed tube heating of the dried residue with 0.25 ml of formic acid (88 percent) at 175°C for 30 minutes.

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** The following abbreviations were used: H^3-TdR, tritiated thymidine; DNA, deoxyribonucleic acid; TCA, trichloroacetic acid; POPOP, 1,4-bis-2-(5-phenyloxazolyl)-benzene; PPO, 2,5-diphenyloxazole.

The formic acid digest was transferred from the incubation-hydrolysis tube (Corning, pyrex, No. 9820) to a liquid scintillation counting vial (Packard Instrument Co.). Formic acid was removed in vacuo. The residue was extracted in the vial with 1 ml of absolute ethanol, at 37°C with shaking. This is the minimal amount of ethanol found necessary to stabilize the counting rate. Eighteen ml of liquid scintillation solution (15 mg POPOP and 4 gm PPO per liter of toluene) were added to the vial. Radioactivity was counted in a Packard automatic scintillation spectrometer.

The validity of this method was established by demonstrating that tritium was not lost through exchange during formic acid hydrolysis in the presence of stated amounts of DNA and protein. The experimental data establishing this fact appears in Table I. Under the experimental conditions of A, B, and C the count rates derived from the H^3-TdR are identical, within the limits of experimental error. This demonstrates that the tritium of H^3-TdR was not lost during formic acid hydrolysis. As seen in D, in the absence of DNA, variations in the counting rates of replicate samples are excessive. It appears from A, B, C, and D that the recovery of counts derived from H^3-TdR following formic acid hydrolysis was favored by the inclusion of DNA in the hydrolysis mixture. The results of E and F indicate that the washing procedure effectively removed any occluded H^3-TdR. Tritiated cytidine, when substituted for H^3-TdR under conditions of A and B of Table I, yielded variations in count rate from replicate samples, which were excessive. This contrast in lability of the tritium label probably results from the difference in the position of the label in the two compounds, thymidine being labeled exclusively in the methyl group (Friedkin, 1960).

By test it was found that the internal standard method (Hayes, 1956) provided precise correction for quenching materials introduced via the formic acid hydrolyzate. Two separate experiments, one in which H^3-TdR was held constant and the quenching material varied, and the other where the amount of

TABLE I

EFFECT OF FORMIC ACID HYDROLYSIS ON TRITIATED THYMIDINE

Procedure	cpm	mean cpm
A. Protein plus DNA; pptn.; hydrolysis; H^3-TdR; counted.	22394, 22081, 22972, 22787	22308
B. Protein plus DNA; pptn.; H^3-TdR; hydrolysis; counted.	22328, 22284, 22499, 22673, 22207, 22405, 21972	22338
C. DNA; pptn.; H^3-TdR; hydrolysis; counted.	23426, 23028, 22482, 22309	22811
D. H^3-TdR; hydrolysis; counted.	7140, 9842, 11731, 22258, 17116, 13313, 17322, 18194, 17322, 18194	14614
E. (Control) Protein plus DNA plus H^3-TdR; pptn.; hydrolysis; counted.	45, 45, 45, 42	44
F. Counting background.		40

The components, under Procedure, were added in the order given. The abbreviated entries signify: Protein, 0.8 mg in 100 µl; DNA, 100 µg in 200 µl; H^3-TdR, 100 µl containing 0.252 mµmoles of specific activity 0.495 Curies per mmole; pptn., acid precipitation and washing as in text; hydrolysis, 0.5 ml formic acid, tubes sealed, and heated at 175°C for 30 minutes; cpm, counts per minute.

a mixture of H^3-TdR and quencher was varied, demonstrated that accurate correction could be made. In both cases quencher was increased over a range four times that normally encountered in routine assays. In several thousand routine assays of H^3-TdR incorporation into DNA, the internal standard correction consistently has been a factor ranging between 10 and 12 percent.

The high reproducibility of this method is revealed by statistical analysis of routine experimental data derived from 87 different complete analyses, i.e., 29 groups in triplicate. Coefficients of variation, 100x σ/μ (where σ is the standard deviation of the three values in a group about their mean, μ) were calculated. These 29 coefficients were distributed as follows: coefficients with a value between 0 and 1.0, 5; between 1.0 and 2.0, 8; between 2.0 and 3.0, 6; between 3.0 and 4.0, 6; between 4.0 and

5.0, 2; between 5.0 and 5.2, 2. Replicate count rates on single samples varied between 0.0 and 1.5% at normal count rates, i.e., 500 to $5x10^4$ cpm.

This method is ideally suited to large numbers of samples; sixty are easily processed simultaneously. A major advantage of this procedure is the requirement of only one quantitative (liquid) transfer.

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