INCORPORATION OF [5-^{3}H] URIDINE INTO DNA

R. L. P. ADAMS

Department of Biochemistry, University of Glasgow, Glasgow, W.2, Scotland

Received 4 November 1968

Hayhoe and Quaglino [1] suggest that [5-^{3}H] uridine can be used as a specific label for RNA, a result quoted on the data sheet supplied with the compound from the Radiochemical Centre, Amersham. Their statement is the result of an autoradiographic study on phytohaemagglutinin stimulated lymphocytes, and it is doubtful whether a firm conclusion can be drawn from their data.

The situation is different however in L929 mouse fibroblasts, where, on incubation with [5-^{3}H] uridine up to one third of the acid insoluble radioactivity is in DNA. That the product is truly DNA is shown by its stability towards incubation in 0.3M NaOH for 16 hr at 37°, and its lability towards deoxyribonuclease.

5 cm plastic dishes were seeded with 3 ml of a suspension of L929 mouse fibroblasts (2×10^5 per ml) in minimum essential medium (Eagle) containing 10% calf serum (Flow Laboratories, Irvine, Scotland). The cells were incubated with [5-^{3}H] uridine (10 μc/ml; 28.8 c/mmole; the Radiochemical Centre, Amersham, Bucks) for 1 hr or for longer periods. The labelled cells were washed three times with cold Earle's balanced salt solution and extracted first with a small volume of 0.5N perchloric acid followed by 15 ml 5% trichloroacetic acid. The cells were then dissolved in 0.3N NaOH (37°, overnight) and acid insoluble radioactivity measured in a sample of the solution. The remainder was precipitated and the radioactivity in DNA assayed as described by Lieberman, Abrams and Ove [2]. The perchloric acid extract was boiled for 10 min to convert all derivatives to the monophosphate level and the neutralised extract chromatographed in ammonium acetate/ethanol/borate [3] to separate ribonucleotides from the monophosphates of deoxyuridine, deoxycytidine and thymidine.

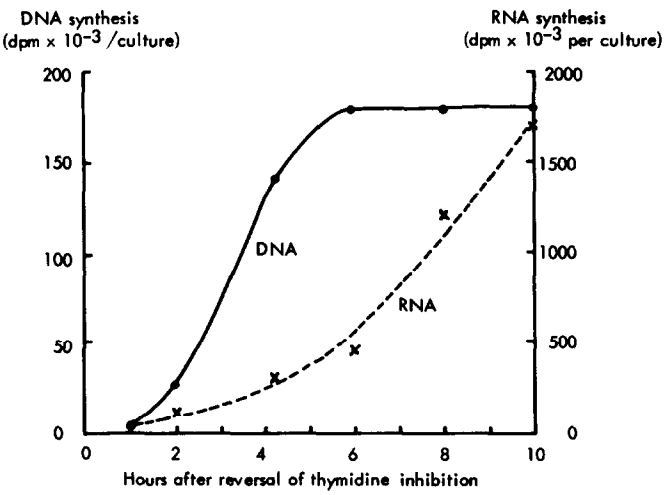

Fig. 1. L929 cells were incubated for 16 hr with 5 mM thymidine. The inhibiting levels of thymidine were removed by washing the cells with warm medium and the cells were incubated in the presence of [5-^{3}H] uridine (10 μc/ml 28.8 c/mmole). The method of assay of the incorporation of radioactivity into DNA and RNA is described in the text.

The kinetics of labelling of a synchronised cell culture were determined by incubating cells for 16 hr in 5 mM thymidine [4]. On removing the inhibitory levels of thymidine the cells were incubated with [5-^{3}H] uridine and fixed at varying times thereafter. Although radioactivity is incorporated into RNA regularly throughout the period of the experiment, tritium is incorporated into DNA primarily from 1–6 hr after reversal of the inhibition (fig. 1). Two hours after reversal of inhibition 80% of the acid soluble nucleotide material is ribonucleotide. Of the deoxyribonucleotide 59% is deoxyuridylate, 31% deoxycytidyl-

late and 10% thymidylate. This last figure is particularly surprising as tritium on the 5-position of uridine should be lost on methylation. The presence of radioactive deoxycytidylate (formed presumably by amination of UTP) explains how [5-^{3}H] uridine is incorporated into DNA as well as into RNA.

The result of this experiment should be contrasted with that of a parallel experiment using [6-^{3}H] uridine. Essentially similar results were obtained for incorporation of radioactivity into DNA, RNA and ribonucleotide. However at 2 hr after reversal of inhibition the distribution of radioactivity among the deoxyribonucleotides is as follows: deoxyuridylate, 49%; deoxycytidylate, 23%; and thymidylate, 28%. These results are consistent with the fact that the tritium on the 6-position of uridine is not lost on methylation.

The conditions in these experiments are perhaps those most likely to accentuate incorporation of [5-^{3}H] uridine into DNA via deoxycytidylate in that, after reversal of excess thymidine inhibition the proportion of cells making DNA is high (90%) and also, during excess thymidine treatment any pools of deoxycytidylate would have been exhausted. Under the conditions used by Hayhoe and Quaglino [1] one would expect that the proportion of tritium incorporated into DNA would be much lower.

The author wishes to thank Professors J.N.Davidson, F.R.S. and R.M.S.Smellie for their support throughout this work and Mrs. K.Mulholland for able assistance. The work was supported in part by funds supplied by the British Empire Cancer Campaign.

References

[1] F.G.J.Hayhoe and D.Quaglino, Nature 205 (1965) 151.
[2] I.Lieberman, R.Abrams and P.Ove, J. Biol. Chem. 238 (1963) 2141.
[3] H.Klenow and E.Lichtler, Biochim. Biophys. Acta 23 (1957) 6.
[4] D.Bootsma, L.Budke and O.Vos, Exptl. Cell Res. 33 (1964) 301.