THE TIMING OF PHOSPHOLIPID SYNTHESIS IN NEOPLASTIC MAST CELLS

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1. Introduction

It is known that DNA synthesis occurs at a discrete time (the S phase) during the life cycle of eukaryotic cells [1,2] whereas RNA and protein are synthesized throughout the intermitotic period, the rate of synthesis increasing during S [3–5]. The timing of phospholipid synthesis is of interest in so far as the event is a prerequisite for the construction of new membranes. Using choline as a specific precursor, we have now investigated phospholipid synthesis in neoplastic mast cells.

2. Materials and methods

P815Y neoplastic mast cells, kindly donated by Dr. G.A.Fischer, were grown as previously described [6]. Cell number and mean cell volume were measured with a Coulter model A counter [6].

Cells were synchronised by the addition of 2 mM thymidine [7] to an exponentially growing culture for 15 hr, which is the mean generation time. Cells were washed free of inhibitor, allowed to recover for approximately 7 hr and thymidine added for a further 15 hr. On removal of inhibitor, synchronous growth commences (fig. 1).

Radioactive precursors (Radiochemical Centre, Amersham, England) were added to cell suspensions (2–5 X 10^5 cells/ml) during exponential or synchronous growth. A sample (0.1–0.3 ml) was filtered (Whatman GF/C paper), washed with 5% trichloroacetic acid and incorporated radioactivity assayed in 7 ml of a solution containing 8 g Scintillator Butyl-PBD (CIBA) and 80 g naphthalene in 1 l of toluene-methyl Cellosolve (3:2, v/v) in a Beckman liquid scintillation spectrometer. Radioactive choline was incorporated solely into phospholipid, as determined by chemical analysis of cells exposed to [1,2-^{14}C] choline and [methyl-^{14}C] choline (unpublished observations).

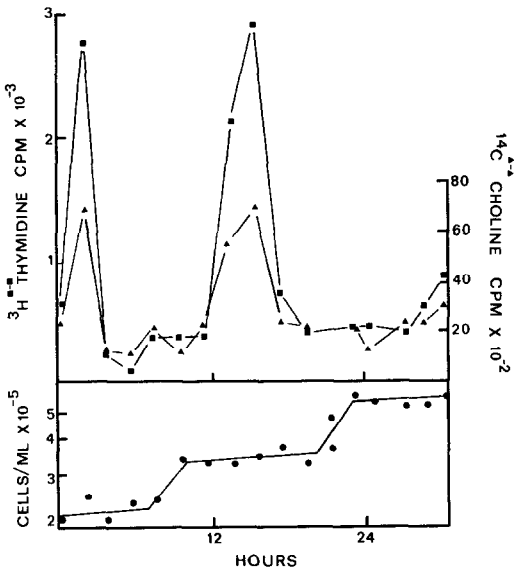

Fig. 1. Rate of DNA and phospholipid synthesis in synchronous P815Y cells. Samples were removed at intervals and exposed to a pulse of [^{3}H] thymidine and [^{14}C] choline for 30 min and analysed as described in Materials and Methods.

The percentage of exponentially growing cells incorporating radioactive precursors during a 15 min pulse (60 min in the case of choline) was determined by conventional autoradiography. Cells which had been exposed to a pulse of [^{3}H] choline were fixed in osmium tetroxide [8].

Gradient centrifugation through Ficoll was used to separate cells according to size and position in the cell cycle [9].

3. Results and discussion

Autoradiography of cells exposed to a pulse of [^{3}H] thymidine showed that 40% of the cells had incorporated the isotope. When exposed to a pulse of [^{14}C] uridine, [^{14}C] valine or [^{3}H] choline, 100% of the cells became labelled, clearly indicating that RNA, protein and phospholipid synthesis are continuous throughout the cell cycle. This result does not distinguish between different rates of synthesis, though some cells were definitely more heavily labelled than others in the case of choline.

In order to examine rates of synthesis, synchronously growing cells were exposed to a pulse of [^{3}H] thymidine, [^{3}H] uridine, [^{3}H] proline or [^{14}C] choline at intervals. Fig. 1 shows that choline uptake increases and decreases at approximately the same time as does thymidine incorporation. However in the case of thymidine the lowest points are not significantly above background whereas with choline they represent true incorporation. Thus the variation in rate is more than 8-fold with thymidine but only 3–4-fold with choline. Uridine and proline incorporation vary 2–3-fold, though the timing again coincides with thymidine uptake. That choline incorporation fluctuates less markedly than thymidine incorporation is confirmed by an experiment in which isotope was present throughout synchronous growth (fig. 2).

If phospholipid synthesis and membrane construction are coupled, an increase in choline incorporation might result in an increase in cell size. It is therefore interesting to note that the mean cell volume increased throughout the intermitotic period of synchronously growing cells.

Results obtained with cells which have been synchronised by thymidine treatment are open to the criticism that the cells pass through an unphysiological milieu, even though recovery from inhibition is rapid. An alternative method of studying the timing of macromolecular synthesis, namely the separation of cells by gradient centrifugation, was therefore used. Fig. 3 shows that cells near the top of the gradient have incorporated less isotope than those near the

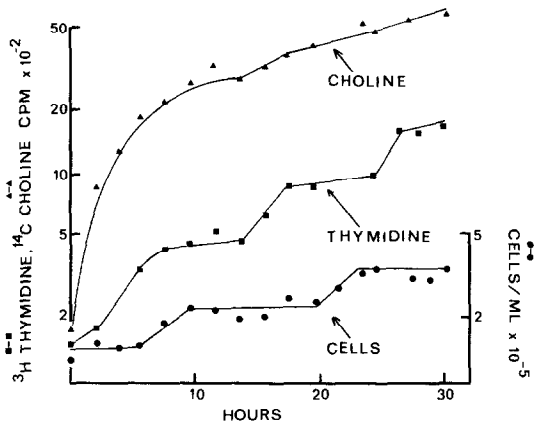

Fig. 2. DNA and phospholipid synthesis in synchronous P815Y cells. [^{3}H] thymidine and [^{14}C] choline were added at 0 time and samples were removed at intervals and analysed as described in Materials and Methods.

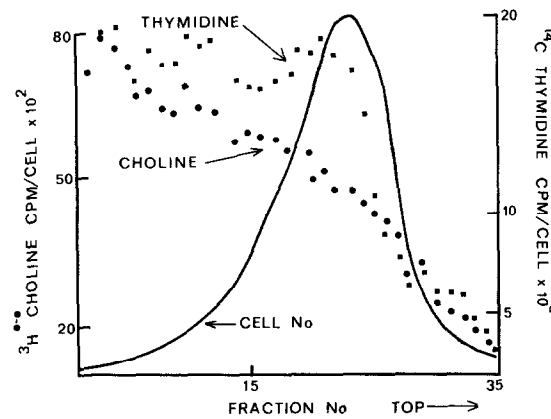

Fig. 3. Gradient centrifugation of P815Y cells. Exponentially growing cells were exposed to a pulse of [^{14}C] thymidine and [^{3}H] choline for 30 min and separated by centrifugation through a 5–10% gradient of Ficoll in growth medium at 80 X g for 7 min in an M.S.E. Mistral Swing-out Rotor (No. 62301) at 0°.

bottom, and that the increase in incorporation is sharper for [^{14}C] thymidine than for [^{3}H] choline. It may be wondered why the rates of incorporation do not decrease towards the bottom of the gradient, in view of the results of fig. 1. The reason is probably that G 2 cells are not well separated from S cells and

that the values obtained – at least in the case of thymidine – may therefore represent a mean of high incoporation (by S cells) and low incorporation (by G 2 cells). The pattern obtained with [^{3}H] uridine and [^{3}H] proline was similar to that of choline. The mean cell volume also increased gradually, like choline incorporation, from the top to the bottom of the gradient, suggesting that phospholipid synthesis and plasma membrane formation may indeed be related.

In general, the results of the experiments with cells separated by gradient centrifugation are in accord with those obtained by autoradiography and with synchronously grown cells. That is, the synthesis of phospholipid, like that of RNA and protein, is continuous throughout interphase, the rate increasing from a low value in G1 to a maximum prior to division.

Acknowledgements

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