'Inhibitor evidence for allosteric interaction in the replitase multienzyme complex' by G. Prem veer Reddy and Arthur B. Pardee, *Nature* 304, 86–88 (1983).

By an unfortunate error in the editorial office, the figures accompanying this article on page 87 were wrongly printed. Fig. 1 was omitted and Fig. 2*C* was labelled as Fig. 1. The page as it should have appeared is reproduced below.

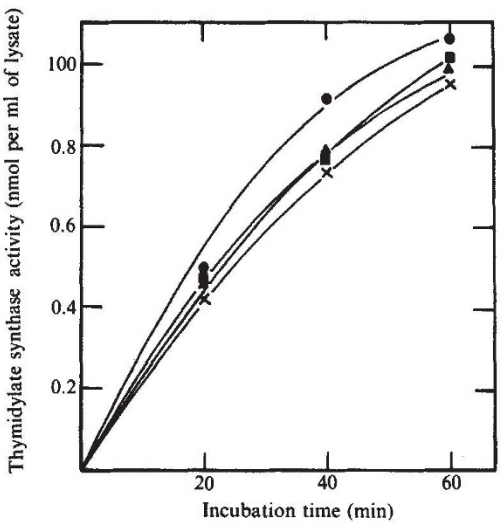

Fig. 1 Effect of hydroxyurea (×), novobiocin (▲) and aphidicolin (■) on thymidylate synthase activity in soluble extracts of S phase CHEF/18 cells. ●, Control. CHEF/18 cells were grown and synchronized by isoleucine starvation as described elsewhere[2]. When the cells were in mid-S phase, 12 h after release from an isoleucine block, they were trypsinized and suspended in ice-cold Dulbecco's modified Eagle's medium (DMEM). These cells were pelleted and resuspended in buffer A (mM): 150 sucrose, 80 KCl, 35 HEPES *p*H 7.4, 5 potassium phosphate *p*H 7.4, 5 $MgCl_2$, 0.5 $CaCl_2$, at a density of 5×10^7 cells ml^{-1} and homogenized in a Potter–Elvehjem glass homogenizer by applying 10 strokes at setting 3 in a Sorvall Omni-mixer (type 17105, DuPont Instruments) with a teflon pestle. The nuclei were obtained by centrifugation at 850*g* for 10 min. The nuclear pellet was suspended in ice-cold buffer A to a density of $\sim5\times10^7$ nuclear particles ml^{-1}, then sonicated in four pulses of 30 s each, using a Branson Sonifier (Model 185) at setting 2. The nuclear sonicate was centrifuged at 25,000 r.p.m. at 4 °C for 1 h in a Beckman SW 50.1 rotor with an adapter which allows the use of 0.8-ml tubes, and the supernatant was used as the soluble extract for thymidylate synthase assays, measured as described elsewhere[1]. Inhibitors were included in the reaction mixture at the following concentrations: hydroxyurea, 0.5 mM; novobiocin, 0.5 mM; aphidicolin, 10 $\mu g\ ml^{-1}$.

As shown in Fig. 1, hydroxyurea, novobiocin and aphidicolin had no inhibitory effects on thymidylate synthase activity in crude soluble extracts of S phase CHEF/18 cells as measured by the ^3H-H_2O formed from added [5-^{3}H]dUMP on its conversion to dTMP[5,6]. Thymidylate synthase, *per se*, is not sensitive to these inhibitors. In striking contrast, as shown in Fig. 2*A–C*, at similar or lower concentrations, they caused dramatic parallel inhibitions of thymidylate synthase activity and DNA synthesis measured in intact cells. These results demonstrate that thymidylate synthase activity is inhibited in intact cells by three agents which affect three other enzymes associated with DNA biosynthesis.

These observations could be accounted for if a metabolite such as dTTP accumulated during inhibition of DNA synthesis, and if this metabolite feedback-inhibited thymidylate synthase, or affected transport of ^{3}H-dUrd into the cell or kinase activity. The first condition seems unlikely because hydroxyurea prevents accumulation of deoxynucleotides[7]. The second condition appears to be ruled out because thymidylate synthase activity in permeabilized cells was not inhibited by excess dTMP or dTTP (50 μM) when the substrate, dUMP, was only 16 μM (ref. 1). Furthermore, hydroxyurea did not limit the accumulation of radioactive thymidine in acid-soluble pools of L929 cells[7].

Thymidylate synthase activity is present throughout the cell cycle, but in intact or permeabilized cells it is high only when the cells are in S phase[1,5]. During this phase thymidylate synthase is part of a sedimentable entity[1]. From these observations

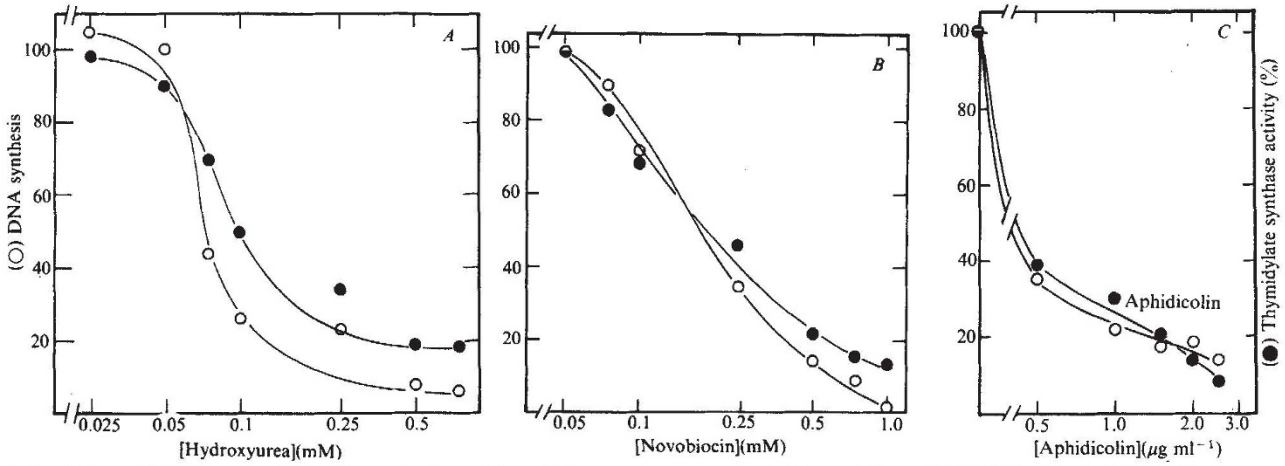

Fig. 2 Effect of inhibitors on DNA biosynthesis and thymidylate synthase activity in intact S phase CHEF/18 cells. *A*, Effect of hydroxyurea; *B*, effect of novobiocin; *C*, effect of aphidicolin. Open symbols, [6-^{3}H]dUrd incorporation; solid symbols, ^3H-H_2O formed from [5-^{3}H]dUrd. **Methods:** CHEF/18 cells were synchronized as described in Fig. 1 legend. Duplicate culture dishes were pulsed for 1 h at 37 °C with undiluted 5 $\mu Ci\ ml^{-1}$ [6-^{3}H]dUrd (24.5 Ci $mmol^{-1}$; NEN) or with 10 $\mu Ci\ ml^{-1}$ [5-^{3}H]dUrd (13 Ci $mmol^{-1}$; Schwarz Mann). Cells pulsed with [6-^{3}H]dUrd were processed to estimate the rate of DNA biosynthesis by washing monolayers twice with ice-cold phosphate-buffered saline (PBS), three times with 10% (w/v) trichloroacetic acid (TCA) and extracting the acid-insoluble material with 1 ml NaOH (0.2 M). Aliquots (100 μl) of alkaline extract were counted to determine moles of deoxyuridine incorporated into DNA. In control culture dishes, 100% [6-^{3}H]dUrd incorporation into acid-insoluble material was equivalent to 6.9 ± 0.2 pmol per 10^6 cells per min. Culture dishes pulsed with [5-^{3}H]dUrd were processed as follows to estimate thymidylate synthase activity in intact cells: the medium from these dishes was collected without disturbing the monolayer and analysed for ^3H-H_2O content formed by synthesis of dTMP from [5-^{3}H]dUMP, within the cell[5]. ^3H-H_2O was separated from [5-^{3}H]dUrd by filtering the medium through a 1-cm charcoal (activated Norit) bed in a Pasteur pipette, which retained >99% of the [5-^{3}H]dUrd. The filtrate contained all the released tritium as ^3H-H_2O; 100 μl of filtrate were counted for radioactivity. In control cultures 100% ^3H-H_2O formed from [5-^{3}H]dUrd was calculated to be 6.0 ± 0.5 pmol per 10^6 cells per min. To test the effect of inhibitors, various concentrations of aphidicolin (Wako), hydroxyurea (Sigma) or novobiocin (Sigma) were added to the culture dishes 30 min before pulsing with radioactive nucleosides, and processed as described above.

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