Conservation of desoxyribonucleic acid phosphorus in animal cells propagated *in vitro*

Recent studies on proteins[1,2,3] and nucleic acids[4,5] in *E. coli* have indicated that there is little or no metabolic renewal of these constituents during the logarithmic phase of bacterial multiplication. As a result, the question has been raised whether the renewal of proteins and nucleic acids observed in animal tissues[6] is, in reality, the result of intracellular turnover in those tissues. In this report we present the results of experiments bearing on the problem of phosphorus renewal in the deoxyribonucleic acid (DNA) fraction of a strain of animal cells propagated *in vitro*.

The cells used in these experiments were derived from Earle's L strain that was isolated from the subcutaneous tissue of a C3H mouse and continued later as the progeny of a single cell[7]. The methods employed for harvesting these cells from stock Kolle flask cultures, and distributing them as replicate cultures in T15 flasks for assay purposes, have been described in detail[8].

In the first type of experiment we wished to determine the kinetics of uptake of inorganic ^{32}P into the cellular DNA fraction from a medium of constant specific activity. The cells from two 7-day-old Kolle flask cultures propagated in a chemically defined medium No. 858[9] supplemented with 20% horse serum were harvested, and the cells washed with medium 635[10]. The cells were finally suspended in 80 ml of medium 635 and screened to remove clumps. While the resulting suspension was stirred, 0.5 ml portions (eventually found to contain $2.4 \cdot 10^5$ cells) were distributed into each of a series of T15 flasks that already contained 2 ml of a mixture comprised of 20% horse serum in medium 858 with a tracer amount of carrier-free inorganic ^{32}P as orthophosphate.

The flasks were incubated at 37° C to allow multiplication of cells, and at each of several intervals two were removed for cell count, two for estimation of DNAP and two for estimation of $DNA^{32}P$. The number of cells was determined by counting the cell nuclei[8]. DNAP was separated by a modified Schmidt and Thannhauser procedure and estimated colorimetrically[11], and $DNA^{32}P$ was measured in a liquid counter[12] after having been separated by the same technique. As shown in Fig. 1, cell multiplication was logarithmic for at least three generations with a generation time of 34 hours. Increase in DNAP was parallel to the increase in cell number and this result was consistent with previous observations[11].

Fig. 1 shows the change in specific activity of cellular DNAP in these cultures. Since the

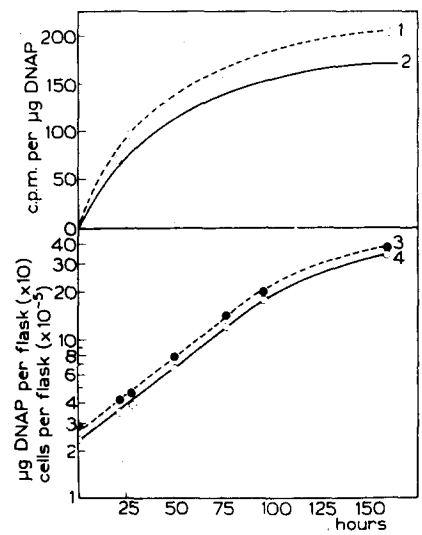

Fig. 1. Curves 1 and 2 show DNAP specific activities calculated according to the considerations given in the text; the points represent experimentally determined values. Curves 3 and 4 represent the amount of DNAP per culture and number of cell nuclei per culture, respectively. Each point shows the average result for two cultures.

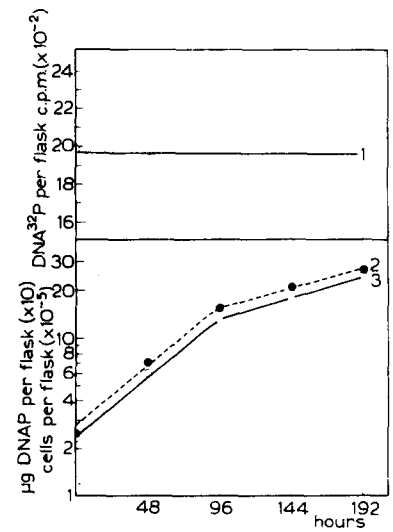

Fig. 2. Curve 1 shows the total ^{32}P in the DNA fraction during cell multiplication. Curves 2 and 3 represent the amount of DNAP per culture and number of cell nuclei per culture, respectively. Each experimental point represents the average for two cultures.

cells multiplied in a medium of constant specific activity, one may calculate the rate of increase in the specific activity of DNAP if it is assumed (a) that there is no renewal of DNAP and (b) that the size of the DNAP precursor pool is negligible. After one generation, the specific activity of cellular DNAP should have reached 50% of that of the medium, after two generations 75%, and after five generations almost 100%. For Curve 1, the specific activity of the medium was assumed to be that of cellular DNAP at the end of the experiment, *i.e.*, after four generations of cells. For Curve 2, the specific activity of the medium was calculated from the measured values of total phosphorus and inorganic ^{32}P. This estimate might be low since organically bound phosphorus in the medium is probably not used as rapidly as the inorganic phosphorus. Nevertheless, the experimental points show that uptake of ^{32}P into DNA followed the expected kinetics for four cell generations and suggest that little or no renewal of DNAP occurred.

In the second type of experiment, the release of ^{32}P from the DNA of labelled cells was measured over several generations. Two Kolle flask cultures were propagated for four days at 37° C in a medium containing 40% horse serum and 2.5% chick embryo extract, in Earle's balanced saline solution, and 0.25 mc of ^{32}P as carrier-free orthophosphate. The cells were then harvested, centrifuged, washed three times in Earle's saline, suspended in Earle's saline to remove clumps, and the resulting suspension, which contained $4.6 \cdot 10^5$ cells per ml, was distributed in 0.5 ml portions into a series of T15 flasks. These flasks already contained 2.0 ml of the horse serum, embryo extract, saline mixture mentioned above. They were then incubated at 37° C, and at 2-day intervals six flasks were withdrawn for duplicate measurement of DNAP and DNA^{32}P, and for cell counts, as in the first experiment. On the fourth day, 1 ml of fluid from each of the remaining cultures was replaced with fresh medium. The results are shown in Fig. 2. There was little loss of ^{32}P from cellular DNA during multiplication in isotope-free medium.

The results of both types of experiment show that when L strain cells from the mouse multiply *in vitro* the phosphorus of the DNA fraction is not renewed to any significant extent. They also demonstrate, for this system at least, that replication of DNA does not occur in the manner postulated by STEVENS *et al.*[13] and by DAOUST *et al.*[14].

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The specific esterification of the carboxyl groups of gelatin with methanol and thionyl chloride

Carboxyl esterified gelatin was desired for studies of physical properties of modified gelatins. The alkyl sulfate and alkali[1] method could not be used as alkali causes extensive hydrolysis and as the method is not selective. The diazomethane[2] method and the methanol-acetic anhydride[3] method are also not selective. The alcohol and hydrochloric acid[4] method was successful in producing a high degree of esterification with methanol but we have found that extensive degradation occurred during the long reaction time (7 days) required. BRENNER AND HUBER[5] have reported that alcohol and thionyl chloride give good yields of amino acid esters while leaving the amino groups intact. We here report the application of the method for the first time to a protein, namely, gelatin.

The esterification of gelatin was largely complete in three hours. The amino groups were not methylated as indicated by Van Slyke amino nitrogen determination. The methoxyl content