Automatic Monitoring of Biochemical Parameters in Tissue Culture

STUDIES ON SYNCHRONOUSLY GROWING MOUSE MYELOMA CELLS

By N. J. COWAN and C. MILSTEIN

Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.

(Received 26 January 1972)

An instrument is described that will maintain a population of mammalian cells at constant cell density while automatically monitoring the growth rate of the culture and the extent of precursor incorporation into a variety of cell products. The apparatus was used in an investigation of cyclic changes in the incorporation of labelled precursors into the DNA, RNA, total protein and myeloma protein synthesized in synchronous cultures of a mouse myeloma line. The incorporation of [^3H]uridine into trichloroacetic acid-insoluble material reveals a slight periodicity, with maxima and minima corresponding to late S phase and the mitotic phases respectively. The incorporation of [^3H]lysine into total intracellular protein also shows a slight oscillation, with maxima and minima occurring during the respective G2 and mitotic phases. Cyclical changes in the synthesis of serologically precipitable myeloma protein were found to vary somewhat according to the conditions used to synchronize the cells. In experiments conducted with 4.0mM-thymidine, maximal incorporation of label took place during S phase or early G2 phase. Experiments with 1.0mM-thymidine revealed a significantly less marked periodicity of myeloma protein synthesis.

Methods of obtaining synchronously dividing populations of mammalian cells generally depend either on a physical selection of cells in a particular phase of the cell cycle (Terasima & Tolmach, 1963; Sinclair & Bishop, 1965; Warmley & Pasternak, 1970; Shall & McClelland, 1971) or on the imposition of a reversible chemical blocking agent that operates at a specific point in the cell cycle (Xeros, 1962; Puck, 1964; Sinclair, 1965; Stubblefield & Klevecz, 1965). The application of chemical blocking procedures necessarily entails a variation in the times that different cells spend in the blocked state, as some cells will reach the block in advance of others. Apart from possible toxic effects of the blocking agent itself, this variation can lead to a subsequent condition of unbalanced growth (Rueckert & Mueller, 1959; Adams, 1969), which might influence the initial events observed in cultures synchronized by chemical procedures. For example, the use of thymidine results in an overall increase in mean cell volume and a decrease in the duration of the cell cycle probably associated with the continued synthesis of RNA and protein during the blocked state (Studzinski & Lambert, 1969; Bergeron, 1971). In order to examine the possibility that cyclical events observed shortly after synchronization by a chemical method are not grossly influenced by the synchronization procedure, it may be necessary to follow the biochemical processes under study for a number of generations after the removal of the blocking agent. Although disruption of growth can be avoided by using a

physical selection procedure, only a small proportion of a total randomly growing population may be selected if a satisfactory degree of synchrony is to be obtained. Hence, if this method is to yield the quantity of synchronously growing cells required for many biochemical studies, an inconveniently large initial bulk of randomly growing culture is required.

In addition to the problem of unbalanced growth after chemical synchronization, a loss of synchrony is commonly encountered after only one synchronous division (Pederson & Robbins, 1971). As this observation imposes a limit on the useful duration of experiments, it is often not clear whether cyclic phenomena are reproducibly observable over a number of generations after synchronization. The loss of synchrony might be due to inherent variability in the generation time of different cells in the culture. A further factor may be that, because each synchronous division involves an overall doubling of cell number, the growth conditions do not remain constant during the course of the experiment. Consequently the cultures rapidly approach a density which, under normal conditions, would no longer permit exponential growth. Further, as the duration of the cell cycle frequently extends over 16h or more, the monitoring of cell number, mitotic index and other criteria of cell synchrony is both inconvenient and tedious, especially over a number of generations.

We have developed an instrument designed to maintain a population of synchronous or randomly

growing mammalian cells at constant cell density while automatically monitoring the incorporation of labelled precursors into the DNA, RNA, total protein and myeloma protein synthesized in synchronous cultures of a mouse myeloma line.

Experimental

Apparatus

The apparatus was constructed by linking a number of commercially available instruments, some of which required the modifications described below. In addition, the spinner flask (Fig. 1, B) and incubation chamber (Fig. 1, I) were made specifically for the apparatus (T. W. Wingent Ltd., Cambridge, U.K.). The complete instrument enables the maintenance of a mammalian cell culture at a chosen cell density (Fig. 1, steady-state culture unit); simultaneously, successive small samples of the culture fluid are automatically withdrawn from the culture bottle and incubated with a chosen isotopically labelled precursor for a preset period of time (Fig. 1, analytical unit). In experiments where a constant cell density is not required, the steady-state culture unit can be switched off.

All flexible tubing used in the apparatus was of uniform-internal-diameter (1.0mm) silicone rubber. The tubing and glassware were presterilized by autoclaving. The spinner flask (Fig. 1, B) and incubation chamber (Fig. 1, I) were maintained at 37°C by a closed-circuit water bath.

Steady-state culture unit. The glass water jacket surrounding the spinner bottle (Fig. 1, B) was interrupted at opposite ends of a diameter beneath the level of the paddle so as to allow the passage of a collimated beam of light (Fig. 1, Q). The absorbance of the cell suspension was monitored by using an adaptation of a Technicon AutoAnalyzer colorimeter (model 1), in which the light-path was increased to 12cm and the original collimator was replaced by a +16 dioptré lens. After traversing the culture, the light-beam is received by the colorimeter photocell (Fig. 1, S). The colorimeter is coupled to a 10mV Technicon potentiometric recorder (Fig. 1, RE). The central axle carrying the recorder slide-wire pulley is fitted with a 2.0mm-thick aluminium vane, which passes through a Mullard vane-switched proximity detector (model 2P/730/33) coupled to a relay switch. The switch in turn operates the LKB peristaltic pump (Fig. 1, P₁), which, when activated, simultaneously adds fresh medium to and withdraws spent fluid from the spinner flask. Thus, when the light-transmission across the spinner flask rises above a value determined arbitrarily by the orientation of the aluminium vane on the recorder axle, the peristaltic pump operates until the set transmission value is once more restored. In addition, the peristaltic pump is fitted with a cam such that, after the completion of every revolution, the output from the photocell (Fig. 1, S) is momentarily short-circuited. Each complete revolution of the peristaltic pump is therefore recorded as an abrupt momentary deviation

Fig. 1. Block diagram of the automatic monitoring apparatus

B, Water-jacketed spinner bottle (1 litre capacity); C, timer; F, fraction collector; G, gas inlet; I, incubation chamber; L, light-proof box; M, top-suspended magnetic paddle; S, photoelectric cell; P₁, peristaltic pump; P₂, syringe pump; Q, collimated light-beam; R₁, medium reservoir (Dulbecco-modified Eagle's medium); R₂, medium reservoir containing radioisotope; R₃, waste reservoir; RE, potentiometric recorder; V₁, V₂, V₃, valves (cf. Fig. 2); W, sterile cotton-wool plugs; Y, mixing junction.

on the recorder trace. Thus the culture can be maintained at a set constant absorbance, while growth is assessed by the rate of renewal of medium required to maintain that value.

Analytical unit. Continuous pulsing of samples taken from the spinner flask was accomplished by using a modification of a Gilson Escargot fraction collector fitted with head model TS15P and its associated syringe pump (Fig. 1, P₂). In the original apparatus, a sample is removed from each successive fraction-collector tube and is redeposited after spectrophotometric analysis. In our instrument, this sequence of operations is modified by introducing a spring-loaded double valve and an intermediate incubation chamber (Fig. 1, I) kept at 37°C. Samples initially withdrawn from the spinner flask (Fig. 1, B) and a reservoir (Fig. 1, R₂) containing radioactive isotope are mixed, incubated and subsequently redeposited in the fraction collector (Fig. 1, F) for subsequent analysis.

Modifications to the Gilson fraction collector were constructed as shown in Fig. 2. D is a platform with a central vertical extension protruding 1.0cm above the existing horizontal deck (Z) of the fraction collector. The platform rests on two coil springs surrounding attached vertical shafts that pass through a fixed platform (X). When the fraction-collector bar (A) descends, the spring-loaded platform (D) is depressed so that tube V₁ is compressed while tubes V₂ and V₃ are opened. Conversely, when the fraction-collector bar (A) ascends, tubes V₂ and V₃ are compressed while tube V₁ is opened.

The cyclic operation of the fraction collector and syringe pump operates as follows. A signal from the timer (Fig. 1, C) is sent to the pump (Fig. 1, P₂), which expels the sample held in the chamber (Fig. 1, I) via V₁ into the fraction collector (Fig. 1, F). The fraction collector advances one position, and the syringe pump (Fig. 1, P₂) simultaneously withdraws culture fluid and medium containing radioisotope through the junction (Fig. 1, Y) so that complete mixing occurs before entry into the incubation chamber (Fig. 1, I). At the end of an arbitrarily fixed period, a signal from the timer activates the next cycle.

As the same proportion of culture fluid and isotopically labelled medium must be mixed before each incubation, hydrostatic-pressure differences must not be allowed to develop between the reservoir (Fig. 1, R₂) and the spinner flask (Fig. 1, B). Such pressure differences are avoided by using a reservoir with a horizontal cross-section identical with that of the spinner bottle.

Materials and Methods

The P3K (MOPC 21) mouse myeloma line was a gift from Dr. K. Horibata (Horibata & Harris,

Fig. 2. Detail of valves V₁, V_{2,3} of Fig. 1 incorporated in the Gilson instrument

A, Fraction-collector bar; D, spring-loaded platform; V₁, V_{2,3}, valves; X, fixed platform; Z, fraction-collector deck; □, existing Gilson apparatus; ■, additions to existing apparatus.

1970). P3 myeloma protein was purified from the sera of tumour-bearing Balb/c mice by J. Svasti.

P3 cells were grown in Dulbecco-modified Eagle's medium (Smith *et al.*, 1960), supplemented with 10% (v/v) of heat-inactivated horse serum (Grand Island Biological Co., London W.11, U.K.); the cultures were maintained under an atmosphere of N₂+O₂+CO₂ (83:7:10). Aceto-orcin stain was obtained as a 1% solution from E. Gurr Ltd., London S.W.14, U.K. In the reservoir containing radioactive precursors (Fig. 1, R₂), HEPES [2-(N-2-hydroxyethyl-piperazin-N'-yl)ethanesulphonic acid] (10mM) was used in place of bicarbonate in the medium. Dulbecco-modified Eagle's medium and HEPES were bought from Biocult Laboratories Ltd., Glasgow, U.K.

[¹⁴C]Thymidine (specific radioactivity 55mCi/mmol), [³H]thymidine (specific radioactivity 26Ci/mmol), [³H]uridine (specific radioactivity 8.1Ci/mmol) and [³H]lysine (specific radioactivity 18.8Ci/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Thiomersal [sodium 2-(ethylmercurithio)benzoate] and the non-ionic detergent Nonidet P40 were obtained from BDH Chemicals Ltd., Poole, Dorset, U.K. Thymidine was purchased from Sigma (London) Chemical Co., London S.W.6, U.K. All other chemicals were of analytical grade.

Synchronization procedure. Cells were synchronized by double thymidine block (Galavazi & Bootsma, 1966). Cultures at a density of 2.0×10^5 – 4.0×10^5 cells/ml were grown in either 1.0mM- or 4.0mM-thymidine for 12h. Cells were harvested, washed by resuspension in fresh Dulbecco-modified Eagle's medium (150ml) and allowed to grow for a further 5½h. Thymidine was then added back to the medium to the original concentration, and the cells were incubated for a further 12h. At the end of the second blocked period the cells were harvested by centrifugation, washed three times by successive resuspension in warm Dulbecco-modified Eagle's medium (100ml) and transferred to the spinner bottle (Fig. 1, B).

Monitoring conditions. In experiments conducted at constant cell density the instrument was adjusted to maintain the population at about 3.0×10^5 cells/ml. The reservoir (Fig. 1, R₂) was filled with HEPES-buffered Dulbecco-modified Eagle's medium containing [¹⁴C]thymidine. In some experiments [³H]-uridine was also added to the reservoir. In other experiments the reservoir contained HEPES-buffered Dulbecco-modified Eagle's medium lacking lysine, [¹⁴C]thymidine and [³H]lysine. In experiments with [¹⁴C]thymidine or with [¹⁴C]thymidine and [³H]uridine alternate fraction-collector tubes were prefilled with thiomersal [0.25ml; 0.1% (w/v) in 2.0M-tris-HCl buffer, pH7.2] and acetic acid (2.0M). In experiments with [¹⁴C]thymidine and [³H]lysine alternate fraction-collector tubes were prefilled with thiomersal and 0.5ml of Nonidet [5% (v/v) in Dulbecco-modified Eagle's medium].

Analytical procedures. Incubated cell suspensions were thus collected in either thiomersal, Nonidet or acetic acid. Cells retained in thiomersal were filtered on to glass-fibre discs and were washed successively with 20ml of 150mM-NaCl, 20ml of chilled 5% (w/v) trichloroacetic acid and 20ml of ethanol. The discs were dried in an oven at 70°C and their radioactivity contents measured by scintillation counting. Cells collected in Nonidet were centrifuged at 2500g for 10min to remove nuclei. The supernatants were divided into equal portions. To one portion, 0.5ml of sheep antibody to purified P3 myeloma protein was added, together with an equivalent proportion of purified P3 protein. As a control for non-specific precipitation of radioactive material, an equivalent proportion of normal sheep serum and rabbit antibody to normal sheep immunoglobulin G was added to the second portion. Both mixtures were incubated for 30min at 37°C and then for 12h at 4°C. The precipitates were collected by filtration on glass-fibre discs, washed with three successive 3ml portions of chilled 150mM-NaCl containing 25mM-sodium phosphate buffer, pH7.2, and dried in the oven at 70°C, and their radioactivity contents were measured by scintillation counting after immersion

in 3ml of 0.4% (w/v) BBOT [2,5-bis-(5-t-butylbenzoxazol-2-yl)thiophen; CIBA (A.R.L.) Ltd., Duxford, Cambridge, U.K.] in toluene.

Cells collected in acetic acid were used in the measurement of mitotic index. The cells were harvested by centrifugation and resuspended in 0.2ml of aceto-orcein. The number of cells fixed in mitosis was assessed by examination of each preparation under the microscope. At least 50 mitoses were scored in each case.

Results

Under the experimental conditions used the incorporation of [¹⁴C]thymidine into trichloroacetic acid-precipitable material was shown to vary linearly as a function of time in incubations of ½–2½h. The incorporation of [¹⁴C]thymidine as a function of cell density is shown in Fig. 3. Thiomersal (but not NaN₃) and Nonidet (Table 1) were found to be totally effective in terminating the incorporation.

Random and synchronous growth of cells

In a randomly growing culture (Fig. 4a) the recorder trace reveals a linear increase in absorbance.

Fig. 3. Incorporation of [¹⁴C]thymidine as a function of cell density

Portions of suspensions of cells at densities of 2.0×10^5 , 4.0×10^5 , 8.0×10^5 and 10.0×10^5 cells/ml were mixed and incubated at 37°C for 30min with 2.5ml of HEPES-buffered Dulbecco-modified Eagle's medium containing 0.02μCi of [¹⁴C]-thymidine/ml. The incubations were terminated by the addition of thiomersal and the cells were prepared for radioactivity measurement as described in the text.

Table 1. *Effect of inhibitors on the incorporation of [³H]thymidine and [³H]lysine*

Samples (2.5ml) of P3 cells at a density of 3.0 cells/ml were mixed and incubated for 30min at 37°C either with 2.5ml of HEPES-buffered Dulbecco-modified Eagle's medium containing [³H]thymidine (0.01 μ Ci/ml) or with 2.5ml of Dulbecco-modified Eagle's medium lacking lysine but containing [³H]lysine (5.0 μ Ci/ml). At the end of the incubation period the mixtures were left at room temperature (19°C) for various times in the presence of NaN₃, thiomersal or Nonidet P40 at the concentrations shown. The amount of radioactivity present as intracellular trichloroacetic acid-precipitable material (for untreated cells and cells treated with either NaN₃ or thiomersal) or as cytoplasmic serologically precipitable myeloma protein (for cells treated with Nonidet P40) was determined as described in the text.

Label	Inhibitor (final concn.)	Duration of exposure to inhibitor (h)	Radioactivity present in intracellular trichloroacetic acid-precipitable material or in serologically precipitable myeloma protein (c.p.m.)
³ H]Thymidine	None	0	1589
		24	10403
		48	17816
	NaN ₃ (0.05 %, w/v)	0	1514
		24	3455
		48	9876
		0	1375
	Thiomersal (0.005 %, w/v)	24	1399
		48	1221
³ H]Lysine	Nonidet P40 (1.0 %, v/v)	0	278*
		24	306*
		48	292*

* Radioactivity recovered in serologically precipitable myeloma protein.

Above a cell density of about 1.0×10^6 cells/ml there is a decline in the rate of increase of absorbance (not shown). There is a corresponding exponential increase in the rate of incorporation of [¹⁴C]thymidine trichloroacetic acid-precipitable material. Exponential growth followed by a stationary phase was confirmed by cell counting.

With the steady-state culture unit (Fig. 1) in operation, a randomly growing culture at a density of 3.8×10^5 cells/ml required a rate of turnover of medium of about 20ml/h (Fig. 4c). Sequential pulsing with [¹⁴C]thymidine yielded a constant incorporation of label under these conditions throughout the course of the experiment.

With a synchronized cell population the recorder trace reveals little or no growth for 6h after the second release from thymidine-containing medium (Fig. 4b). Thereafter there is a sharp increase in absorbance, which becomes less marked after about 9h. A second rise in the rate of increase of absorbance occurs at about 19h. The sequential pulsing of cell samples with [¹⁴C]thymidine under these conditions reveals a periodic oscillation, reflecting the synthesis of most of the cellular DNA in the S phase.

There is a rise in the mitotic index and cell number corresponding to those periods in which incorporation into DNA is minimal.

A synchronous culture maintained under steady-state conditions gave the result shown in Fig. 4(d). There is a slight periodic oscillation in cell number, which declines in magnitude during the experiment. Two bursts of division, indicated by the increased mitotic index and rate of turnover of medium in the culture, occur during the 7–10 and 20–23h periods. Pulsing with [¹⁴C]thymidine yielded a periodic curve essentially similar to that obtained without the imposition of steady-state conditions, with minima corresponding to the periods of maximal rate of turnover of medium and increased mitotic index.

Incorporation of [³H]uridine

The incorporation of [³H]uridine into total trichloroacetic acid-precipitable material in a synchronized culture maintained under steady-state conditions is shown in Fig. 5. Incorporation rises initially from almost zero, reaching a peak in the G2 phase. Thereafter a slight periodicity is maintained

Fig. 4. Random and synchronous growth under normal and steady-state conditions

With the steady-state culture unit switched off, a randomly growing culture of cells (2.1×10^5 cells/ml) (a) or a culture synchronized by double thymidine block (2.2×10^5 cells/ml) (b) was introduced into the spinner bottle and the number of cells/ml (○), mitotic index (i.e. the percentage of cells in the mitotic phase) (▲), optical extinction (----) and extent of [^3H]thymidine incorporation (●) were measured as described in the text. Correspondingly, under steady-state conditions, a randomly growing culture of cells (3.8×10^5 cells/ml) (c) or a synchronized culture (4.0×10^5 cells/ml) (d) was monitored in the same manner. The rate of turnover of medium (ml/h) required to maintain steady-state conditions is indicated by the continuous line.

Fig. 5. Incorporation of [³H]uridine in a synchronous culture maintained under steady-state conditions

A culture of cells synchronized with 4.0mM-thymidine was grown under steady-state conditions (3.6×10^5 cells/ml) and the incorporation of [¹⁴C]thymidine (0.01 μ Ci/ml) (●) and [³H]uridine (5.0 μ Ci/ml) (○) was followed automatically. Cells were prepared for radioactivity measurement as described in the text. —, Rate of turnover of medium (ml/h).

with minima and maxima occurring during the mitotic and late S phase respectively.

Incorporation of [³H]lysine into total protein and myeloma protein

The incorporation of [³H]lysine into total trichloroacetic acid-precipitable protein and myeloma protein in a synchronized culture under steady-state conditions is shown in Fig. 6. In cultures synchronized either with 1.0mM-thymidine (Fig. 6b) or 4.0mM-thymidine (Fig. 6a), incorporation into total protein rises initially and thereafter shows a slight periodicity, with maxima occurring in the G₂ period and minima roughly corresponding to periods of cell division. Incorporation into myeloma protein, however, varies somewhat according to the concentration of thymidine used in the synchronization procedure. In experiments conducted with 4.0mM-thymidine (Fig. 6a) there is slight periodicity, with maxima in late S phase or early G₂ phase. In contrast, experiments conducted with 1.0mM-thymidine (Fig. 6b) revealed that, although the degree of synchrony was only slightly inferior, the periodicity of myeloma protein synthesis was much less marked.

Discussion

The automatic system described in this paper embodies a number of advantages over previously described techniques (Schindler *et al.*, 1970; Periano *et al.*, 1970). The spinner flask is simple in construction, and the monitoring of absorbance *in situ* utilizes a maximum optical path with a consequently enhanced sensitivity. The rapid withdrawal and mixing of samples of culture fluid and medium containing label precludes the possibility of cell settling, a problem inherent in continuous-flow systems. The instrument also incorporates a high degree of flexibility. The culture may be maintained at any pre-selected cell density; the duration of each pulse can be varied over a broad range; any precursor or combination of radioisotopes may be included in the incubation mixtures; and experiments may be performed over any length of time.

In our synchronized populations, successive measurements of cell number, [¹⁴C]thymidine incorporation and mitotic index (Figs. 4a and 4b) reveal a reasonable initial degree of synchrony, which declines progressively during the course of the experiment. The rate of turnover of medium required to maintain the culture at a constant absorbance

Fig. 6. *Synthesis of total protein and myeloma protein in synchronized cultures*

A culture of cells synchronized with either 4.0mM-thymidine (a) or 1.0mM-thymidine (b) was grown under steady-state conditions (4.8×10^5 cells/ml), and the incorporation of [^{14}C]thymidine ($0.01 \mu\text{Ci/ml}$) and [^3H]lysine ($5.0 \mu\text{Ci/ml}$) was monitored automatically as described in the text. ●, [^{14}C]Thymidine incorporation; ○, [^3H]lysine incorporated into trichloroacetic acid-precipitable material; ▲, [^3H]lysine incorporated into detergent-releasable serologically precipitated myeloma protein; —, rate of turnover of medium (ml/h).

reflects the degree of synchrony somewhat less markedly (Fig. 4d). In experiments performed with the imposition of steady-state culture conditions, there is an increase in absorbance during periods when there is a smaller corresponding increase in cell number (Fig. 4b). This observation can be ascribed to the increase in mean volume of cells as they traverse the cell cycle (Anderson & Peterson, 1967; Anderson

et al., 1969), with a consequently enhanced propensity to scatter incident light. Thus, during experiments with synchronized cultures maintained in a steady state, there is a slight oscillation of cell density during each cell cycle in which the maximum deviation from the mean is about $\pm 10\%$. The extent of radioisotope incorporation in such experiments can be appropriately corrected to constant cell number, the

correction becoming smaller as the degree of synchrony declines.

The finding that the amount of labelled thymidine incorporated into synchronized cells never falls below 28% of the amount incorporated during the initial burst of synthesis (Figs. 4b and 4d) probably reflects a relatively imperfect degree of synchronous growth. However, the picture is complicated by the observation that in human liver cells (Koch & Stokstad, 1967) and in L 5178Y cells (Bosmann, 1971) mitochondrial DNA is synthesized in the G2 phase shortly before cell division.

The low initial incorporation and lower initial maximum of [³H]uridine incorporation relative to subsequent maxima (Fig. 5) presumably reflects unbalanced growth as a result of the synchronization procedure. This interpretation is consistent with the observation that RNA synthesis continues during exposure of cells to high concentrations of thymidine (Studzinski & Lambert, 1969; Bergeron, 1971). Thus there may be an initial lag period during which the accumulated RNA is depleted and its synthesis *de novo* is relatively low. Thus the variation in the extent of uridine incorporation in successive cell cycles after chemical synchronization emphasizes the desirability of long-term measurements. An increase in the rate of RNA synthesis after synchronization by mechanically selecting mitotic cells has been described in HeLa S3 cells (Scharff & Robbins, 1965) and in Chinese-hamster cells (Enger & Tobey, 1969). However, the latter studies did not include measurements showing a decrease in incorporation during the subsequent mitotic phase. Our results support the contention that such a decrease does indeed occur (Sinclair, 1967; Bergeron *et al.*, 1970; Warmley & Pasternak, 1970).

Some 20% of the protein-synthesizing capacity of myeloma cells is devoted to the production of homogeneous myeloma protein (Schenkein & Uhr, 1970). There is a delay of at least 1 h before newly synthesized myeloma protein appears extracellularly (Schubert, 1970; Melchers, 1970; Choi *et al.*, 1971). As we have used 30 min pulses with labelled lysine, the secretory lag allowed us to follow the production of both total intracellular trichloroacetic acid-precipitable protein and serologically identifiable myeloma protein in a synchronized culture. Our experiments (Fig. 6a) revealed only a slight periodicity in total intracellular protein production, a result similar to that found in synchronized populations of some cell lines (Pfeiffer & Tolmach, 1968; Martin *et al.*, 1969), but not in others (Bergeron *et al.*, 1970; Warmley & Pasternak, 1970). However, we failed to observe the distinct maximum of myeloma protein synthesis during late G1 and early S phase described in culture-adapted mouse myeloma cells by Byars & Kidson (1970). As the latter authors synchronized their cells with 1.0 mM-thymidine, we

repeated our experiment under these conditions (Fig. 6b), but once more failed to detect a dramatic periodicity of myeloma protein synthesis. Cytochemical examination of synchronized human lymphoid-cell lines by Buell & Fahey (1969) revealed that during mitosis there is a marked decrease of specific intracellular protein. However, as the mitotic phase extends over only a small proportion of the total cell cycle, a partial degree of synchrony would result in a relatively prolonged period during which different cells in the culture pass through the mitotic phase. Hence, even if no specific protein is produced during cytokinesis, this fact could be obscured by production of protein by cells about to enter, or just emerging from, the mitotic period. Alternatively, it is possible that in P3 cells the production of myeloma protein is decreased, but does not altogether cease, during cell division.

We thank Dr. U. Arndt for helpful discussions on the design of our instrument, and Mr. F. P. Walker for assistance in its construction.

References

- Adams, R. L. P. (1969) *Exp. Cell Res.* **56**, 55
- Anderson, E. C. & Peterson, D. F. (1967) *Biophys. J.* **7**, 353
- Anderson, E. C., Bell, G. I., Peterson, D. F. & Robey, R. A. (1969) *Biophys. J.* **9**, 246
- Bergeron, J. J. M. (1971) *Biochem. J.* **123**, 385
- Bergeron, J. J. M., Warmley, A. M. H. & Pasternak, C. A. (1970) *Biochem. J.* **119**, 489
- Bosmann, H. B. (1971) *J. Biol. Chem.* **246**, 3817
- Buell, D. N. & Fahey, J. L. (1969) *Science* **164**, 1524
- Byars, N. & Kidson, C. (1970) *Nature (London)* **226**, 648
- Choi, Y. S., Knopf, P. M. & Lennox, E. S. (1971) *Biochemistry* **10**, 668
- Enger, M. D. & Tobey, R. A. (1969) *J. Cell Biol.* **42**, 308
- Galavazi, G. & Bootsma, D. (1966) *Cell Res.* **41**, 438
- Horibata, K. & Harris, A. W. (1970) *Exp. Cell Res.* **60**, 61
- Koch, J. & Stokstad, E. L. R. (1967) *Eur. J. Biochem.* **3**, 1
- Martin, D., Jr., Tomkins, G. M. & Granner, D. (1969) *Proc. Nat. Acad. Sci. U.S.A.* **62**, 248
- Melchers, F. (1970) *Biochem. J.* **119**, 765
- Pederson, T. & Robbins, E. (1971) *J. Cell Biol.* **49**, 942
- Periano, C., Bocchetti, G. & Eisler, W. J. (1970) *Science* **169**, 204
- Pfeiffer, S. E. & Tolmach, L. J. (1968) *J. Cell Physiol.* **71**, 77
- Puck, T. T. (1964) *Cold Spring Harbor Symp. Quant. Biol.* **29**, 167
- Rueckert, R. R. & Mueller, G. C. (1959) *Cancer Res.* **19**, 1584
- Scharff, M. D. & Robbins, E. (1965) *Nature (London)* **208**, 464
- Schenkein, I. & Uhr, J. W. (1970) *J. Cell Biol.* **46**, 42
- Schindler, R., Ramseier, L., Schaer, J. C. & Griedler, A. (1970) *Exp. Cell Res.* **59**, 90
- Schubert, D. (1970) *J. Mol. Biol.* **51**, 287
- Shall, S. & McClelland, A. J. (1971) *Nature New Biol. (London)* **229**, 59

- Sinclair, N. K. (1967) *Proc. Int. Congr. Radiat. Res.* 3rd. 607
- Sinclair, R. & Bishop, D. H. L. (1965) *Nature (London)* **205**, 1272
- Sinclair, W. K. (1965) *Science* **150**, 1729
- Smith, J. D., Freeman, G., Vogt, M. & Dulbecco, R. (1960) *Virology* **12**, 185
- Stubblefield, E. & Klevecz, R. (1965) *Exp. Cell Res.* **40**, 660
- Studzinski, G. P. & Lambert, W. (1969) *J. Cell. Physiol.* **73**, 109
- Terasima, T. & Tolmach, L. J. (1963) *Exp. Cell Res.* **30**, 344
- Warmley, A. M. H. & Pasternak, C. A. (1970) *Biochem. J.* **119**, 493
- Xeros, N. (1962) *Nature (London)* **194**, 682