Kinetics of Polyamine Synthesis and Turnover in Mouse Fibroblasts

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Kinetics of polyamine synthesis and degradation were studied in mouse fibroblasts growing in suspension culture. The approach was to prelabel cells with radioactive polyamines and to observe the rate of loss of radioactivity and the rate of decrease in specific activity of these compounds in cells. Radioactive putrescine declined with a half-life of 1.5–2 h, whether derived directly from exogenous putrescine or indirectly from ornithine. Much of this turnover was due to excretion, the kinetics of which suggested that a steady-state was being established between putrescine inside and outside the cells. Within 5 h of medium change, cells growing at a density of 5×10^5 cells/ml had supplied putrescine to the medium to a concentration of about $1 \mu\text{M}$. When cells were prelabelled with either putrescine or spermidine, radioactivity in cell spermidine declined with a half-life of 60 h. This rate of turnover is sufficient to provide all the spermine required by the cell. Spermine synthesis was the only observed reaction of spermidine, although some excretion into the growth medium was detected. Spermine was not degraded at a detectable rate as long as cells were growing exponentially; in stationary phase, degradation to spermidine, which was excreted, became significant. The half-lives of the specific activities of spermine, spermidine and putrescine were 24, 15 and 1.5 h respectively. From these values, the rate of synthesis of each was calculated. Spermidine was synthesized at 6.8 times the rate of spermine, and putrescine was synthesized at $0.46 \text{ nmol}/10^6$ cells per h, twice the rate of spermidine. The significance of these kinetic parameters is discussed.

The pathway for the biosynthesis of the polyamines spermidine and spermine in animal cells is relatively well established (Cohen, 1971; Pegg, 1970; Williams-Ashman *et al.*, 1973), and has received much attention in recent years. The pathways of degradation, however, are poorly understood. It is known that enzymes present in the serum of various animal species, particularly ruminants, catalyse the oxidation of spermidine and spermine (Blaschko, 1966) to products that are highly toxic (Alarcon *et al.*, 1961; Bachrach *et al.*, 1967). Polyamine oxidation has also been observed in peroxisomes of rat liver (Hölttä, 1977). This oxidation results in the conversion of spermine into spermidine, and of spermidine into putrescine. These interconversions have also been reported by Sturman *et al.* (1976), working on retinal cells.

Acetylation of polyamines occurs *in vitro*, with preparations of chromatin from rat liver or kidney, but acetylated polyamines have not been found in extracts from these tissues. This may be due to the presence of enzymes catalysing further metabolism of these derivatives (Blankenship & Walle, 1977) or excretion from cells. Monoacetylspermidine has been

found in the urine of normal and cancer patients (Walle, 1973; Tsuji *et al.*, 1975; Abdel-Monem *et al.*, 1975), suggesting that acetylation may be involved in spermidine excretion, as for some bacterial systems (Cohen *et al.*, 1967). Other polyamine conjugates have also been detected in the urine of cancer patients, as reviewed by Bachrach (1976), and this has led to discussion of the use of polyamine derivatives as markers in the diagnosis and therapy of cancer (Cohen, 1977). Nothing is known about the synthesis of these conjugates.

The catabolism of putrescine is better understood than that of spermidine and spermine. In animal cells it is known to be a substrate for diamine oxidases from serum and some tissues (Zeller, 1965; Atkins & Beavan, 1975). In addition, the monoacetyl derivative has been found in various vertebrate tissues (Perry *et al.*, 1967; Seiler, 1973). The metabolism of this compound in brain has been described by Seiler & Al-Therib (1974); they found that it can either be deacetylated to free putrescine, or deaminated by monoamine oxidase. In the latter case, it serves as a precursor to γ -aminobutyric acid.

The search to define roles for the polyamines has revealed that they take part in a great variety of reactions, many involved with nucleic acid structure and function (Cohen, 1971; Bachrach, 1973). Esti-

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mates of the concentrations of these compounds in different cell compartments showed that both spermidine and spermine are concentrated in the cell nucleus (about 6 and 1.3 mM respectively), the concentration of each being about 3-fold lower in the cytoplasm (McCormick, 1977). In view of the relatively high concentrations of these compounds, and their involvement in such a large range of reactions, the question arises as to which of these reactions are actually sensitive to changes in the concentration of cell polyamines. A regulatory role has been suggested because in virtually all systems in which cells are induced to grow at an increased rate, one of the earliest events is a sharp increase in polyamine synthesis. Examples include regeneration of liver following partial hepatectomy (Hannonen *et al.*, 1972; Raina *et al.*, 1973), stimulation of lymphocytes by concanavalin A (Fillingame & Morris, 1973) and stimulation of 3T3 cell proliferation by growth hormone (Clark, 1974).

The ability of polyamines to regulate biochemical reactions depends on the ability of cells or organelles to alter polyamine concentrations at the sites of these reactions in response to appropriate stimuli. This in turn may depend to a large extent on the turnover rates of these compounds at these sites, since the time required to alter the steady-state concentration of a compound is a function of its rate of degradation or excretion (Berlin & Schimke, 1965). In this paper, I have studied the turnover of mouse fibroblast polyamines to elucidate the significance of these compounds as regulatory molecules, and to clarify details of their catabolic pathway.

Materials and Methods

Growth of cells

Cultures of mouse fibroblasts (strain L929, supplied by Dr. J. Kates, State University of New York at Stony Brook) were grown in suspension in Joklik-modified minimal essential medium (Grand Island Biological Co., NY, U.S.A.) supplemented with 5% horse serum (Flow Laboratories, Rockville, MD, U.S.A.). Horse serum was used since it contains little or no spermine oxidase activity. Cultures were free of mycoplasma contamination by the test of Hayflick (1971).

Estimation of polyamines

Polyamines were estimated by a modification of the procedure of Dion & Herbst (1971). Cell pellets were washed twice with phosphate-buffered saline (0.15 M-NaCl, 0.02 M-KCl, 0.02 M-NaH₂PO₄, pH 7.2) and polyamines were extracted with 2% HClO₄. Solid Na₂CO₃ was added to 200 μ l samples of acid extracts until effervescence ceased and carbonate was in excess (about 50 mg required). 5-Dimethylamino-naphthalene-1-sulphonyl chloride (400 μ l) (dansyl

chloride; Calbiochem, Spring Valley, NY, U.S.A.) in acetone (30 mg/ml) was added, mixed and the reaction tubes were left unsealed in a dark fume-hood overnight. This permits selective evaporation of the acetone. Benzene (22 μ l) was added and 50 μ l samples of the benzene extract were layered on Quantaglam LQ6D silica-gel plates; these plates have a stacking region that permits up to 100 μ l to be spotted if necessary. Since the acetone was allowed to evaporate, the volume of the benzene extract remains 200 μ l after mixing with the sample. This improves sensitivity and reproducibility. Dansyl-polyamines were separated by t.l.c. in cyclohexane/ethylacetate (2:1, v/v), and were estimated by scanning the plate with a Farrand t.l.c. scanner, and comparing fluorescence of samples with standards on the same plate. Radioactive derivatives were scraped from the plate and counted in 3a70 scintillation fluid (Research Products International, Elk Grove Village, IL, U.S.A.), at an efficiency of 7.2% for dansyl-[³H]polyamines or 19% for dansyl-[¹⁴C]polyamines.

Labelling conditions

All radioactive compounds were obtained from New England Nuclear, Boston, MA, U.S.A. Radioactive polyamines were checked for purity by the modified dansyl procedure described above. All of the compounds used contained less than 1% contamination. Specific radioactivities were: [4,7-¹⁴C]-spermine, 12 mCi/mmol; [aminopropyl-³H]spermidine, 500 mCi/mmol; [1,4-¹⁴C]putrescine, 50 mCi/mmol; DL-[5-¹⁴C]ornithine, 9.8 mCi/mmol; [methyl-³H]thymidine, 10 Ci/mmol.

These compounds were added to 50 ml of cell culture in the exponential phase of growth. After 6–10 h of labelling, the cells were centrifuged at 1000g for 10 min at 37°C. The pellets were washed twice with phosphate-buffered saline and resuspended in 100 ml of fresh medium at a cell density of 1–2 $\times 10^5$ cells/ml.

Under these conditions of labelling, the radioactive isotopes added to cells did not account for more than 1% of the total cell polyamine.

Results

Interpretation of data

The decrease of radioactive compounds, A, from cells following removal of exogenous label is expected to follow first-order kinetics, provided that the cells do not contain radioactive precursors to this compound.

$$\text{Rate of decrease of radioactive A} = -k_d \cdot [A^*] \quad (1)$$

where k_d is the rate constant for the decrease of the compound and $[A^*]$ is the amount of radioactive A in cells per unit volume of culture. Thus total radioactive A in cells per unit volume of culture at any

time, t , after removal of exogenous label ($t=0$) is given by the simple equation:

$$[A^*]_t/[A^*]_0 = e^{-k_d t} \quad (2)$$

and the time required to decrease the radioactivity in A by one-half is $(\ln 2)/k_d$.

If cells are in the exponential phase of growth, total unlabelled A accumulates as follows:

$$\text{Rate of accumulation of A} = k_s \cdot [A] \quad (3)$$

where k_s is a rate constant for accumulation of A, and $[A]$ is the amount of A in cells per unit volume of culture. If the amount of A per cell remains constant, it will obviously increase at the same rate as cell numbers. The amount of A in the culture at any given time is given by:

$$[A]_t/[A]_0 = e^{k_s t} \quad (4)$$

and the time required to double the amount of A in cells per unit volume culture is $(\ln 2)/k_s$.

The specific radioactivity of A ($[A^*]/[A]$) is obtained by dividing eqn. (2) by eqn. (4):

Specific radioactivity of A_t /specific radioactivity of

$$A_0 = e^{-(k_d + k_s)t} \quad (5)$$

and the time required to decrease the specific radioactivity by one-half ($t_{1/2SA}$) is:

$$t_{1/2SA} = (\ln 2)/(k_d + k_s) \quad (6)$$

If radioactive A is lost in exactly the same ways as unradioactive A, i.e. they are part of the same pool, then eqn. (1) holds for total A in the cell, and:

$$\text{Rate of loss of A} = k_d \cdot [A]$$

The rate of accumulation of A, as defined in eqn. (3), is a function of the rate of synthesis and the rate of loss of A:

$$k_s \cdot [A] = (\text{rate of synthesis of A}) - k_d \cdot [A]$$

From this equation, rate of synthesis of A = $[A] \cdot (k_s + k_d)$ and:

$$k_s + k_d = (\text{rate of synthesis of A})/[A]$$

Substituting this in eqn. (6), and rearranging gives:

$$\text{Rate of synthesis of A} = (\ln 2) \cdot [A]/t_{1/2SA}$$

$[A]$ was defined as the amount of a compound in cells per unit volume at any given time. If it is assumed that the amount of A per cell (α) is constant during the exponential phase, then by dividing the equation above by the number of cells per unit volume, we obtain:

$$\text{Rate of synthesis of A per cell} = (\ln 2) \cdot \alpha/t_{1/2SA}$$

This provides a simple means of determining the rate of synthesis of a metabolite in a growing cell culture, provided that the cell concentration of the metabolite

is known to be constant. The specific radioactivity is determined at various times after removal of radioactive isotope, and, knowing the amount of the metabolite per cell (α) under the conditions of the experiment, the rate of synthesis is determined directly. This technique has the advantage that since specific radioactivity is being measured, problems of inefficient recovery and sampling errors are avoided. Furthermore, the specific radioactivity need not be measured in absolute terms, since only the rate of decline is required; this is most conveniently expressed in terms of the time required to decrease the specific activity by one-half.

Spermine turnover

After 12 h of prelabelling with $[4,7-^{14}\text{C}]$ spermine, 91% of the radioactivity in cells was recovered as free spermine; 8% was in spermidine, and the remaining traces of radioactivity were in compounds that, after treatment with dansyl chloride, were not extracted into benzene as were dansyl-polyamines. These were not identified.

Fig. 1 shows that, after removal of exogenous radioactive spermine, there is essentially no detectable decrease in total radioactive spermine in the culture

Fig. 1. Turnover of radioactive spermine. Rapidly growing cells (at about 2×10^5 cells/ml) were labelled with $[4,7-^{14}\text{C}]$ spermine ($0.1 \mu\text{Ci/ml}$) for 10 h. After being washed twice with phosphate-buffered saline, cells were resuspended in 100 ml of fresh medium supplemented with 5% dialysed horse serum. Samples (7.5 ml) of the cell suspension were taken at various times, cell numbers were counted (●) with a Coulter counter, and the cells were sedimented by low-speed centrifugation. Radioactivity in cell spermine was determined (▲) as described in the Materials and Methods section.

Fig. 2. Rate of decrease in specific radioactivity of cell spermine

Total spermine was determined in extracts obtained as in the experiment described in the legend to Fig. 1; from this the specific radioactivity was calculated. $t_{1/2}$ for spermine was 24 h.

during the early period of the experiment, at which time cells were in the exponential phase of growth. Within the limits of sensitivity of this system (with 5–8% error on each estimation, and 95% recovery of spermine) it appears that there is no degradation of spermine in growing cells.

As cells approached the stationary phase, they lost radioactive spermine (Fig. 1); all of this radioactivity was recovered as acid-soluble material in the medium. Analysis of this medium after 60 h showed that 50–60% of the radioactivity was present in spermidine and the remainder as spermine. The source of this spermidine is unclear; it appears that radioactive spermine lost from cells has been converted into spermidine, although there is no evidence of this conversion within cells. The amounts of radioactivity in cell spermidine showed no increase over this period. In fact, the small amount of labelled spermidine present in cells at the end of the prelabelling period was lost with a similar time course to spermine. The possibility that spermine was converted into spermidine by enzymes present in the growth medium (from the horse serum) was discounted by control experiments in which radioactive spermine was incubated with medium alone for up to 48 h. The maximum loss of spermine observed was 14% over this period.

Since radioactivity in spermine does not change over the first 30–40 h of the experiment, the decrease in specific radioactivity of cell spermine is only a result of synthesis *de novo* of unlabelled spermine

Fig. 3. Release of radioactivity from cell DNA into growth medium

Cells were prelabelled with [^3H]thymidine (1 $\mu\text{Ci/ml}$) for 6 h, washed twice with phosphate-buffered saline and resuspended in fresh growth medium. At the times shown, 5 ml samples of the culture were centrifuged (1000g for 5 min), and the radioactivity in the supernatant was measured by mixing 0.1 ml samples in 3a70 liquid-scintillation fluid. The efficiency of radioactive counting was 14%.

(Fig. 2). The time required to double the total cell spermine in the culture, and thus to half its specific radioactivity, was 24 h. This is close to the doubling time of the cells (22 h in this experiment).

'Stationary phase' in this system is defined as the period during which there is no increase of cell number with time. It could either represent a cessation of growth, analogous to contact inhibition of monolayer cultures, or it could represent continued cell division accompanied by an increased rate of cell death. Cell viability paralleled cell numbers during exponential and stationary phases in these cultures (data not shown), as observed by other workers (e.g. Ortiz *et al.*, 1972), but the possibility remains that, after cell death, rapid cell breakdown renders cells too small to be counted. To distinguish between these two possible meanings of 'stationary phase', cells were labelled with [^3H]thymidine for 6 h, and the fate of [^3H]DNA was followed over a 72 h period. The results are shown in Fig. 3. While cells were in stationary phase, there was a significant increase in the rate of appearance of radioactivity in the medium. At the time this occurred, 90% of the radioactivity in cells was acid-insoluble, presumably as DNA. However, 60% of the radioactivity in medium after 70 h was acid-soluble. The release of radioactivity therefore appears to be from breakdown of cell DNA to acid-soluble components. The experiment was repeated with cultures labelled with [^3H]thymidine and [^{14}C]spermine. The release of spermine paralleled the breakdown of DNA and is, therefore, a result of cell death and degradation rather than excretion.

Fig. 4. Turnover and decrease in specific radioactivity of cell spermidine

This experiment was conducted exactly as in Figs. 1 and 2, except that cells were prelabelled with [^3H]-spermidine ($0.5 \mu\text{Ci/ml}$) instead of spermine. Symbols: $\circ$, specific radioactivity ($t_4=15\text{h}$); $\triangle$, radioactivity/ml of culture ($t_4=60\text{h}$).

Fig. 5. Turnover of radioactive putrescine

Details are as in Figs. 1 and 2, except that cells were prelabelled with [$1,4\text{-}^{14}\text{C}$]putrescine ($0.1 \mu\text{Ci/ml}$) for 6 h. The total putrescine in cell extracts did not change during this short time course. Thus the specific radioactivity decreases as total radioactivity.

total spermidine in the culture. This is the same as the doubling time of the cells; thus it appears that cellular spermidine concentrations remained constant during exponential growth.

Spermidine turnover

Total radioactivity in cell spermidine declined with a half-life of 58–60h after removal of exogenous [^3H]spermidine (Fig. 4). Of the radioactive spermidine lost 80% was recovered in cells as spermine, and the remainder appeared as free spermidine in the medium. At late times in the experiment (45–55h), an unidentified compound also appeared in the medium; this did not extract into benzene after treatment of extracts with dansyl chloride. These data suggest that spermine is the only major metabolite of spermidine in these cells. Also, it can easily be calculated that if one-half of the cell spermidine is turned over in 60h, one-fifth will be turned over in 20h, the generation time of the cells in this experiment. This turnover is sufficient to provide all the spermine required by the cell, since spermine concentrations are about one-fifth of spermidine concentrations in these cells (McCormick, 1977). Spermidine is, therefore, the only major precursor of spermine.

As discussed above, the decline in specific activity is a function of loss of radioactivity (by excretion and metabolism) and accumulation of unlabelled material. Knowing the half-life of the specific radioactivity (15h; Fig. 4), and the half-life of radioactive spermidine, the rate constant for spermidine accumulation can be calculated by using eqn. (6). The value obtained, 0.034h^{-1} , gives a doubling time of 20h for

Putrescine turnover

After 6h of labelling cells with [^{14}C]putrescine, radioactivity in cell polyamines was distributed in the following proportions; putrescine/spermidine/spermine, 100:250:22.5. The specific radioactivities were 2000, 1450 and 505 d.p.m./nmol respectively. No other radioactive compounds were detected in the cell extracts and <0.5% of the total radioactivity in cells appeared in acid-precipitable material.

Within 5h of removing exogenous radioactive putrescine, 95% of the radioactive putrescine in the cells had disappeared (Fig. 5). Of this radioactivity, 40% was recovered in the growth medium. The concentration of putrescine excreted into the fresh growth medium by cells at a density of 5×10^5 cells/ml was calculated to be approx. $0.5\text{--}1 \mu\text{M}$; this calculation was based on the initial specific radioactivity of cell putrescine and the radioactivity appearing in medium.

This rapid loss of putrescine from cells could have been due to an artifact of labelling conditions used. For example, exogenous putrescine might enter a separate pool to endogenous putrescine. To test this, cells were prelabelled with [^{14}C]ornithine instead of putrescine. The experiment was carried out in exactly the same way as with the putrescine label. It was found that putrescine synthesized endogenously was lost from cells in exactly the same way as was

Fig. 6. Exchange of putrescine between cell pools and growth medium

Cells were labelled with [5- ^{14}C]ornithine for 12h, washed twice with phosphate-buffered saline and resuspended in fresh growth medium containing [^3H]putrescine (1 $\mu\text{Ci}/\text{ml}$). Symbols: $\circ$, ^3H radioactivity; $\triangle$, ^{14}C radioactivity.

putrescine from exogenous label (Fig. 6). This experiment was repeated with the addition of [^3H]putrescine in the fresh growth medium. Fig. 6 shows that this label was taken up with a time course similar to that obtained for loss of [^{14}C]putrescine, implying that putrescine in cells equilibrates with putrescine in the growth medium.

The rapid turnover of putrescine ($t_{1/2}=1.5\text{--}2\text{h}$), and the relatively high levels of radioactivity already in spermidine after the prelabelling period, make it hard to assess the metabolic relationship between these compounds in quantitative terms. However, since no radioactive compounds other than polyamines were detected, it appears that polyamine synthesis is the only major pathway of putrescine metabolism in these cells.

Since radioactive putrescine is lost so rapidly from cells, the data obtained for spermidine turnover can be verified by using this system. Within 5 h of removal of exogenous putrescine, radioactivity in putrescine is <5% that of spermidine. This is insufficient to contribute to the pool of radioactive spermidine. The half-life of radioactive spermidine derived from putrescine was 58 h (data not shown), confirming the value obtained above and implying that exogenous spermidine enters the same pool as spermidine synthesized endogenously. Furthermore, the spermidine turnover data described in the previous section were obtained from spermidine labelled in the amino-propyl group, and would not have revealed any reconversion of spermidine into putrescine.

No evidence of this conversion was obtained from analysis of cell extracts, but in late times of the experiment (55–65 h after removal of label) radioactive putrescine appeared in the medium, comprising 40% of the radioactivity present.

Rates of polyamine synthesis

The rate of synthesis of a compound can be determined from its amount per cell, and the half-life of its specific radioactivity, as described above. The concentration of spermine in mouse fibroblasts growing exponentially was 1.1 nmol/ 10^6 cells. The half-life of its specific radioactivity (Fig. 2) was found to be 24 h. This gives a rate of synthesis of 0.035 nmol/ 10^6 cells per h. The rate of spermidine synthesis was also calculated, on the basis of a cell concentration of 5.2 nmol/ 10^6 cells and a half-life of specific radioactivity of 15 h. The rate obtained was 0.24 nmol/ 10^6 cells per h. These two rates are not strictly comparable, since cells grew at somewhat different rates during the determinations (doubling times of 22 and 20 h respectively).

The very rapid turnover of putrescine, and the variability of cell putrescine concentrations made estimates of the rate of synthesis of this diamine difficult. However, an approximation can be made by using a $t_{1/2}$ of specific radioactivity of 1.5 h and a concentration of 1 nmol/ 10^6 cells. These values give a rate of synthesis of 0.46 nmol/ 10^6 cells per h, of which more than one-half is for synthesis of spermidine and the remainder for excretion and provision of cell putrescine.

Discussion

Estimation of the rate of turnover of a cell or tissue component yields information concerning both its function and its metabolism. Such estimates are generally carried out with the use of trace radioactive isotopes, whose rates of disappearance are taken to represent the rates of metabolism and excretion of the unlabelled homologues. In many cases, it is difficult to follow the rate of disappearance quantitatively because of problems of recovery and sample preparation. It is more convenient to measure the decline in specific radioactivity of a compound, since this measurement is independent of recovery and variations between sample sizes. For example, Russell *et al.* (1970) attempted to estimate rates of turnover of the polyamines by measuring the decline in specific radioactivity of polyamines in rat liver and brain following removal of exogenous radioactive isotope. The present work shows that in one cellular system this decline in specific radioactivity is a function of the rate of synthesis rather than of degradation of a compound. In many tissues, the rate of synthesis may be equal to the rate of degradation, but in rapidly

growing cells in culture this is clearly not the case. Thus there is no detectable turnover of spermine in cells during exponential growth, but the specific radioactivity declines with a half-life of 24 h.

Excretion of spermine was insignificant during the exponential phase of growth, but increased dramatically as the cells approached the stationary phase. This was a result of extensive cell death and degradation, as measured by breakdown of labelled cell DNA. Some conversion of spermine into spermidine was also observed under these conditions, but such a conversion could not be detected in rapidly growing cells.

On the other hand, turnover of spermidine was almost entirely due to conversion into spermine. The rate of turnover was sufficient to provide all the spermine required by the cell. There was also detectable excretion of spermidine during cell growth, and cells in the stationary phase appeared to convert some spermidine into putrescine. In these experiments, cells were grown in the presence of horse serum instead of calf serum; this was to prevent oxidation of polyamines released from cells into the medium. It is well known that oxidized spermidine or spermine is toxic to tissue culture cells (e.g. Otsuka, 1971), and so it seems likely that production of toxic products from excreted polyamines may be a normal feature of tissue culture systems using calf serum to support cell growth.

Degradation of spermine to spermidine and spermidine to putrescine only occurred in the stationary phase, which with mouse fibroblasts signified an increased rate of cell death and breakdown. This degradation of polyamines may be brought about by the enzyme described by Hölttä (1977), as a result of release of oxidases from peroxisomes during cell breakdown.

The distribution of radioactivity derived from labelled putrescine, as with spermidine and spermine, did not reveal the presence of metabolites other than free polyamines. However, it must be emphasized that the sensitivity of the methods employed in the present paper would be insufficient to detect metabolites present at less than about 2% of polyamine concentrations. Similar results have recently been obtained in HeLa cells and during infection of HeLa cells with herpes simplex virus (F. McCormick, unpublished work).

The rapid loss of putrescine from cells after medium change has not been described in detail before, although it has been known for several years that some cells supply free putrescine to their growth medium (Pohjanpelto & Raina, 1972). More attention has been directed towards the rapid uptake of putrescine into cells after medium change (Pohjanpelto, 1976). The significance of the rapid loss of putrescine from cells to medium is that to maintain cell putrescine concentrations in the face of this loss, it would

appear necessary for cells to synthesize putrescine at a greatly increased rate. In the system described in the present paper, as in many others, a dramatic increase in ornithine decarboxylase activity accompanies medium change, or even dilution with fresh medium (Hogan, 1971; McCormick, 1977). Furthermore, it is well established that addition of putrescine to fresh medium prevents the increase in ornithine decarboxylase activity (Jänne & Hölttä, 1974). In the present paper it is shown that cells actually supply putrescine to the culture under normal conditions, and it is possible that this putrescine contributes to the regulation of cell ornithine decarboxylase activity.

In a kinetic analysis of polyamine turnover, it is necessary to consider cells as if they contained precursors and products in a single homogeneous compartment. If this were not so non-linearity in the exponential plots of decrease in radioactivity or specific radioactivity with time would be expected. For example, if a significant fraction of the spermidine in the culture was not available for further metabolism owing to compartmentation in the cell, a biphasic decline in radioactive spermidine would be expected. This was not found, and so to a first approximation cell spermidine appears to be as one pool in terms of its activity as a precursor to spermine. This approach would not, however, detect small localized variations in rates of metabolism. It was shown that spermidine is concentrated in the cell nucleus, and that this may be the site of polyamine synthesis (McCormick, 1977), but it appears that the equilibration of spermidine between nucleus and cytoplasm is sufficiently rapid relative to the rate of spermidine utilization that spermidine from both compartments acts as one pool serving as a precursor to spermine.

The rates of turnover of spermidine and spermine are very low, with half-lives much longer than the generation time of the cells. This imposes considerable restrictions on the ability of cells to bring about rapid variations in polyamine concentrations throughout the cell, because the time required to alter the steady-state concentration of a compound is a function of its rate of degradation. For example, by the approach of Berlin & Schimke (1965), it can be calculated that to double the concentration of cell spermidine ($t_{\frac{1}{2}}$, 60 h), in a period of 1 h, it would be necessary to increase the rate of synthesis by 27-fold. To bring about this increase in spermidine concentration in 4 h would require a 14-fold increase in rate of synthesis. The concentration of spermidine could also be increased by decreasing the rate of conversion into spermine, but this of course would extend the time required to bring about a spermine increase. It must be stressed that these calculations assume an even distribution of spermidine throughout the cell and refer to the time required to increase the total cell content of polyamines. Rapid localized changes

in polyamine concentration at biologically significant sites are by no means excluded.

In conclusion, the data presented here indicate that in these rapidly growing animal cells, the conversion of putrescine into spermidine and spermine is essentially unidirectional, and no other reactions of these compounds occur at detectable rates. The pathway is reversed in cells in the stationary phase, and the products of the reverse reaction appear in the growth medium. These observations differ from those of Siimes (1967), who observed simultaneous synthesis and degradation of polyamines in slices of rat liver.

The low turnover rates of spermidine and spermine imply that rapid fluctuations in total cell polyamine concentrations are unlikely to occur. However, it is well established that increased polyamine concentrations are associated with increased rates of growth; when these increases in polyamine concentrations are necessary, such as in long-term effects on cellular growth rates and proliferation, it appears that dramatic increases in the rate of polyamine synthesis are essential to effect these increases in a short time. Such increases in the activities of the enzymes of polyamine synthesis are well documented in the literature (Cohen, 1971; Bachrach, 1973).

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References

- Abdel-Monem, M. M., Ohno, K., Fortuny, K. & Theologides, A. (1975) *Lancet* **ii**, 1210
- Alarcon, R. A., Foley, G. E. & Modest, E. J. (1961) *Arch. Biochem. Biophys.* **94**, 540-541
- Atkins, F. L. & Beavan, (1975) *Biochem. Pharmacol.* **24**, 736-768
- Bachrach, U. (1973) *Function of Naturally Occurring Polyamines*, Academic Press, New York
- Bachrach, U. (1976) *Ital. J. Biochem.* **25**, 77-93
- Bachrach, U., Abzug, S. & Bekierkunst, A. (1967) *Biochim. Biophys. Acta* **135**, 174-181
- Berlin, C. M. & Schimke, R. T. (1965) *Mol. Pharmacol.* **1**, 149-154
- Blankenship, J. & Walle, T. (1977) *Arch. Biochem. Biophys.* **179**, 235-242
- Blaschko, H. (1966) *Pharmacol. Rev.* **18**, 39-45
- Clark, J. L. (1974) *Biochemistry* **13**, 4668-4674
- Cohen, S. S. (1971) *Introduction to the Polyamines*, Prentice-Hall, Englewood Cliffs
- Cohen, S. S. (1977) *Cancer Res.* **37**, 939-942
- Cohen, S. S., Hoffner, N., Jansen, M., Moore, M. & Raina, A. (1967) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1626-1630
- Dion, A. S. & Herbst, E. J. (1971) *Ann. N.Y. Acad. Sci.* **171**, 723-724
- Fillingame, R. H. & Morris, D. R. (1973) *Biochem. Biophys. Res. Commun.* **52**, 1020-1025
- Hannonen, P., Jänne, J. & Raina, A. (1972) *Biochem. Biophys. Res. Commun.* **46**, 341-348
- Hayflick, L. (1971) *Tex. Rep. Biol. Med.* **23**, 284-303
- Hogan, B. L. M. (1971) *Biochem. Biophys. Res. Commun.* **45**, 301-307
- Hölttä, E. (1977) *Biochemistry* **16**, 91-100
- Jänne, J. & Hölttä, E. (1974) *Biochem. Biophys. Res. Commun.* **61**, 449-456
- McCormick, F. (1977) *J. Cell. Physiol.* **93**, 285-292
- Ortiz, P. J., Manduka, M. J. & Cohen, S. S. (1972) *Cancer Res.* **32**, 1512-1517
- Otsuka, H. (1971) *J. Cell Sci.* **9**, 71-84
- Pegg, A. E. (1970) *Ann. N.Y. Acad. Sci.* **171**, 977-987
- Perry, T. L., Hansen, S. & MacDougall, L. (1967) *J. Neurochem.* **14**, 775-782
- Pohjanpelto, P. (1976) *J. Cell Biol.* **68**, 512-520
- Pohjanpelto, P. & Raina, A. (1972) *Nature (London)* **235**, 247-249
- Raina, A., Jänne, J., Hannonen, P., Hölttä, E. & Ahonen, J. (1973) in *Polyamines in Normal and Neoplastic Growth* (Russell, D. H., ed.), pp. 167-180, Raven Press, New York
- Russell, D. H., Medina, V. J. & Snyder, S. H. (1970) *J. Biol. Chem.* **245**, 6732-6739
- Seiler, N. (1973) in *Polyamines and Normal and Neoplastic Growth* (Russell, D. H., ed.), pp. 137-156, Raven Press, New York
- Seiler, N. & Al-Therib, M. J. (1974) *Biochem. J.* **144**, 29-35
- Siimes, M. (1967) *Acta Physiol. Scand. Suppl.* **298**, 1-66
- Sturman, J. A., Ingoglia, N. A. & Lindquist, T. D. (1976) *Life Sci.* **19**, 719-724
- Tsuji, M., Nakajima, T. & Sano, I. (1975) *Clin. Chim. Acta* **59**, 161-167
- Walle, T. (1973) in *Polyamines in Normal and Neoplastic Growth* (Russell, D. H., ed.), pp. 355-366, Raven Press, New York
- Williams-Ashman, H. G., Coppoc, G. L., Schenone, A. & Weber, G. (1973) in *Polyamines in Normal and Neoplastic Growth* (Russell, D. H., ed.), pp. 181-198, Raven Press, New York
- Zeller, E. A. (1965) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **24**, 766