A comparison of polyamine metabolism in normal and transformed baby-hamster-kidney cells

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Transformed baby-hamster-kidney cells contain higher intracellular concentrations of polyamines than do normal cells. The difference is greatest in high-density confluent cultures. Transformed cells incorporate exogenous putrescine into the cells at a faster rate than do normal cells. They also show a marked increase in the rate of spermine biosynthesis compared with normal cells. Transformed cells grown to high cell densities released about 10% of their polyamines into the culture medium in a non-specific manner. In contrast, normal cells, under the same culture conditions, release up to 50% of their intracellular polyamines into the medium almost exclusively as free or conjugated spermidine. The elevated levels of polyamines found in transformed cells therefore appear to be the result of altered transport of polyamines across the cell membrane and of increased rates of biosynthesis.

The naturally occurring oligoamines, putrescine, spermidine and spermine, commonly referred to as polyamines, are found in all living organisms (Tabor & Tabor, 1976), where they have been implicated in the regulation of cell growth and development (for recent review, see Heby, 1981). In general, high concentrations of polyamines correlate with rapid cell-growth rates, and low concentrations with low cell-growth rates. It is now well established that tumour cells, or cells transformed by chemical carcinogens or oncogenic viruses, contain higher levels of polyamines than do normal cells (for review, see Jänne *et al.*, 1978), and it has been suggested that these elevated levels of polyamines facilitate the rapid growth of these cells. The activities of two of the enzymes responsible for the biosynthesis of the polyamines, ornithine decarboxylase (EC 4.1.1.17) and *S*-adenosylmethionine decarboxylase (EC 4.1.1.50), are known to increase markedly on transformation of the cells (Russell & Levy, 1971; Bachrach, 1976; Kilton & Gazdar, 1978), but whether increased biosynthesis alone can account for the elevated polyamine content of these cells is not clear.

The mechanisms for the control of intracellular polyamine concentrations in non-transformed cells are complex. In BHK-21/C13 cells the intracellular polyamine content is regulated not only at the level of biosynthesis (Howard *et al.*, 1974) but also at the

level of transport of polyamines both into and out of the cell. When polyamines are required by the cell, for example after a growth stimulus, such as addition of serum, BHK-21/C13 cells increase the rates of polyamine uptake and biosynthesis and markedly decrease the rate of polyamine excretion (Wallace & Keir, 1981*a*). In contrast, when the cell-growth rate is decreased the rates of polyamine uptake and biosynthesis are decreased, whereas the rate of excretion is increased (Melvin & Keir, 1978*a*; Wallace & Keir, 1981*a*).

In the present paper we have compared the rate of uptake, biosynthesis and excretion of polyamines in a cell line (PyY) transformed by polyoma virus and in its parent non-transformed cell line (BHK-21/C13) to determine the contribution of polyamine transport to the high intracellular concentrations of polyamines found in the transformed cell.

Experimental

Materials

[1,4(n)-³H]Putrescine dihydrochloride (sp. radioactivity 19 000 Ci/mol) was purchased from Amersham International, Amersham, Bucks., U.K.

Dansyl chloride, hypoxanthine, putrescine dihydrochloride, spermidine trihydrochloride and spermine tetrahydrochloride were from Sigma (London) Chemical Co., Poole, Dorset, U.K., silica gel 60 t.l.c. plates (without fluorescent indicator; 20 cm × 20 cm) from E. Merck, Darmstadt, Ger-

Abbreviation used: BHK cells, baby-hamster-kidney cells.

many, sterile tissue culture vessels from Sterilin, Teddington, Middx., U.K., and horse serum from Flow Laboratories, Irvine, Ayrshire, Scotland, U.K.

Cell culture

BHK-21/C13 (normal) cells and PyY (polyoma virus-transformed BHK-21) cells (Stoker & Macpherson, 1964) were grown routinely at 37°C in monolayer culture in an atmosphere of CO₂/air (1:19) in Dulbecco's modification of Eagle's medium supplemented with 10% (v/v) horse serum and 0.1 mM-hypoxanthine (DH₁₀Hx medium).

Determination of polyamines

Cells were grown in 9 cm-diameter Petri dishes for either 24 h or until confluence was attained (approx. 48 h). The cell sheet was washed twice in Dulbecco's phosphate-buffered saline, the cells harvested and the polyamines were extracted in 0.2 M-HClO₄. Polyamines were determined by fluorescence assay after dansylation and separation of the dansyl derivatives on t.l.c. (Dion & Herbst, 1973). Protein was determined by the method of Lowry *et al.* (1951).

Determination of polyamine uptake and excretion

Uptake. Cells were seeded at a density of 5.5×10^4 cells/cm² and grown for 24 h in DH₁₀Hx medium. The medium was then changed to Dulbecco's medium containing 2% (v/v) dialysed horse serum, 0.1 mM-hypoxanthine and [³H]putrescine dihydrochloride (1 µCi/ml). At various times thereafter cells were harvested, the polyamines extracted in 0.2 M-HClO₄ and the total acid-soluble radioactivity determined. Protein was extracted from the acid-insoluble pellet in 0.3 M-NaOH and determined quantitatively as described above.

Excretion. Cells were seeded at a density of 5.5×10^4 cells/cm² and grown in Dulbecco's medium supplemented with 10% (v/v) dialysed horse serum, 0.1 mM-hypoxanthine and [³H]putrescine dihydrochloride (1 µCi/ml) for 18–20 h. Cells were then washed twice in Dulbecco's medium, warmed to 37°C and incubated in DH₁₀Hx medium for a further 24 h. The medium was then changed to fresh DH₁₀Hx and samples were taken as described previously (Wallace & Keir, 1981a).

Radioactivity in each polyamine was determined by liquid-scintillation spectrometry after dansylation and separation of the dansyl derivatives on t.l.c. as described above. The radioactivity in each spot was determined by using a toluene/Triton-based scintillation fluid. Samples were counted over a period of 10 min with an efficiency for ³H of 25–30%.

Results

Polyamine content of BHK-21/C13 and PyY cells

At both high and low cell densities the transformed PyY cells contained higher intracellular concentrations of polyamines than the normal BHK-21/C13 cells (Table 1). All three polyamines were elevated in the transformed cells with the largest difference occurring in spermidine, the concentration of which was about 1.5 times that found in normal cells. In agreement with previous results (Melvin & Keir, 1980), the intracellular concentrations of polyamines in normal BHK-21/C13 cells decreased as the cell-growth rate decreased and the cells attained confluence. In this experiment the intracellular polyamine content of BHK-21/C13 cells had decreased by 50% at 30 h after confluence (Table 1). Over a similar time scale, the polyamine content of the transformed PyY cells

Table 1. Polyamine content of BHK-21/C13 and PyY cells

BHK-21/C13 cells were seeded at a density of 1.2×10^4 cells/cm² and were grown in DH₁₀Hx medium for either 24 h (low density) or 72 h (high density). The time at which confluence was attained was determined by microscopic examination of the cell cultures. PyY cells were seeded at a density of 1.6×10^4 cells/cm² and were grown in DH₁₀Hx medium for either 24 h or 72 h. Polyamines were extracted and determined quantitatively as described in the Experimental section. Values are means $\pm$ S.D. ($n = 3$).

Cell density	Polyamine content (nmol/mg of protein)				Putrescine
	Spermine	Spermidine	Putrescine	Total polyamines	spermidine
(a) BHK-21/C13 cells					
Low	4.48 ± 0.25	7.24 ± 0.58	2.46 ± 0.18	14.30 ± 0.98	0.34
High	3.47 ± 0.19	3.24 ± 0.10	0.42 ± 0.07	7.14 ± 0.35	0.13
(b) PyY cells					
Low	6.78 ± 0.25	10.72 ± 0.75	2.76 ± 0.13	20.26 ± 1.42	0.26
High	7.36 ± 0.26	7.13 ± 0.45	2.32 ± 0.22	16.83 ± 0.43	0.32

had decreased by only 17%. In contrast with the normal cells, where all three polyamines decreased, in the transformed cultures, putrescine and spermidine decreased whereas spermine increased.

Uptake of exogenous putrescine

At all times the transformed cells incorporated more [^3H]putrescine than did the normal BHK-21/C13 cells (Fig. 1). As early as 3 h after addition of label, PyY cells accumulated five times as much [^3H]putrescine intracellularly as BHK-21/C13 cells. Analysis of the distribution of ^3H radioactivity in each polyamine showed that there was little difference in the rate of conversion of putrescine into spermidine in the two cell types (Fig. 2). The half-life of putrescine calculated from these experiments was of the order of 2 h. On the other hand, PyY cells synthesized spermine from spermidine three times faster than did BHK-21/C13 cells (Fig. 2). The calculated half-life of spermidine was approx. 17 h for PyY cells and 50 h for BHK-21/C13 cells. Both cell lines were undergoing cell growth and division as determined by the increase in protein content over the time course of the experiment (Fig. 1, insert).

Not all the radioactivity could be accounted for as free polyamines (Fig. 2). As we have described previously (Melvin *et al.*, 1980) a significant proportion of the radioactivity remains at the origin of the chromatogram. This material was partially purified by ion-exchange chromatography and was tentatively identified as monoacetylspermidine by thin-

Fig. 1. Uptake of exogenous putrescine by BHK-21/C13 cells and PyY cells

Cells were grown as described in Experimental section. Radioactive putrescine was added to the medium and samples were taken at various times thereafter. The results are means $\pm$ S.D. ($n = 6$). $\square$, BHK-21/C13 cells; $\blacksquare$, PyY cells.

Fig. 2. Distribution of ^3H -labelled polyamines in BHK-21/C13 cells (a) and PyY cells (b)

Cells were grown as described in the legend to Fig. 1. Acid-soluble extracts were dansylated, the dansyl derivatives separated by t.l.c. and the radioactivity in each polyamine determined as described in Experimental section. Since the total counts per min on each plate were not the same the results are presented as $100 \times (\text{c.p.m. per polyamine}) / (\text{total intracellular c.p.m.})$. At zero h, 100% was equal to $(0.5 \pm 0.02) \times 10^4 \text{ c.p.m.}$ for BHK-21/C13 cells and $(2.0 \pm 0.2) \times 10^4 \text{ c.p.m.}$ for PyY cells. Values are means $\pm$ S.D. ($n = 3$). $\blacksquare$, Putrescine; $\circ$, spermidine; $\triangle$, spermine.

Fig. 3. Excretion of polyamines from BHK-21/C13 cells and PyY cells

Intracellular polyamines were radioactively labelled by incubating the cells for 18–20 h in [^3H]-putrescine dihydrochloride ($1\mu\text{Ci/ml}$). Cells were grown to high cell densities as described in the Experimental section and samples were taken at various times to determine the total acid-soluble radioactivity in both the cells and extracellular medium. As the total amount of radioactivity incorporated into each culture was not the same the results are expressed as:

$$\frac{\text{c.p.m. in cells or medium}}{\text{total c.p.m. (cells + medium)}} \times 100$$

Values are means $\pm$ s.d. ($n = 3$). At zero h 100% corresponds to $(2.35 \pm 0.22) \times 10^5$ c.p.m. for BHK-21/C13 cells and to $(10.65 \pm 1.16) \times 10^5$ c.p.m. for PyY cells (means $\pm$ s.d., $n = 7$). Symbols: Δ , radioactivity in BHK-21/C13 cells; $\square$, radioactivity in medium surrounding BHK-21/C13 cells; $\blacktriangle$, radioactivity in PyY cells; $\blacksquare$, radioactivity in medium surrounding PyY cells.

layer and paper chromatography (H. M. Wallace, unpublished work). In these experiments this material amounted to 10% of the total intracellular polyamines for BHK-21/C13 cells and 14% for PyY cells. The results for monoacetylspermidine have been omitted from Fig. 2 for the sake of clarity.

Excretion of polyamines from BHK-21/C13 cells and PyY cells

Confluent cultures of BHK-21/C13 cells contained less polyamines than growing cultures (Table 1). Radioactive labelling experiments revealed that the polyamines were released into the extracellular medium. BHK-21/C13 cells lost 51% of their intracellular polyamines over the 24 h period of the experiment. In contrast, PyY cells lost only 11% of their polyamines in the same time (Fig. 3). Analysis of the intracellular distribution of radioactivity revealed that in both cell lines spermine was the major intracellular polyamine (Table 2). Putrescine accounted for less than 1% of the total intracellular polyamine content.

Spermidine was the major polyamine in the extracellular medium from BHK-21/C13 cells (Fig. 4) with spermine and putrescine forming only a small percentage (15%) of the total radioactivity. On the other hand, the pattern of radioactivity in the medium from PyY cells reflected that found intracellularly; spermine accounted for the major proportion of the radioactivity (Fig. 4). In this case, only 20% of the total radioactivity was present as spermidine.

As before, there was some radioactivity present at the origin of the chromatogram. This material amounted to approx. 17% of the total cellular polyamines and to about 25% of the total extracellular polyamines in each of the cell lines. There was little difference in the relative proportions of this

Table 2. Intracellular distribution of ^3H -labelled polyamines in BHK-21/C13 cells and PyY cells

Cells were grown as described in the legend to Fig. 3. The acid-soluble extract from the cell pellet was used to determine the radioactivity in each polyamine as described in the Experimental section. Values are means $\pm$ s.d. ($n = 3$).

		$10^{-4} \times \text{Radioactivity (c.p.m.)}$			
		Time (h)	Spermine	Spermidine	Putrescine
(a) BHK-21/C13 cells					
	0		10.6 ± 0.4	9.5 ± 0.2	0.2 ± 0.02
	4		11.5 ± 0.3	8.0 ± 0.3	0.3 ± 0.02
	8		12.5 ± 0.3	7.7 ± 0.2	0.3 ± 0.02
	12		11.8 ± 0.7	7.6 ± 0.5	0.3 ± 0.09
	24		13.7 ± 0.3	5.8 ± 0.1	0.3 ± 0.02
(b) PyY cells					
	0		71.7 ± 2.7	14.5 ± 0.4	0.7 ± 0.10
	4		69.5 ± 2.8	14.7 ± 0.2	0.7 ± 0.07
	8		66.7 ± 3.1	11.9 ± 0.4	0.5 ± 0.03
	12		75.2 ± 2.1	12.7 ± 0.3	0.5 ± 0.06
	24		70.2 ± 1.7	13.4 ± 0.2	0.6 ± 0.03

Fig. 4. Distribution of ^3H -labelled polyamines in the extracellular medium from BHK-21/C13 cells (a) and PyY cells (b). Cells were grown as described in the legend to Fig. 3. Acid-soluble extracts of the medium were used to determine the total radioactivity in each polyamine as described in the Experimental section. Values are means $\pm$ S.D. ($n = 3$). ■, Putrescine; ○, spermidine; △, spermine.

material between the two cell lines (results not shown). Preliminary experiments have again identified this material as acetylspermidine (H. M. Wallace, unpublished work).

Discussion

High concentrations of polyamines are found in a variety of systems involving malignant disease and cell transformation. One or more of the polyamines has been shown to be elevated in several experimental tumours, in cells transformed by chemical carcinogens or oncogenic viruses and in a large number of cases of clinical cancer (for reviews, see Jänne *et al.*, 1978; Russell & Durie, 1978). Transformation of normal BHK cells with polyoma virus (PyY cells) also resulted in increased intracellular concentrations of polyamines (Table 1). The concentration of all three polyamines was higher in the PyY cells than in the parent BHK-21 cells. High concentrations of polyamines therefore seem to be a consequence of transformation, but the way in which these high concentrations are attained is not clear.

One way to increase the intracellular content of polyamines is to increase the rate of biosynthesis. Transformed PyY cells showed a marked increase in the rate of formation of spermine compared with the normal BHK-21 cells (Fig. 2). This could be the result of an increase in the activity of either *S*-adenosylmethionine decarboxylase and/or spermine synthase. An increase in the activity of the former enzyme would also lead to an increase in the rate of catabolism of putrescine to form spermidine. We did not observe such an increase in transformed

PyY cells. The rate of spermidine synthesis from putrescine is rapid; the half-life of putrescine was less than 2 h in both normal and transformed cells. Thus it may be that we cannot detect a small change in the rate of this already very fast reaction. Increased activities of both ornithine decarboxylase and *S*-adenosylmethionine decarboxylase have been observed in the L1210 leukaemic tumour in mice (Russell & Durie, 1978) and in chick embryo fibroblasts infected with Rous sarcoma virus (Don & Bachrach, 1975); thus increased rates of polyamine biosynthesis may explain the high levels of spermidine and spermine found in transformed cells.

Elevated concentrations of putrescine are also found in transformed cells, especially in high-density cell cultures (Table 1). In transformed rat kidney cells the high levels of putrescine have been attributed to alterations in the activities of the polyamine-degrading enzymes (Quash *et al.*, 1979). The activity of diamine oxidase, the enzyme that degrades putrescine to γ -aminobutyric acid and CO_2 (Andersson *et al.*, 1980), is lower in normal rat kidney cells than in transformed cells. In contrast, the activity of polyamine oxidase, which degrades spermine and spermidine to putrescine is increased in the transformed cell. When taken together, these changes in polyamine degradation would result in an increase in the intracellular level of putrescine.

Intracellular concentrations of polyamines can also be increased by alterations in the transport of polyamines across the cell membrane. Transformed cells exhibited a pattern of uptake and excretion of polyamines that resulted in optimal intracellular concentrations. In other words transformed cells have a faster rate of polyamine uptake than normal

cells (Fig. 1) and, in high-density cultures, a lower rate of excretion (Fig. 3). Increased uptake of polyamines has recently been observed in Rous-sarcoma-virus-transformed chick embryo fibroblasts (Bachrach & Seiler, 1981). These authors also reported an increase rate of spermidine metabolism in these cells, particularly in the direction of *N*-acetylputrescine and *N*¹-acetylspermidine. We too observed an increase in the amount of radioactivity present as conjugated polyamines in transformed cells (results not shown).

By preventing the excretion of polyamines the transformed cell (Fig. 3) can maintain higher intracellular levels of polyamines. This conservation phenomenon has also been observed in BHK-21/C13 cells infected with herpes simplex virus type-1 (Wallace & Keir, 1981*b*) and in cells whose intracellular content of polyamines has been depleted by drugs that inhibit polyamine biosynthesis (Melvin & Keir, 1978*b*; Wallace *et al.*, 1979). In each of these cases, polyamines are conserved, presumably because they are required for the continuation of cell or virus replication.

Finally, the pattern of results presented in this paper for transformed cells bears a striking resemblance to those reported previously for normal BHK-21 cells stimulated to grow by the addition of fresh serum (Wallace & Keir, 1981*a*). Thus, it may be that any 'shift-up' in the growth rate of the cell, whether it be by a temporary growth stimulus or by transformation by an oncogenic virus such as polyoma virus, requires an increase in the intracellular content of polyamines. This increase is produced by a combination of increased rates of biosynthesis and uptake on the one hand and decreased rates of excretion and oxidation on the other. The degree and time span of the increase will depend on the requirements of the cells and therefore a stably transformed cell line (e.g. PyY cells) will maintain high intracellular levels of polyamines indefinitely.

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