Diamine Oxidase and Polyamine Oxidase Activities in Normal and Transformed Cells

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1. The activity of diamine oxidase (EC 1.4.3.6) in normal rat kidney cells and in normal rat kidney cells transformed by avian sarcoma virus (B77 strain) growing in tissue culture varies with the stage of growth. There is an initial stimulation of activity by 24 h after seeding, followed by a steep decline during exponential growth (48–72 h). Enzyme activity decreases even further as the cells reach saturation density (confluence) after 4 days in culture when the activity in normal rat kidney cells is twice as high as that in transformed cells. 2. Differences of about the same order of magnitude are observed between transformed human cells HeLa, HEP2 (a human epithelioid carcinoma) and normal human fibroblasts, in chicken cells between normal myeloblasts and leukaemic myeloblasts, and in rats between biopsy material from normal mammary tissue and 9,10-dimethylbenz[*a*]anthracene-induced mammary tumours. 3. Polyamine oxidase activity also varies with the growth of transformed rat kidney cells, but shows no significant variation with the growth of normal rat kidney cells between 24 and 96 h after seeding. The activity in cells at confluence is from 3- to 5-fold lower in the transformed than in the normal rat kidney cells. 4. A similar 5–10-fold decrease in activity has been found in 9,10-dimethylbenz[*a*]anthracene-induced mammary tumours in rats and in human oesophageal tumours. 5. Possible reasons for these observations and the contribution of these two enzymes to cellular putrescine concentrations are discussed.

Pig kidney diamine oxidase (EC 1.4.3.6) shows multi-substrate specificity. It can oxidize aliphatic amines with the general formula $\text{NH}_2[\text{CH}_2]_n\text{NH}_2$ where $n = 3-6$, the monoamino-substituted derivatives of these aliphatic amines and aromatic amines such as histamine (Bardsley & Ashford, 1972; Zeller, 1963).

Its activity can be modulated by metabolic derivatives of amino acids. For example it has been found that 2-oxosuccinamate, which is an intermediate of asparagine decarboxylation in baby hamster kidney cells transformed by polyoma virus, is an activator of the enzyme (Quash *et al.*, 1976). On the other hand, agmatine, a guanidine derivative and the product of arginine decarboxylation (Gale, 1946; Morris & Koffron, 1969), like aminoguanidine itself, is an inhibitor of diamine oxidase (Ballet *et al.*, 1975). On adding agmatine at a concentration of $1\mu\text{M}$ to human lymphocytes stimulated by tetanus toxoid, the incorporation of [^3H]thymidine into the DNA of these lymphocytes was increased (Ballet *et al.*, 1975). In this context the accelerated despiralization of chromosomes in *Vicia faba* root tips by the addition of diamine oxidase may be relevant (Ziemba-Zak *et al.*, 1965). These observations suggest that some

product(s) of diamine oxidase action is associated with DNA synthesis and/or cell growth by a mechanism as yet unidentified.

As regards substrates of this enzyme 0.25 mM-putrescine was found to be a growth factor for human fibroblasts in culture (Pohjanpelto & Raina, 1972). The inhibition of putrescine synthesis by α -methylornithine (Mamont *et al.*, 1976) or by *trans*-3-dehydro-DL-ornithine (Relyea & Rando, 1975) resulted in a concomitant inhibition of the growth in tissue culture of rat hepatoma cells and chick embryo muscle cells respectively. Diaminopropane, another substrate for diamine oxidase (Quash & Taylor, 1970), is also a potent inhibitor of ornithine decarboxylase activity in Ehrlich ascites cells (Kallio *et al.*, 1977a) and in rat ovary (Guha & Jänne, 1977) and markedly impairs DNA synthesis in regenerating rat liver (Pösö & Jänne, 1976; Kallio *et al.*, 1977b). It is therefore possible that its effect on DNA synthesis could be due to either diaminopropane competing with another substrate for diamine oxidase itself or the action of its oxidation product, 3-aminopropionaldehyde, as previously suggested (Quash & Taylor, 1970).

To try to understand the apparent association

between diamine oxidase activity, DNA synthesis and cell growth, and eventually the action of modulators and substrates of the enzyme such as 2-oxosuccinamate and diaminopropane, we first determined whether there exists a direct relationship between diamine oxidase activity and growth by comparing the diamine oxidase activity of normal and transformed cells in exponential growth and at confluence when cell growth had decreased. The results are reported along with evidence for differences between these two cell types in polyamine oxidase activity, which is also involved in putrescine metabolism, since this enzyme oxidizes spermine to spermidine and spermidine to putrescine (Hölttä, 1977).

Materials and Methods

Reagents

[1,4- ^{14}C]Putrescine dihydrochloride (sp. radioactivity 60 mCi/mmol) and [^{14}C]spermidine [*N*-(3-aminopropyl)[1,4- ^{14}C]tetramethylene-1,4-diamine trihydrochloride; sp. radioactivity 111 mCi/mmol] were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. *N*-Methylbenzothiazol-2-one hydrazone hydrochloride and 2,5-diphenyloxazole were purchased from Koch-Light, Colnbrook, Bucks., U.K. 1,4-Bis-(4-methyl-5-phenyloxazol-2-yl)benzene was obtained from Packard Instrument International, Zurich, Switzerland. Putrescine dihydrochloride, spermidine trihydrochloride, dithiothreitol and diamine oxidase were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. DEAE-cellulose DE-52 was purchased from Whatman, Maidstone, Kent, U.K.

Cells

The cells used were normal rat kidney cells and normal rat kidney cells transformed by avian sarcoma virus (B77 strain). Chick embryo cells were prepared from 10-day chick embryos. Secondary cultures were infected with Rous sarcoma virus at a multiplicity of infection of about 0.5 foci-forming units per cell and used 13 days later, at which time about 90% of the cells were transformed, as judged by morphology and 2-deoxyglucose uptake (Kawai & Hanafusa, 1971). Human fibroblasts (MRC5 strain) were used at passage 39. HEP2 cells, a continuous cell line derived from a human epithelioid carcinoma, were used as the transformed counterparts.

Eagle's minimum essential medium containing glutamine as supplied by Grand Island Biological Co., Grand Island, NY, U.S.A., supplemented with 3% NaHCO_3 , 10% tryptose phosphate broth from Difco Laboratories, Detroit, MI, U.S.A., and 10% foetal calf serum was used for all cultures; for chick-embryo

cells 5% foetal calf serum was used. Roux bottles or Petri dishes were incubated at 37°C in a humid atmosphere of air/ CO_2 (19:1, v/v).

Preparation of homogenates from biopsy material

Biopsy material from rats was first dissected macroscopically to obtain tumour material as devoid of normal tissue as possible. For all biopsies the degree of malignancy was checked by histological examination. Tissues were pulverized in liquid N_2 in a freeze clamp. The powder was collected in tubes and after adding 3 ml of 0.14M-NaCl the cells were disrupted by ultrasonic disintegration in a Bronson sonifier (model S75, Dansbury, CT, U.S.A.), fitted with a microprobe, for 2 s at output 2.

Preparation of homogenates from cells in tissue culture

The cell sheets were washed twice with cold phosphate-buffered saline containing 0.14M-NaCl and 0.014M-sodium phosphate buffer, pH 7.2, after which the cells were scraped off with a rubber-covered rod, transferred to tubes and washed by centrifugation at 600g for 5 min. The cell pellet was stored at -20°C until use. Cells were disrupted by freezing and thawing or by sonication in a Bronson sonifier for 2 s at output 2 with a microprobe and finally suspended in 0.1M-sodium phosphate buffer, pH 7.0, for the assay of diamine oxidase activity.

Purification of diamine oxidase

Sigma grade II diamine oxidase was dissolved (50 mg/ml) in 0.016M-sodium phosphate buffer, pH 7.3. Under the standard assay conditions this solution of crude enzyme oxidized 3 nmol of putrescine/h per mg of protein at 37°C. The solution was heated at 66°C for 8 min and centrifuged. The precipitate was discarded and solid $(\text{NH}_4)_2\text{SO}_4$ was added to the supernatant to obtain 30% saturation. After standing at 4°C for 1 h, the precipitate was centrifuged off and more solid $(\text{NH}_4)_2\text{SO}_4$ was added to the supernatant to give 50% saturation. After standing at 4°C for 2 h the precipitate was centrifuged and washed with 50%-satd. $(\text{NH}_4)_2\text{SO}_4$. The pellet was dissolved in 5 ml of water and extensively dialysed against 0.015M-sodium phosphate buffer to remove ammonium ions.

After centrifuging at 4000g for 10 min to remove any precipitate formed during dialysis, the enzyme was put on a column (17 cm $\times$ 2 cm) of DEAE-cellulose DE-52 equilibrated with the same buffer. Elution was carried out with a linear gradient of putrescine from 0 to 0.05M dissolved in the same buffer, as described by Kluetz & Schmidt (1977). Protein was monitored by its A_{280} , and enzymically formed aldehyde by the reaction with *N*-methylbenzothiazol-2-one hydrazone in the presence of FeCl_3 as described by Bachrach & Reches (1966).

After extensive dialysis to eliminate reaction products, the activity of the different fractions was assessed with [1,4-¹⁴C]putrescine. Fractions able to oxidize more than 300nmol of putrescine/h per mg of protein were pooled. The active fractions contained less than 1% polyamine oxidase activity as assessed by the capacity to oxidize spermidine.

Assay of diamine oxidase activity

To 0.5ml of cell homogenate containing various amounts of protein in screw-cap culture tubes was added 0.1 μ Ci of [1,4-¹⁴C]putrescine (5nmol) in 0.1ml and the volume was brought to 2.0ml with 0.1 M-sodium phosphate buffer, pH 7.0.

After incubation at 37°C for 1h, the reaction was stopped by the addition of 0.2ml of 2% Na₂CO₃ and the reaction product extracted with 10ml of toluene scintillant [4g of 2,5-diphenyloxazole plus 0.1g of 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene in 1 litre of toluene] as described by Kobayashi (1963).

Control tubes contained the same constituents, except the cell homogenate, which was replaced by the phosphate buffer. Control values were subtracted for each determination.

Samples were counted on an Intertechnique liquid-scintillation spectrometer SL30 at an efficiency of 80%. Enzyme activity, expressed as μ units, refers to pmol of 1-pyrroline formed/h. All determinations were in duplicate and all duplicates agreed within 6%. The number of experiments or cultures for each value is given in the Figures and Tables.

Assay of polyamine oxidase activity

The assay developed was based on the fact that the action of polyamine oxidase on [1-¹⁴C]spermidine as substrate yields stoichiometric amounts of [1-¹⁴C]-putrescine (Hölttä, 1977). This can be measured by the addition of purified exogenous diamine oxidase and determining the amount of 1-pyrroline formed. As the cell extracts used for determining polyamine oxidase activity also contain diamine oxidase (Tables 3 and 5) we investigated whether the endogenous diamine oxidase was sufficient to oxidize the putrescine formed from spermidine. Accordingly 200 μ g of protein from normal and transformed rat kidney cells at 24h post seeding were incubated in the presence of 2nmol of [1-¹⁴C]spermidine (0.1 μ Ci), for 2h at 37°C. At the end of this period diamine oxidase in amounts varying from 249 to 1027 μ units were added and incubation was continued for a further 1h. 1-Pyrroline was extracted in the presence of Na₂CO₃ and toluene scintillant as described earlier.

Table 1 shows that the addition of exogenous diamine oxidase up to 1027 μ units does not significantly increase polyamine oxidase activity. This means that in these cells at 24h after seeding the endogenous diamine oxidase is sufficient to

Table 1. *Effect of exogenous diamine oxidase on polyamine oxidase activity*

Origin of cell homogenate	Diamine oxidase added (μ units)	1-Pyrroline formed (pmol/h)
Normal rat kidney cells	0	0.99
	247	1.00
	527	1.15
	1027	1.16
Transformed rat kidney cells	0	2.52
	247	2.67
	527	2.49
	1027	2.75

Fig. 1. *Diamine oxidase activity as a function of cell growth* 1-[¹⁴C]Pyrroline formation from [1,4-¹⁴C]putrescine (sp. radioactivity 0.1 μ Ci/5nmol) by 100 μ g of homogenate from normal (NRK) and transformed (NRKB77) rat kidney cells at different times after seeding. Assays were carried out as described in the Materials and Methods section. The number of separate cultures investigated at each time is indicated and points represent mean values $\pm$ S.E.M. Symbols: $\square$, $\blacksquare$, normal rat kidney cells (NRK); $\triangle$, $\blacktriangle$, transformed rat kidney cells (NRKB77). —, 1-Pyrroline formed; ---, protein content/dish.

oxidize the putrescine formed from spermidine. Even at confluence (96h) when diamine oxidase activity decreases (Fig. 1) the endogenous activity of this enzyme in these cell types is greater than the polyamine oxidase activity. Nevertheless, as a precautionary measure, 390 μ units of diamine oxidase were routinely added to all assays for polyamine oxidase.

For the normal assay, the homogenate was prepared as described and then dialysed against a buffer containing 0.1 M-glycine/NaOH (pH 8.5) and 0.005 M-dithiothreitol for 30 min at 4°C. At the end of this period the dialysis buffer was renewed and dialysis was continued for a further 1 h. The possibility of any isotopic dilution of the substrate by free endogenous unlabelled spermidine in the cell extract was thus eliminated. Dithiothreitol was included in the dialysis buffer, since it has been shown (Hölttä, 1977) that the activity of the enzyme depends on the presence of free SH groups. A pH value of 8.5 was chosen instead of 9.5 (the optimum pH of the enzyme) as this latter pH was unsuitable for diamine oxidase activity. The assay mixture in screw-cap culture tubes contained increasing amounts (10–300 μ g of protein) of cell homogenate in volumes ranging from 0.05 to 0.5 ml, 390 μ units of purified diamine oxidase, 2 nmol of [14 C]spermidine (0.1 μ Ci) in a final volume of 2 ml of glycine/NaOH buffer, pH 8.5, containing 0.005 M-dithiothreitol. Incubation was for 2 h at 37°C, after which 0.2 ml of 2% Na₂CO₃ in water was added to favour 1-pyrroline formation. Extraction with toluene scintillant was carried out as described for the diamine oxidase assay.

Control tubes contained all constituents except cell homogenate and the values obtained were subtracted for each assay. All determinations were in duplicate and duplicates agreed within 13%.

Enzyme activity was expressed as μ units when pmol quantities of 1-pyrroline were measured.

Protein determination

The protein content of the homogenates was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Results

Diamine oxidase activity

Preliminary diamine oxidase assays demonstrated that 1-pyrroline formation was linear with time up to at least 3 h with 0.3 or 0.5 mg of protein for a homogenate of normal rat kidney cells. Enzyme activity over a 1 h period was also linear with protein concentration (50–600 μ g) for homogenates of normal and transformed rat kidney cells.

Effect of cell growth. Normal rat kidney and transformed cells were seeded into 10 cm plastic

dishes at a density of 8×10^5 cells/dish. Duplicate dishes were harvested at zero time when cells were seeded, and after 24, 48, 72 and 96 h of culture; the cells were washed with phosphate-buffered saline and stored at –20°C so as to carry out all the assays at the same time.

Fig. 1 shows for both cell types that diamine oxidase activity rises during the lag phase and shows maximum activity at 24 h after seeding. The activity in transformed cells is 50% greater than in normal rat kidney cells at this time. It then decreases during the exponential phase of growth, reaching a minimum value when the cells have reached saturation density between 3 and 4 days. The decrease in activity between 1 and 2 days is greater in transformed than in normal rat kidney cells.

At saturation density, normal rat kidney cells show about twice the activity of transformed cells.

It has been shown that the protein content of cells varies with the cell cycle (Tsuboi *et al.*, 1976). It was possible that the expression of enzyme activity on a 100 μ g of protein basis may have biased the results. To verify this, the protein content of cells in exponential growth and at confluence was determined. Table 2 shows the results obtained. In transformed cells, enzyme activity expressed per 10^6 cells is still about half that in normal rat kidney cells at confluence.

Comparative study in normal and transformed cells. The decrease in diamine oxidase activity found for both normal and transformed cells with exponential growth and the even lower activity found in transformed cells at confluence prompted us to ask whether this observation was also valid for other cell types at confluence when cell growth had decreased.

The importance of this question is underlined by the fact that one major characteristic common to all transformed cells in culture is that they attain higher saturation densities than those of normal cells (Pollack *et al.*, 1968). Accordingly, diamine oxidase activity was measured on normal and transformed

Table 2. *Protein content and diamine oxidase activity as a function of cell number*

Assays, as detailed in the Materials and Methods section, were carried out in duplicate and the value for each time represents the mean $\pm$ S.E.M. for 7 different cultures.

Cells	Time after seeding (h)	Protein content (μ g/ 10^6 cells)	1-Pyrroline formed (pmol/ 10^6 cells)
Normal rat kidney	48	222	27.4 ± 3.16
	96	258	8.1 ± 0.42
Transformed rat kidney	48	462	58.8 ± 5.43
	96	470	4.5 ± 0.43

rat kidney cells at confluence, i.e. 4 days after seeding, and extended to other cell types grown in tissue culture that had attained confluence. It can be seen that diamine oxidase activity is about 3–4-fold lower in all the transformed compared with the normal cells (Table 3).

Effect of intracellular putrescine. Before ascribing any physiological significance to those results, one possible source or error was considered. Transformed cells have been shown to possess a higher putrescine content than normal cells (Don *et al.*, 1975); therefore the lower diamine oxidase activity of the former might simply reflect an isotopic dilution of the [^{14}C]putrescine by unlabelled intracellular putrescine.

Published values for putrescine in transformed cells vary from 0.06 nmol/100 μg of protein in Ehrlich ascites carcinoma cells (Kallio *et al.*, 1977a) to 0.32 nmol/100 μg of protein in chick-embryo cells transformed by Rous Sarcoma Virus (Don *et al.*, 1975). Therefore, if transformed rat kidney cells are typical of other transformed cells, then the maximum dilution of the 5 nmol of putrescine added by intracellular putrescine would be about 10%.

Nevertheless, in the absence of exact values for transformed rat kidney cells, the contribution of free intracellular putrescine to the assay was determined by dialysing the cell homogenate before assay. Samples (2 ml) of cell homogenates containing 2.4 mg of protein were dialysed twice against 250 ml of buffer containing 0.14 M-NaCl and 0.01 M-phosphate, pH 7.2 at 4°C, for 1 h. Table 3 shows that with dialysed homogenates, there is still a 2–3-fold difference in diamine oxidase activity between normal and

transformed cells. Thus the presence of free intracellular putrescine is not responsible.

Lowered diamine oxidase activity therefore can partially account for the increased putrescine concentrations found in transformed cells. Of three other enzymes involved in putrescine metabolism, ornithine decarboxylase, spermidine synthase and polyamine oxidase, the first increases free cellular putrescine concentrations through the decarboxylation of ornithine to putrescine, whereas the second decreases it through the addition of the propylamine moiety of *S*-adenosylpropylamine to form spermidine. Abundant evidence exists in the literature to substantiate these pathways (Herbst & Bachrach, 1970; Russell, 1973; and for a review see Tabor & Tabor, 1976). Polyamine oxidase can increase putrescine concentrations by oxidizing spermine to spermidine, which is itself further oxidized to putrescine, with the production of equivalent amounts of 3-aminopropionaldehyde at each oxidative step (Hölttä, 1977).

Table 3. Determination of diamine oxidase activity of different cell types

Diamine oxidase activity was measured as described in the Materials and Methods section. The numbers of cultures examined are shown in parentheses. Mean values $\pm$ S.E.M. are given.

Cells	1-Pyrroline formed (pmol/h per 100 μg of protein)
Chick-embryo cells	0.57 $\pm$ 0.10 (8)
Chick-embryo cells transformed by Rous sarcoma virus	0.15 $\pm$ 0.04 (2)
Normal myeloblasts (chick)	0.83 $\pm$ 0.25 (2)
Leukaemic myeloblasts (chick)	0.17 $\pm$ 0.03 (3)
Human fibroblasts (MRC5)	1.16 $\pm$ 0.10 (4)
HEp2	0.27 $\pm$ 0.04 (10)
HeLa	0.19 $\pm$ 0.02 (5)
Normal rat kidney, non-dialysed	3.12 $\pm$ 0.16 (11)
Normal rat kidney, dialysed	3.12 $\pm$ 0.07 (2)
Transformed rat kidney, non-dialysed	1.16 $\pm$ 0.08 (12)
Transformed rat kidney, dialysed	1.57 $\pm$ 0.02 (2)

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Fig. 2. Polyamine oxidase activity as a function of cell growth. 1-[^{14}C]Pyrroline formation from [^{14}C]spermidine (sp. radioactivity 0.1 $\mu\text{Ci}/2\text{ nmol}$) by 50 μg of homogenate from normal (NRK) and transformed (NRKB77) rat kidney cells was measured at different times after seeding. Assays were carried out as described in the Materials and Methods section. The number of separate cultures investigated at each time is indicated and points represent mean values $\pm$ S.E.M. Symbols: $\blacksquare$, normal rat kidney cells (NRK); $\blacktriangle$, transformed rat kidney cells (NRKB77).

Polyamine oxidase activity

For both normal rat kidney cells and transformed kidney cells polyamine oxidase activity was linear with the amount of protein up to 200 μ g. Normal cells showed about 5 times the activity of transformed cells. Enzyme assays on homogenates from cells in different phases of growth could be carried out on microgram quantities of protein; this was a great advantage when only limited amounts of biopsy material were available.

Effect of cell growth. Normal rat kidney cells and transformed kidney cells were seeded at 8×10^5 cells/dish and harvested at the same intervals as described for diamine oxidase. Fig. 2 shows that whereas enzyme activity per 50 μ g of protein shows a small initial rise at 24h and then remains relatively constant with the growth of normal cells, it is increased in transformed cells at 24h after seeding. This activity drops off rapidly (24–48h) during the exponential phase of growth. Between 48 and 72h the slope again changes resulting in activities lower than those observed for normal cells as confluence is reached.

Comparative study in normal and transformed cells. The polyamine oxidase activity of cells at confluence was measured on 50 μ g of protein homogenate, at which concentration the assay was linear. Polyamine oxidase activity is 2–5-fold lower in the transformed compared with the normal cells (Table 4).

Polyamine oxidase and diamine oxidase activities on biopsy material

We investigated whether the differences observed in diamine and polyamine oxidase activities were a special feature of cells growing in artificial culture conditions, or whether similar differences also applied to biopsy material of rat and human origin. In addition, to see whether such differences were also characteristic of chemically induced tumours, biopsies from rats with tumours induced by 9,10-

Table 5. *Determination of diamine and polyamine oxidase activities on biopsy material*

Diamine oxidase and polyamine oxidase activities were measured as described in the Materials and Methods section. The number of experiments is shown in parentheses. Mean values $\pm$ S.E.M. are given.

	1-Pyrroline formed (pmol/h per 50 μ g of protein)	
	Diamine oxidase	Polyamine oxidase
Normal rat mammary gland	1.06 $\pm$ 0.030 (2)	0.14 $\pm$ 0.003 (2)
Rat mammary tumour I	0.07 $\pm$ 0.009 (6)	0.03 $\pm$ 0.008 (6)
Rat mammary tumour II	0.16 $\pm$ 0.030 (4)	0.04 $\pm$ 0.018 (2)
Normal human oesophageal epithelium	1.72 $\pm$ 0.090 (6)	0.17 $\pm$ 0.029 (3)
Human carcinoma of oesophagus	0.62 $\pm$ 0.080 (5)	0.09 $\pm$ 0.004 (5)

dimethylbenz[a]anthracene were also included in this comparative study.

Table 5 shows that diamine oxidase activity shows differences between normal and tumour cells of the same order of magnitude as those obtained for cells at confluence growing in tissue culture. Polyamine oxidase activity is 5-fold lower in tumour extracts than in extracts from normal tissues.

Discussion

From the results presented, it is apparent that variations in the activities of diamine oxidase and polyamine oxidase are associated with the growth of the normal and transformed cells.

As cells approach confluence normal cells show about twice the diamine oxidase activity of their transformed counterparts (Fig. 1). This inhibition of putrescine degradation could contribute to the rise in putrescine concentrations found when cells are transformed by oncogenic viruses (Don *et al.*, 1975). But similar observations were made on chemically induced tumours (Table 5). Therefore the differences in diamine oxidase activity do not seem to be linked to viral transformation only, but rather to the transformed state itself. A much larger systematic study will be necessary, however, on all the different types of tumours presently available before it can be concluded that diminished diamine oxidase activity is a characteristic of all types of transformed cells.

Polyamine oxidase can degrade spermine to spermidine and spermidine to putrescine, yielding equimolar amounts of 3-aminopropionaldehyde

Table 4. *Determination of polyamine oxidase activity of different cell types*

Polyamine oxidase activity was measured as described in the Materials and Methods section. The number of cultures examined is shown in parentheses. Mean values $\pm$ S.E.M. are given.

Cells	1-Pyrroline formed (pmol/h per 50 μ g of protein)
Normal rat kidney at confluence	0.22 $\pm$ 0.01 (18)
Transformed rat kidney at confluence	0.09 $\pm$ 0.01 (14)
Normal myeloblasts (chick)	1.27 $\pm$ 0.10 (2)
Leukaemic myeloblasts (chick)	0.15 $\pm$ 0.08 (2)

at each oxidation step. The increased activity found in transformed rat kidney cells in exponential growth could also favour a rise in cellular putrescine concentrations, whereas at confluence the decrease in activity would contribute to a decline. These observations may represent a mechanism characteristic of transformed cells, since in normal rat kidney cells no marked fluctuations in the activity of this enzyme have been observed with growth. This constant enzyme activity in normal cells confirms previous findings in rat liver, where no variations in enzyme activity were observed during regeneration after liver damage by carbon tetrachloride (Hölttä, 1977). Other transformed cells, such as HEp2, HeLa and 9,10-dimethylbenz[*a*]anthracene-induced tumours, also show a 5-fold decrease in activity when compared with their normal counterparts (Tables 4 and 5).

As H_2O_2 is formed by the action of both polyamine oxidase and diamine oxidase, it was possible that H_2O_2 could inactivate polyamine oxidase or oxidize the γ -aminobutyraldehyde formed to γ -aminobutyric acid, which would not then be cyclized. However, the addition of catalase to the reaction mixture did not increase the amount of 1-pyrroline formed. Apparently the H_2O_2 formed in the reaction was not responsible for the decreased enzyme activity and confirms previous findings that polyamine oxidase is insensitive to H_2O_2 (Hölttä, 1977).

These arguments suggest that in homogenates of transformed cells at confluence the decrease in enzyme activity could be due to the absence of activators, to the presence of inhibitors or to some property of the enzyme itself.

Polyamine oxidase can be stimulated by low-molecular-weight aliphatic and aromatic aldehydes, with benzaldehyde and pyridoxal showing 74% and 23% activation respectively (Hölttä, 1977). The addition of 2 mM-pyridoxal phosphate to the assay system did not increase enzyme activity, however, with homogenates from transformed cells (results not shown).

When considering possible inhibitors it must be borne in mind that polyamine oxidase is inhibited by the products of the reaction. Putrescine at 1 mM inhibits the enzyme by 23% (Hölttä, 1977). But this concentration is at least a thousand times greater than that formed in our assay system, and further any putrescine formed would be oxidized to γ -aminobutyraldehyde by the excess diamine oxidase present. Thus it seems unlikely that it is the accumulation of end-product putrescine that is responsible for the lower enzyme activity observed with homogenates from transformed cells. It remains to be investigated whether enzyme activity can also be affected by the γ -aminobutyraldehyde so formed, or by 3-aminopropionaldehyde, the other direct product of polyamine oxidase action.

Finally, to detect a difference in the enzyme itself, one experimental approach could be to use antibodies specific for the polyamine oxidase of the cells being studied. With such antibodies any differences in the structure of the enzyme from normal and transformed cells should be detectable by immunochemical and immunoenzymic methods.

Regardless of the detailed mechanism(s) controlling the activity of polyamine oxidase, it seems clear from the results presented that the activity of this enzyme during the growth of transformed cells shows fluctuations that are not shown by their normal counterparts. On the other hand, for diamine oxidase both cell types show quantitative variations with growth. As these two enzymes are involved in putrescine metabolism, present explanations for the increase in putrescine on cell transformation, based entirely on the activation of ornithine decarboxylase, must be expanded to include the consideration of a role for polyamine and diamine oxidases.

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