The Polyamine Content in Two Slow-Growing Rat Hepatomas

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The spermidine concentration in proliferating normal eukaryotic cells increases before and during the increase in RNA content (Raina *et al.*, 1966; Calderara *et al.*, 1965), whereas the spermine content roughly parallels DNA biosynthesis (Russell *et al.*, 1969). The activity of ornithine decarboxylase, which appears to be a key regulatory enzyme in the biosynthetic pathway of the polyamines, increases dramatically in resting cells after the application of a proliferative stimulus, or after treatment with the appropriate anabolic hormones (Janne & Raina, 1969; Russell *et al.*, 1970; Cavia & Webb, 1972). In view of the relationship of the polyamines to hypertrophy and hyperplasia in normal tissue, and consequently the possibility that the rate of tumour growth and indeed the uncontrolled growth of tumour cells could be dependent on elevated polyamine concentrations, this parameter has been measured in two rat hepatomas which are considered to have a minimum of those lesions that are not essential to the neoplastic transformation *per se*, but rather that correlate with the growth rate or invasiveness of the tumour.

Experimental

The Morris hepatomas 5123D and 7800 were selected for this study since they have a slow growth rate (a transplant generation time of approx. 2 months) and are highly differentiated (Morris & Wagner, 1968). The tumours were carried in the hind legs of male rats of the Buffalo strain that weighed approx. 350g. Partial hepatectomy was performed by the method of Higgins & Anderson (1931). The spermine and spermidine contents of the normal liver and the hepatomas were determined by the method of Raina (1963), except that after electrophoresis the polyamines were determined by use of a special ninhydrin reagent (Heilman *et al.*, 1959). The recoveries of spermine and spermidine were 97 and 103% respectively. The relationship between the polyamine content and ornithine decarboxylase activity was also investigated. The latter activity was measured by radioassay with [^{14}C]-ornithine as substrate (see Cavia & Webb, 1972).

Results and discussion

The results in Table 1 indicate that the molar ratio of spermidine to spermine in the resting liver of the
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350g rat (12-16 months old) of the Buffalo strain is approx. 0.5; the corresponding ratio in a 200g rat was 1.20 (results not shown). These results correlate with and extend earlier results for the Wistar strain (Janne *et al.*, 1964), which showed that the molar ratios for the livers of 61g (1 month old), 213g (2 months old) and 328g (9 months old) rats of the latter strain were 1.95, 1.28 and 0.84 respectively. Since the ratios for the 250g rats of both strains were essentially identical, the present results indicate that this ratio continues to decrease in the rat liver as the animal ages.

The results for the regenerating liver of the aged (350g) rats are presented in Table 1 for comparative purposes. The spermine content, the molar ratio of spermidine to spermine and the ornithine decarboxylase activity increase dramatically after partial hepatectomy; the latter results are comparable with those reported previously for young rats (Russell *et al.*, 1969; Pegg & Williams-Ashman, 1970). However, the essential new finding of the present study is that, despite the fact that both the regenerating and neoplastic livers are proliferating tissues, the polyamine concentrations in the hepatomas are 3- to 6-fold lower in the neoplastic liver than in the normal proliferating tissue. Further, the results indicate that the ratio of the polyamines in hepatoma 5123D (1.70) and hepatoma 7800 (0.84) is significantly lower than the corresponding ratio in the regenerating liver (1.97) and more closely resembles the ratios in the resting livers of 2-month-old (1.69) and 9-month-old (0.84) rats respectively (Janne *et al.*, 1964; E. Cavia, unpublished work). This correspondence of the ratio of the polyamines in the two hepatomas to different stages of development of rat liver is relevant to the concept that oncogeny represents blocked ontogeny (Potter, 1969).

The elevated activity of ornithine decarboxylase in the hepatomas may be due to high concentrations of circulating corticosteroids released in response to the stress of the tumours, since this enzyme is induced in resting liver in response to the administration of cortisol (Cavia & Webb, 1972). The moderately high activity of ornithine decarboxylase in the hepatomas suggests that these tumours are deficient in other enzymes, possibly *S*-adenosyl-L-methionine decarboxylase, or one of the two propylamine transferases involved in the biosynthesis of spermine and spermidine (Raina & Hannonen, 1971; Janne & Williams-Ashman, 1971). In conclusion, the results of the

Table 1. *Concentration of polyamines and ornithine decarboxylase activity in normal, regenerating and neoplastic rat liver*

The concentrations of the polyamines are expressed as nmol/g wet wt. and the ornithine decarboxylase activity is expressed as c.p.m./h of incubation at 36°C per mg of soluble cell protein (determined by the method of Gornall *et al.*, 1949). The activity of ornithine decarboxylase was maximal (2200 c.p.m./h per mg) at 8h after the operation, then decreased to 1400 at 24h after the operation. The s.e.m. values, based on four to six rats, were in all cases less than 10%. As a standard for comparison the concentration of either spermine or spermidine in the resting liver of the 210g rat is 880 nmol/g wet wt.

Tissue	Concn. of polyamines			Ornithine decarboxylase activity
	Spermidine	Spermine	Ratio [spermidine]/[spermine]	
Normal liver (350g rat; age 14 months)				
Resting	382	760	0.50	30
60h regenerating	1360	690	1.97	800
Neoplastic liver				
Hepatoma 5123D	400	240	1.70	730
Hepatoma 7800	220	260	0.84	274

present study indicate that the high concentrations of polyamines reported (Russell & Levy, 1971) for some tumours are not an essential lesion in the cancer cell and therefore are not a necessary characteristic of a tumour. However, the low polyamine content of hepatomas 5123D and 7800 could, in fact, serve as a restraint on their growth rate. Coincidentally, the present finding that the spermidine content of hepatoma 5123D is approximately double that of hepatoma 7800, whereas the spermine content is approximately the same, provides further support for a recent proposal (Raina & Hannonen, 1971; Janne & Williams-Ashman, 1971) that separate propylamine transferases are involved in the synthesis of spermidine and spermine, one specific for putrescine and the other specific for spermidine.

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Calderara, C. M., Barbiroli, B. & Moruzzi, G. (1965) *Biochem. J.* **97**, 84
 Cavia, E. & Webb, T. E. (1972) *Biochim. Biophys. Acta* **262**, 546

Gornall, A. G., Bardawill, C. J. & David, M. (1949) *J. Biol. Chem.* **177**, 751
 Heilman, J., Barrolier, J. & Watzke, E. (1959) *Hoppe-Seyler's Z. Physiol. Chem.* **309**, 219
 Higgins, G. M. & Anderson, R. M. (1931) *Arch. Pathol.* **12**, 186
 Janne, J. & Raina, A. (1969) *Biochim. Biophys. Acta* **174**, 769
 Janne, J. & Williams-Ashman, H. G. (1971) *Biochem. Biophys. Res. Commun.* **42**, 222
 Janne, J., Raina, A. & Siimes, M. (1964) *Acta Physiol. Scand.* **62**, 352
 Morris, H. P. & Wagner, B. P. (1968) *Methods Cancer Res.* **4**, 125
 Pegg, A. E. & Williams-Ashman, H. G. (1970) *Arch. Biochem. Biophys.* **137**, 156
 Potter, V. R. (1969) *Proc. Can. Cancer Res. Conf.* **8**, 9
 Raina, A. (1963) *Acta Physiol. Scand. Suppl.* **218**, 1
 Raina, A. & Hannonen, P. (1971) *FEBS Lett.* **16**, 1
 Raina, A., Janne, J. & Siimes, M. (1966) *Biochim. Biophys. Acta* **123**, 197
 Russell, D. H. & Levy, C. C. (1971) *Cancer Res.* **31**, 248
 Russell, D. H., Snyder, S. H. & Medina, V. J. (1969) *Life Sci.* **8**, 1247
 Russell, D. H., Medina, V. J. & Snyder, S. H. (1970) *Endocrinology* **86**, 1414