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POLYAMINE DISTRIBUTION IN THE RAT INTESTINAL MUCOSA*

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SUMMARY

As the first step in a study of mucosal polyamine metabolism during intestinal adaptation, we have measured mucosal polyamine concentrations at different sites along the normal rat intestine Putrescine, spermidine, spermine and cadaverine were measured by spectrofluorometric analysis after thin-layer chromatography of their dansylated derivatives Spermidine was present in the largest amounts at each of the sampling sites The ratio of the concentration of spermidine to that of spermine paralleled the established pattern of cellular proliferation in the normal intestine as did the putrescine concentration (nmol per 10 cm) which decreased from duodenum to colon These results provide the essential background to an assessment of the role of polyamines in the intestinal adaptive response

INTRODUCTION

Following small bowel resection in the rat, the residual intestinal mucosa undergoes adaptive changes in structure and function which are more marked in the ileum than the jejunum [1] Luminal nutrition, pancreatic and biliary secretions and hormonal factors have all been implicated in the control of intestinal structure and function However, the tissue regulatory mechanisms, that is the processes by which the mucosal tissue responds to the trophic stimuli, are unkown

Recently Luk et al [2] have suggested that the polyamines putrescine,

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spermidine and spermine play an important role in the adaptive response of the intestinal mucosa. Transient increases in polyamine concentrations have been observed in the developing rat intestine, during recovery from intestinal injury and in the adaptation of the ileum following jejunectomy [2–4]

As the first step in a study of mucosal polyamine metabolism during intestinal adaptation, we have measured polyamine concentrations at different sites along the normal intestine We report here our methods and findings.

EXPERIMENTAL

Male Wistar rats [weighing 252–286 g, mean ± standard error of the mean (S E.M.) 263 ± 10 5, n = 13, nine weeks old] were housed individually and fed commercial chow (No 1 chow, Special Diets Service, Essex, U.K.) Following a 20-h fast, during which water was allowed, the animals were anaesthetised with phenobarbitone (intraperitoneally, 4 mg per 100 g body weight) and blood was withdrawn by cardiac puncture. The small bowel was removed from the abdomen, stripped of its mesentry, flushed with ice-cold isotonic saline, stretched with a 5-g weight and its length measured against a vertical scale The duodenum, 5 × 10 cm segments of jejunum, 5 × 10 cm segments of ileum, the colon and the caecum were separated, placed on glass plates over ice, split longitudinally and washed with ice-cold saline. Excess moisture was removed by blotting with filter paper and the mucosa scraped into tared vials. The mucosal samples were then stored at −70°C until analysed for their protein, DNA, α-D-glucosidase and polyamine content.

Reagents

Putrescine dihydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride, cadaverine dihydrochloride, proline and dansyl chloride were all obtained from Sigma (Poole, U.K.) [1,4-^{14}C] Putrescine dihydrochloride, specific activity 100 μCi/mmol and [^{14}C] spermine tetrahydrochloride, specific activity 100 μCi/mmol, were obtained from Amersham International. Precoated 0 2-mm Merck thin-layer chromatography (TLC) silica gel 60 plates without fluorescent indicator and all other chemicals were obtained from BDH (Poole, U.K.)

Polyamine measurement

The mucosal polyamines were extracted and measured as follows.

(i) Each mucosal sample was thawed and immediately homogenised in 5 ml ice-cold 0.1 *M* sodium phosphate buffer pH 7 2 (containing 1 m*M* dithioerythritol) using an ultra-Turrax homogeniser and an MSE 1600 sonicator (3 × 15 s bursts).

(ii) Chilled 2% perchloric acid (3 ml) was added to 2 ml of the homogenate The sediment obtained on centrifugation (1000 *g* for 15 min) was washed three times with 5 ml of 2% perchloric acid and the supernatant and washing were combined and applied directly to a column of Dowex-50 (40 × 10 mm) [5].

(iii) The column was washed with 40 ml of 0 1 *M* sodium phosphate buffer pH 8.0, containing 0 7 *M* sodium chloride, followed by 10 ml of 1 *M* hydrochloric acid The polyamines were eluted with 15 ml of 6 *M* hydrochloric acid.

(iv) The volume of each eluate was measured and 0 3 ml were evaporated at 55° C using a rotary evaporator

(v) The residue was dissolved in 0.3 ml of 0.6 *M* sodium carbonate solution. Dansylation was achieved by the addition of 0.3 ml dansyl chloride (10 mg in dry acetone) [6, 7]. Following vortex-mixing some 2 mg of sodium carbonate were added and the mixing was repeated. The dansylation reaction was allowed to continue in the dark at room temperature overnight

(vi) A 0 1-ml volume of 100 mg/ml proline in water was added and the mixture allowed to stand for 1 h at 37° C

(vii) Following the addition of 3 ml of 0.5 *M* sodium hydroxide, the dansylated polyamines were extracted by shaking with 3 × 6 ml diethyl ether. The ether extracts were pooled and evaporated to dryness under a stream of air.

(viii) The residues were dissolved in 200 μl chloroform (containing 1% ethanol).

Thin-layer chromatography

Standard solutions of putrescine, spermidine, spermine and cadaverine were prepared in 6 *M* hydrochloric acid such that the concentrations of each polyamine ranged from 0 to 40 μmol/l (see Fig 1) Of each of these solutions 300 μl were evaporated to dryness, dansylated as described above and the residue was dissolved in 200 μl chloroform. Of these solutions and of the sample extracts 50 μl were applied to activated (1 h at 110° C) silica gel plates Ten extracts including at least two standard solutions were routinely spotted on each TLC plate The plates were run in ethylacetate–cyclohexane (2.3, v/v) [8] Following location under UV light (340 nm), the areas of each dansylated polyamine were cut and placed in small stoppered glass cuvettes with chloroform (2 ml for putrescine, 3 ml for others) and left in the dark for 24 h at room temperature. The strips were then removed and the fluorescence of the cuvette contents was read at 355 nm excitation and 505 nm emission in a Perkin-Elmer spectrofluorimeter (MPF 3-L)

Because dansylated polyamines are unstable, standard solutions of polyamines were dansylated and chromatographed with each batch of tissue analyses

Other assays

Protein content of the homogenates was estimated by the method of Lowry et al [9], DNA as described by Prasad et al. [10] and α-D-glucosidase using a modification [11] of the method of Baudhim et al [12].

RESULTS

Recovery and reproducibility experiments

(i) Recoveries of [^{14}C] putrescine and [^{14}C] spermine added to tissue samples during their homogenisation are shown in Table I Losses of putrescine were appreciable during the dansylation step and both putrescine and spermine were poorly recovered from the thin-layer plates

(ii) The recoveries of standard solutions of each of the polyamines were reproducible and independent of the original concentration (Fig 1)

TABLE I

RECOVERY OF [^{14}C]PUTRESCINE AND [^{14}C]SPERMINE ADDED TO TWO DIFFERENT TISSUE SAMPLES DURING THE HOMOGENISATION STEP

Stage	Tissue sample	Polyamine recovery (%)	
		[^{14}C]Putrescine*	[^{14}C]Spermine*
Perchloric acid digestion	1	88 8	105 1
	2	87 1	105 8
Dowex column chromatography	1	89 0	98 5
	2	93 8	89 8
Dansylation and ether extraction	1	62 1	95 3
	2	73 0	91 1
Thin-layer chromatography	1	50 1	51 3
	2	56 7	51 5

* Activity 20 nCi, approx 0 25 nmol

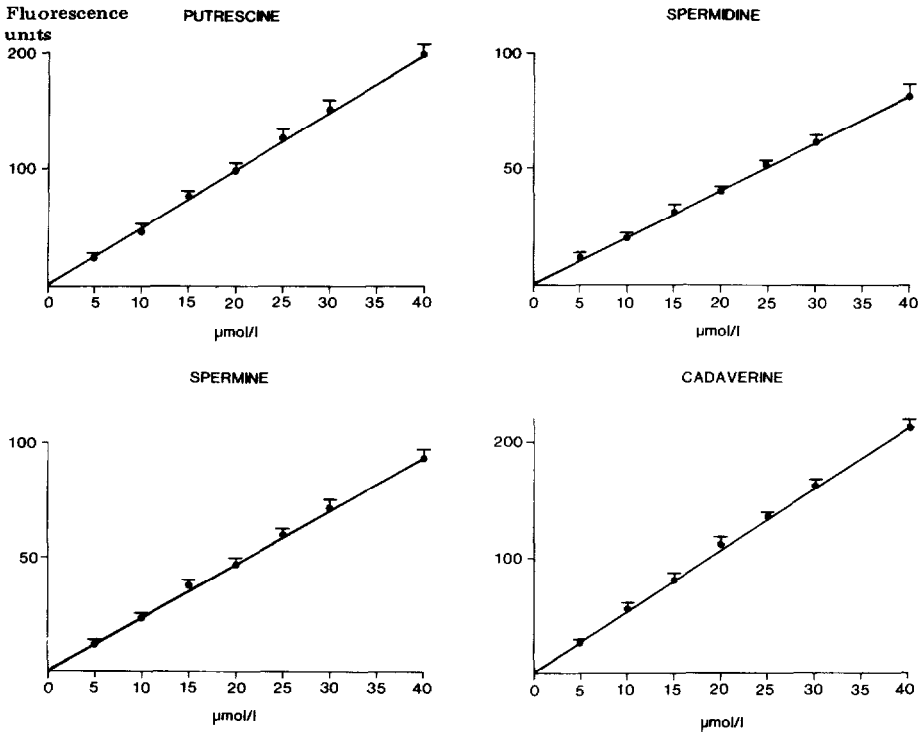

Fig 1 Reproducibility of standard curves (mean ± S E M , n = 15) for each of the polyamines Arbitrary units of fluorescence versus concentration (μmol/l)

(iii) The precision of the R_F values for each of the polyamine fractions within different tissue samples (expressed relative to the R_F of excess dansylated reagent in each lane) was (mean ± S D) putrescine, 46 7 ± 2 0, spermidine, 32.0 ± 1.7, spermine, 22 3 ± 1 7, cadaverine, 51 5 ± 2 1

(iv) All tissue analyses were performed in duplicate and an analysis of the

TABLE II

PRECISION OF DUPLICATE DETERMINATIONS OF POLYAMINE CONCENTRATIONS IN TISSUE SAMPLES ANALYSED IN FIFTEEN DIFFERENT BATCHES

Concentration added (μmol/l)	*n*	Concentration found (mean ± S D) (μmol/l)	Coefficient of variation (%)
< 25	90	16 0 ± 1 0	6 2
26– 49	120	41 0 ± 1 4	3 4
50– 74	71	64 0 ± 2 2	3 4
75– 99	41	89 5 ± 2 2	2 5
> 99	19	122 9 ± 2 3	1 9

data obtained to date showed the method to be reproducible from batch to batch (Table II)

Intestinal mucosal polyamine distribution

Of the polyamines in the normal rat intestine, spermidine was present in the highest concentrations in all of the intestinal segments (Fig 2). Putrescine, spermidine and spermine were present in much greater amounts than the bacteria-associated cadaverine There was a proximal-to-distal gradient in putrescine concentrations when the results were expressed per unit length

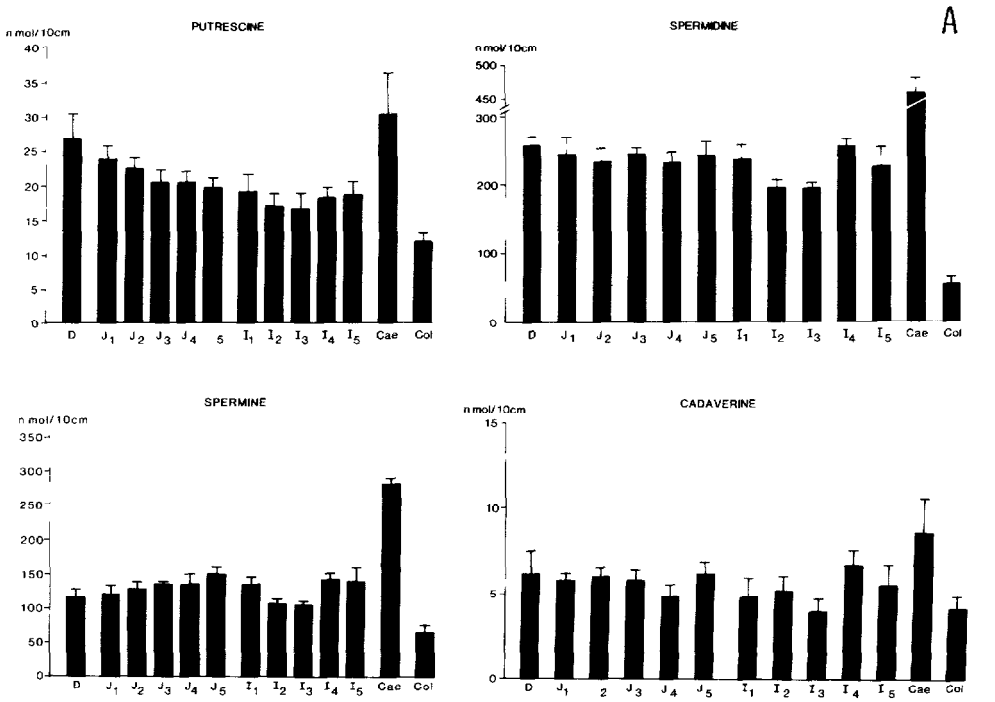

Fig 2

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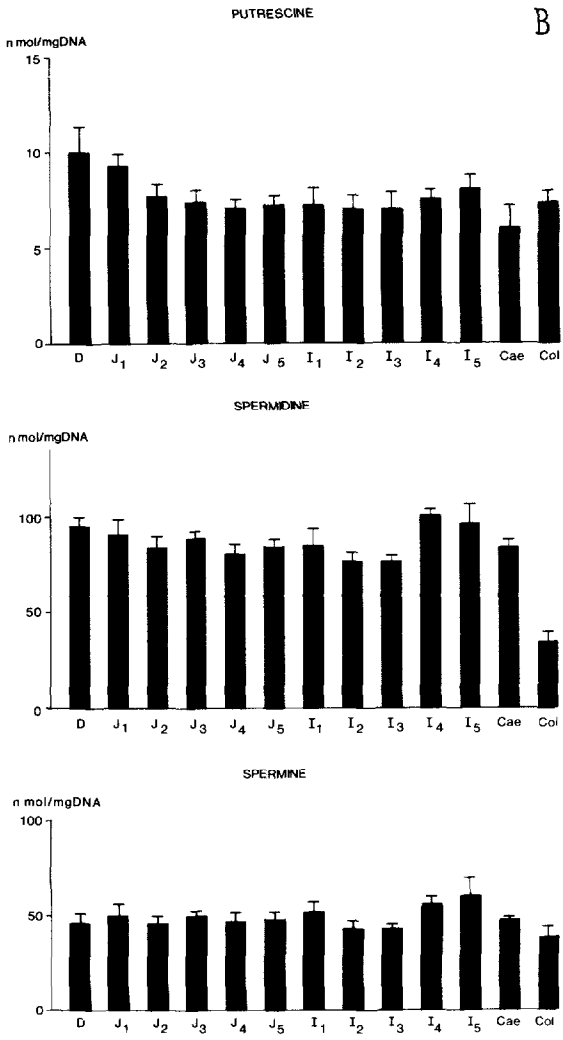

Fig 2 Polyamine distribution in mucosa of normal rat intestine D = Duodenum, J = jejunum, I = ileum, Cae = caecum, Col = colon (A) Concentrations expressed nmol per 10 cm (mean ± S E M , n = 13), (B) concentrations expressed nmol per mg DNA (mean ± S E M , n = 13)

intestine There was also a gradient in the corresponding DNA and protein results (Table III) The distribution of the α-D-glucosidase activity was as previously described [13]

DISCUSSION

This is the first report of the distribution of polyamines throughout the intestine of the normal rat The analytical procedure described makes use of different aspects of earlier methods To avoid lengthy extraction and concomitant poor recovery, the polyamines were isolated from homogenised

TABLE III

INDICES (MEAN ± S E M) OF INTESTINAL MUCOSAL MASS AND α-GLUCOSIDASE IN THIRTEEN NORMAL RATS

		Mucosal wet weight (mg per 10 cm)	DNA (mg per 10 cm)	Protein (mg per 10 cm)	α-Glucosidase (units* per 10 cm)
Duodenum		313 3 ± 16 6	2 69 ± 0 13	30 74 ± 1 24	194 4 ± 24 2
Jejunum	1	298 0 ± 19 2	2 74 ± 0 14	31 78 ± 0 28	230 3 ± 16 2
	2	295 9 ± 15 5	2 78 ± 0 30	30 74 ± 1 46	331 6 ± 32 6
	3	298 0 ± 11 5	2 78 ± 0 18	30 60 ± 1 33	367 8 ± 30 3
	4	293 8 ± 10 9	2 75 ± 0 19	30 59 ± 1 54	365 4 ± 34 0
	5	290 0 ± 12 3	2 72 ± 0 18	29 45 ± 1 01	334 5 ± 27 9
Ileum	1	290 0 ± 12 9	2 66 ± 0 22	29 19 ± 1 01	308 5 ± 17 4
	2	288 2 ± 15 7	2 46 ± 0 14	28 42 ± 1 27	218 1 ± 22 7
	3	286 1 ± 17 4	2 41 ± 0 15	27 95 ± 1 59	125 4 ± 15 3
	4	275 0 ± 15 1	2 47 ± 0 09	27 67 ± 0 83	72 2 ± 10 0
	5	257 5 ± 15 9	2 40 ± 0 15	25 92 ± 1 44	36 8 ± 5 3
Caecum**		290 ± 9 6	5 38 ± 0 60	58 95 ± 5 00	24 9 ± 0 5
Colon		258 2 ± 13 6	1 64 ± 0 19	17 98 ± 0 96	12 0 ± 1 1

*1 Unit of activity = 1 nmol substrate converted to product per minute
**Whole mucosa of each caecum taken for assay

tissue using a small Dowex column as described by Inoue and Mizutani [5], the polyamines were dansylated [6] using the procedure of Dion and Herbst [7] and the TLC separation was that of Dreyfuss et al [8]. With this procedure we obtained consistent recoveries, a sensitive fluorimetric end point and measured cadaverine (which is usually considered to be the product of bacterial decarboxylation of lysine [14] and could be used as an index of bacterial contamination [15]). However, although to date lysine decarboxylase has only been isolated from plants and bacteria, some authors consider that cadaverine may be produced in mammalian cells with a high ornithine decarboxylase activity [16] In the present study, the cadaverine concentrations were lower than those of the other polyamine concentrations and did not vary with the site of tissue sampling Nevertheless, the possibility that the mucosal preparations were contaminated by bacteria (particularly in the caecum) cannot be entirely ruled out The concentrations of putrescine (expressed as nmol per 10 cm) decreased from duodenum to colon and paralleled the well established gradient in mucosal mass from duodenum to colon [1] (Fig. 2A).

Spermidine was the polyamine present in the largest amounts at each of the sampling sites This finding is similar to those of Luk et al [2], Porter et al [17] and Luk and Baylin [4]

The concentrations of spermine found in the present study were always less than those of spermidine although higher than those of putrescine This finding does not agree with those of previous studies [2, 4, 15]. Our results are more in

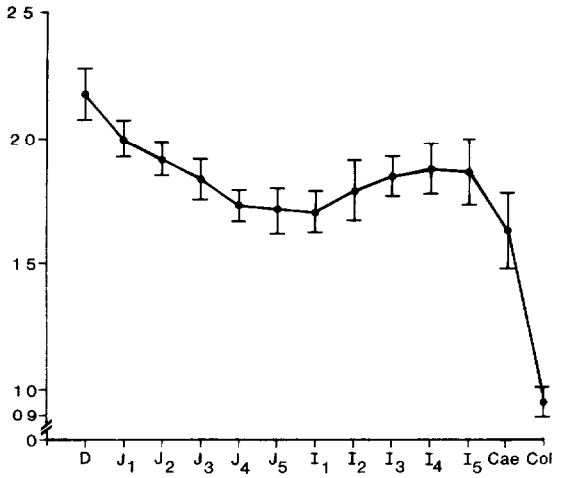

Fig 3 Ratio spermidine/spermine concentrations in mucosa of normal rat intestine (mean ± S E M , n = 13)

keeping with those of Jänne et al [18] Spermine concentration per cell tends to remain constant or increase with age and the ratio spermidine/spermine is also considered to be age-dependent and may be regarded as an index of cellular proliferation [18] The rats in the present study weighed 263 ± 10 5 g and the spermidine/spermine ratios were highest in the duodenum and lowest in the colon (Fig. 3) Thus in our study, the ratios did parallel the established pattern of cellular proliferation in the normal rat intestine

Polyamine concentrations and metabolism have long been of interest to clinicians monitoring the effects of therapy on tumour growth in man [19], studying maturation [20, 21] and tissue regeneration [22, 23]. Recent studies of the link between polyamines and cellular proliferation have been extended to the intestine [2] We have described a convenient method for measurement of the four common polyamines The results provide the essential background for an assessment of the role of polyamines in intestinal adaptive response

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