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Poly(Adenosine Diphosphate Ribose) Synthetase Activity in Nuclei of Dividing and of Non-Dividing but Differentiating Intestinal Epithelial Cells

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Poly(ADP-ribose) synthetase activity is found in nuclei of regenerating epithelial cells in the lower half of the crypts of guinea-pig small intestine. Nuclei from non-dividing but differentiating and maturing cells in the upper crypts and on the villi contain no more than about 10% of the synthetase activity of lower-crypt cell nuclei. The product in the active nuclei is shown to be 80% poly(ADP-ribosylated) protein and 20% mono-(ADP-ribosylated) protein; 60% of the total labelled product was attached to acid-soluble proteins (including histones), and 40% to acid-insoluble (non-histone) proteins. The average number of ADP-ribosyl units in the oligomeric chains of the poly(ADP-ribosylated) proteins was 15 but the range of sizes of (ADP-ribose) oligomers attached to nuclear proteins was smaller in villus than in crypt cell nuclei.

Histological and radioautographic evidence (Wright *et al.*, 1972; Cheng & Leblond, 1974; Al-Dewachi *et al.*, 1974; van Dongen *et al.*, 1976; Specht, 1977), together with cytochemical and microanalytical evidence (Dahlqvist & Nordström, 1966; Nordström *et al.*, 1968; Nordström & Dahlqvist, 1970; Webster & Harrison, 1969; Grey & LeCount, 1970; Weiser, 1973; de Both *et al.*, 1974, 1975), provides the following picture of the epithelium of mammalian small intestine: (i) the entire epithelium is arranged as a continuous and ordered palisade of cells lining the villi (which project into the lumen of the intestine) and the crypts of Lieberkühn; crypt cells account for about 25%, villus cells for about 75% of total epithelium; (ii) there is evidence for the presence of two populations of regenerating cells in the crypts, one possessing a characteristically longer cycle time than the other, but DNA replication and subsequent mitotic division of cells is confined to cells lining the lower half of the crypts; (iii) a gradual process of cell differentiation is already apparent in the cells of the upper crypts and continues as cells emerge on to the villi and progress in ordered array along the villi; senescent cells desquamate from the tips of the villi and are degraded in the intestinal lumen; (iv) the crypt cell population is dominated by regenerating and differentiating columnar epithelial cells (mucin-secreting, endocrine and exocrine cells together make up the remaining 10–12% of the crypt cells), and the villus cell population is dominated by columnar absorptive epithelial cells (mucin-secreting, tuft and microfold cells together make up the remaining 5–10% of this population). Columnar cells make up 90% of the total epithelium of small intestine. It is thus a reasonable simplification to regard intestinal epithelium as being composed of two related populations of cells: the regenerating cells of the lower half of the crypts, and the differentiating cells of the upper crypts and villi. This assumption is made for the purpose of the work to be presented here.

The ordered separation of intestinal epithelial cells into two related populations, one dividing and one differentiating but not dividing, should provide opportunities for discovering changes associated with cessation of DNA replication and the onset of differentiation. It is known that ADP-ribosylated nuclear proteins occur in all eukaryotic cells so far examined, that the donor of the ADP-ribose group is NAD^+, that DNA and histones are essential for the reaction to proceed and that the reaction is catalysed by poly(ADP-ribose) synthetase; the products include mono- and poly-(ADP-ribosyl)-substituted nuclear proteins (Hilz & Stone, 1976; Hayaishi & Ueda, 1977). It has been proposed that this covalent modification of nuclear proteins plays a role in DNA synthesis and repair (Hilz & Stone, 1976; Shall *et al.*, 1977) and in RNA synthesis (Müller *et al.*, 1974; Furneaux & Pearson, 1978). An investigation of the possible distribution of poly(ADP-ribose) synthetase activity between the two related populations of intestinal epithelial cells seemed timely and is presented; some characteristics of the products formed have also been determined.

Materials and Methods

Chemicals

Bovine serum albumin (fraction V) and hyaluronidase (type II) were supplied by Sigma (London)

Chemical Co., Poole, Dorset BH17 7NH, U.K. Snake-venom phosphodiesterase (Sigma, type V) was purified as described by Keller (1964). [*adenosine*-^{14}C]NAD^+ (300mCi/mmol) and [*methyl*-^{3}H]thymidine (50Ci/mmol) were from The Radiochemical Centre, Amersham, Bucks., U.K.

Animals

Male guinea pigs of the Dunkin–Hartley strain were supplied by A. Tuck and Son (Battlesbridge, Essex, U.K.) and maintained on a standardized laboratory diet with water *ad libitum*; they were used at live weights of between 500 and 650g and killed by neck fracture.

Isolation of sequential populations of cells

Intestinal epithelial cells were isolated as four sequential batches of cells by a method based on those described by Evans *et al.* (1971), Weiser (1973) and Towler *et al.* (1978). The jejunum was excised rapidly immediately after death, washed out with 50ml of 1.32% NaCl at room temperature and filled with cell isolation medium (185mM-mannitol, 96mM-NaCl, 8mM-KH_2PO_4, 5.6mM-Na_2HPO_4, 1.5mM-KCl, 5mM-EDTA, 5mM-EGTA, 0.5mM-dithiothreitol, 2.5mg of bovine serum albumin/ml, pH6.8). The lumen was closed at both ends and the filled intestine submerged in 1.32% NaCl at 37°C for 5min. Epithelial cells were released (after removing the intestine from the incubation bath) by discarding the isolation medium, filling the intestinal lumen with cell suspension medium (0.2M-sucrose, 19mM-KH_2PO_4, 76mM-Na_2HPO_4, pH7.4) and patting the length of filled intestine. The suspension of cells was emptied from the lumen of the intestine, the lumen refilled with fresh cell isolation medium, the intestine closed and reincubated for 3–5min. Repetition of this procedure removed sequentially upper villus cells, lower villus cells, upper crypt cells and lower crypt cells. Each batch of cells was sedimented (100*g*, 1min), the supernatant solution discarded and cells were resuspended in 0.2M-sucrose/76mM-Na_2HPO_4/19mM-KH_2PO_4, pH7.4, at 0°C.

Preparation of nuclei

Nuclei were prepared by homogenizing cells in 0.32M-sucrose/0.5mM-$MgCl_2$/1mM-KH_2PO_4/0.2% (w/v) Triton N101, pH6.8, by using a Tri-R homogenizer at 0°C. Nuclei were pelleted by centrifuging (1000*g*, 3min), resuspended in the same fresh medium without Triton N101 and pelleted by centrifuging as before. Phase-contrast microscopy revealed a clean preparation of nuclei with only minor contamination by endoplasmic reticulum, brush borders and cell-surface membranes (Porteous, 1968, 1972).

Thymidine incorporation into DNA in vivo; enzyme activities in vitro

Incorporation of [*methyl*-^{3}H]thymidine into epithelial cells *in vivo* was determined by injecting animals intraperitoneally with 100μCi of [^{3}H]-thymidine 2h before they were killed. The DNA fraction was prepared from each population of isolated cells by the Schmidt–Thannhauser procedure as modified by Munro & Fleck (1966). Samples of the DNA fraction were counted for radioactivity in xylene (2vol.)+Triton X-100 (1vol.)+0.8% 2,5-diphenyloxazole+0.003% 1,4-bis-(5-phenyloxazol-2-yl)benzene. Radioactivities are expressed as c.p.m./mg of nuclear protein.

Alkaline phosphatase activity was determined in cell homogenates as described by Weiser (1973). Protein was determined in cell homogenates and in isolated nuclei by the method of Miller (1959), with bovine serum albumin (fraction V) as standard.

Poly(ADP-ribose) synthetase activity was determined by incubating 1×10^6–5×10^6 nuclei at 25°C for 1min in a final volume of 0.2ml containing 50mM-Tris/HCl (pH8.0), 5mM-$MgCl_2$, 1μM-[^{14}C]NAD^+ and 1mM-dithiothreitol. Reaction was terminated by addition of 0.2ml of 10% trichloroacetic acid containing 10mM-NAD^+. Samples of the acidified suspension were filtered on GF/C discs, washed with 2×5ml of cold 5% trichloroacetic acid followed by 5ml of 70% ethanol, then dried; radioactivity in the discs was determined in toluene containing 0.4% 2,5-diphenyloxazole and 0.005% 1,4-bis-(5-phenyloxazol-2-yl)benzene. Since histone H1 is often removed from tissues by repeated extraction with 5% trichloroacetic acid, it is possible that the procedure described would fail to precipitate this particular protein, but preliminary experiments showed that neither the supernatant from the terminated reaction system, nor the washing from the filtration step, contained significant amounts of radioactively labelled macromolecular material; by the same token, it seems reasonable to conclude that ADP-ribosylation of proteins did not prevent their precipitation by trichloroacetic acid and trapping on filters in the procedure detailed above. Enzyme activity is given as pmol of ADP-ribose incorporated/mg of nuclear protein.

Characterization of the products of poly(ADP-ribose) synthetase activity

Identity of the labelled product. Phosphodiesterase digestion of ADP-ribosylated (trichloroacetic acid-insoluble) nuclear proteins involved all the steps described in the preceding paragraph up to and including termination of the reaction with trichloroacetic acid/NAD^+. The trichloroacetic acid-insoluble material was dissolved in 0.1ml of 0.1M-NaOH in order to remove mono- and poly-(ADP-ribose) units

from proteins; after 15min at 20°C, 0.1ml of 0.1M-HCl was added, followed by 0.05ml of 0.2M-Tris/HCl (pH7.0), 0.05ml of 0.1M-$MgCl_2$ and 0.02ml of snake-venom phosphodiesterase (1mg/ml). After 1h at 37°C, the reaction was terminated by adding cold $HClO_4$, the suspension neutralized with KOH and $KHCO_3$, centrifuged (4000*g*, 10min), and the supernatant solution concentrated by evaporation under N_2. Samples (0.02ml) were chromatographed (Stone *et al.*, 1973) on plastic-mounted poly(ethyleneimine) thin-layer plates with 0.9M-acetic acid/0.3M-LiCl; 5mm-wide strips were analysed for radioactivity in the toluene/Triton scintillant described above. The alkali extraction procedure described above removed 94–96% of the radioactivity, which had been incorporated from [^{14}C]NAD^+ into trichloroacetic acid-insoluble material; all of this extracted radioactivity was subsequently found in the two materials separated by poly(ethyleneimine) chromatography. The chromatographic system separated 5′-AMP completely from phosphoribosyl-5′-AMP.

Distribution of label between monomer and polymer units of ADP-ribose attached to nuclear proteins; mean size of poly(ADP-ribose) chains. The proportions of mono(ADP-ribosyl) and poly(ADP-ribosyl) units incorporated into trichloroacetic acid-insoluble products after incubating nuclei with [^{14}C]NAD^+ were determined by hydroxyapatite chromatography. The trichloroacetic acid-insoluble material was treated with 0.1ml of 0.1M-NaOH; after 15min at 20°C the solution was brought to pH6.8 with 0.1ml of 0.1M-HCl and 0.2ml of 2mM-sodium phosphate buffer (pH6.8), applied to a hydroxyapatite column (5mm×30mm, equilibrated with 1mM-sodium phosphate buffer) and eluted with a linear gradient of sodium phosphate buffer, pH6.8 (1–400mM). Eluted fractions were sampled for determination of orthophosphate concentration and for radioactivity measurements in the toluene/Triton scintillant described above.

ADP-ribosylation of acid-soluble and acid-insoluble nuclear proteins. To determine the distribution of ADP-ribosyl units between acid-soluble and acid-insoluble proteins, nuclei were incubated with [^{14}C]NAD^+ as before, the reaction was terminated by adding HCl to a final concentration of 0.25M, and insoluble material was sedimented (10000*g*, 15min); the acid-insoluble pellet was re-extracted with 0.25M-HCl. The residual pellet (taken to contain non-histone nuclear proteins) was suspended in 20% trichloroacetic acid. The combined HCl extracts (taken to contain histones) were treated with an equal volume of 40% trichloroacetic acid containing 10mM-NAD^+; samples of this suspension, and of the resuspended residual pellet, were filtered on GF/C discs, washed with cold 20% trichloroacetic acid, then with 70% ethanol and dried. Radioactivity in the discs was determined as before.

Results and Discussion

Cell-isolation procedure

Results in Table 1 show that the populations of cells obtained in the early stages of the cell-isolation procedure were predominantly villus cells, the later populations mainly crypt cells. Thus the early batches of cells contained the highest alkaline phosphatase activity, the later batches low activity (Table 1, columns i, ii, iii); this is consistent with histochemical and micro-analytical information showing that the enzyme is characteristically present in the brush borders of villus cells only (Nordström *et al.*, 1968; Grey & LeCount, 1970; de Both *et al.*, 1974). Conversely, labelling of cell DNA with [^{3}H]thymidine occurred predominantly in those cells that were isolated last (Table 1, columns iv, v, vi); this is consistent with radioautographic and histological evidence that only cells in the lower half of the crypts synthesize DNA and undergo mitotic division (Cheng & Leblond, 1974; van Dongen *et al.*, 1976). Cells were clearly released in sequence starting at villus tips and ending in lower crypts, but cross-contamination between the major populations of cells is nevertheless evident and is to be expected. Adjacent intestinal epithelial cells are quite firmly attached to each other by tight junctions, desmosomes and interdigitations of the lateral cell-surface membranes (Porteous & Clark, 1965; Trier & Rubin, 1965; Toner, 1968; Pritchard & Porteous, 1977) and are necessarily isolated as small sheets of cells (Towler *et al.*, 1978); some sheets of cells remain trapped in the intestinal lumen after they have been released from the substratum by treatment with the cell-isolation medium, and may then contaminate cells released by a subsequent incubation with cell-isolation medium. Similarly, intestinal crypts are not of exactly uniform geometry and some may release their cells more readily than others, so contaminating what are predominantly villus cell fractions. The [^{3}H]DNA present in the cell fractions (1), (2) and (3) is taken as a quantitative measure of the contamination of these cell populations by cells from the lower half of the crypts (Table 1).

Distribution of poly(ADP-ribose) synthetase activity between the major cell populations

Table 1 (columns vi and vii) shows that most but not quite all of the poly(ADP-ribose) synthetase activity in cell populations (1), (2) and (3) is accounted for by the contamination of these cells with cells from the lower half of the crypts. The mean synthetase activity of cell populations (1), (2) and (3) is about 10% in excess of that which could be accounted for by contamination of each of these three populations by population (4). The synthetase activity is thus almost entirely associated with those cells that are

Table 1. *Poly(ADP-ribose) synthetase activity in nuclei isolated from different populations of epithelial cells of guinea-pig small intestine*

Four sequential populations of cells were isolated (as described in the Materials and Methods section) 2h after animals had received an intraperitoneal injection of [*methyl*-^{3}H]thymidine. Since the crypt cell-cycle time is of the order of 12h and the cell transit time from crypt to villus tip is of the order of 40h (Cheng & Leblond, 1974), only nuclei in the lower-crypt cells would be labelled under these experimental conditions. In three experiments (A), (B) and (C), the alkaline phosphatase activity of each population of isolated cells was determined (columns i, ii and iii). In four independent experiments (D), (E), (F) and (G) poly(ADP-ribose) synthetase activity and thymidine incorporation into DNA were determined (paired determinations) in each population of isolated cells (columns iv–vii). Isolated cell populations 1, 2, 3 and 4 correspond to upper villus, lower villus, upper-crypt and lower-crypt cell populations respectively. Percentage values are given to the nearest whole unit. One unit of alkaline phosphatase activity releases 1 μmol of orthophosphate during a 15min incubation under the conditions given by Weiser (1973); 1 unit of poly-(ADP-ribose) synthetase activity incorporates 1 pmol of ADP-ribose per min into nuclear protein under the conditions given in the Materials and Methods section.

(i)	(ii)	(iii)		(iv)	(v)	(vi)		(vii)	
		Alkaline phosphatase activity *in vitro*				[^{3}H]Thymidine incorporation into DNA *in vivo*		Poly(ADP-ribose) synthetase activity in isolated nuclei *in vitro*	
Expt.	Isolated cell population	(units/mg of protein)	(%)	Expt.	Isolated cell population	(c.p.m./mg of protein)	(%)	(units/mg of protein)	(%)
(A)	1	2.21	100	(D)	1	2120	17	16.7	34
	2	2.65	120		2	3320	27	16.5	34
	3	1.85	84		3	5730	47	30.5	63
	4	0.65	29		4	12100	100	48.8	100
(B)	1	3.08	100	(E)	1	2110	13	9.3	26
	2	3.10	101		2	3660	22	11.5	32
	3	1.74	56		3	8070	49	18.7	52
	4	0.36	12		4	16400	100	36.1	100
(C)	1	2.84	100	(F)	1	4700	11	10.4	20
	2	2.90	102		2	7430	17	11.0	21
	3	1.62	57		3	23800	54	39.2	74
	4	0.28	10		4	43800	100	52.7	100
				(G)	1	2470	13	15.8	25
					2	4760	25	19.3	30
					3	10800	56	36.3	56
					4	19300	100	64.4	100

known to synthesize DNA and undergo mitotic division, and is almost absent from or inactive in upper-crypt and villus cells which are known not to synthesize DNA but which are known to be differentiating. Substantiation of this deduction is given in a statistical treatment of the results in the Appendix to this paper.

The synthetase activity has been measured (Table 1) by following (net) incorporation of ADP-ribose from [^{14}C]NAD^+ into mono- and poly-(ADP-ribosylated) proteins (Figs. 1 and 2). Net incorporation of labelled ADP-ribose units could be the resultant effect of two opposing activities, poly(ADP-ribose) synthetase activity and the hydrolase activities responsible for the degradation of the ADP-ribosylated proteins in nuclei (Hayaishi & Ueda, 1977; Shall *et al.*, 1977). The lower incorporation of label from [^{14}C]NAD^+ into trichloroacetic acid-insoluble products by villus cell nuclei (Table 1) could thus have resulted from a higher hydrolase activity in these cells. This possibility is discounted because, as will be shown, the average number of ADP-ribosyl units per poly(ADP-ribose) chain was the same in nuclei prepared from populations of isolated cells that were predominantly villus cells as it was in nuclei prepared from isolated cells that were mainly crypt cells.

Products of poly(ADP-ribose) synthetase activity

The total (trichloroacetic acid-insoluble) labelled products formed after incubation of nuclei with [^{14}C]NAD^+ were resistant to ribonuclease and deoxyribonuclease digestion. Snake-venom phosphodiesterase digestion of the labelled products yielded

only 2′-(5″-phosphoribosyl)-5′-AMP and 5′-AMP (Fig. 1) as expected from mono- and poly-(ADP-ribosylated) proteins (Hayaishi & Ueda, 1977; Shall *et al.*, 1977).

Two estimates were made of the mean chain length of oligo(ADP-ribose) chains attached covalently to total nuclear proteins. The first depended upon determination of the proportions of labelled 5′-AMP and labelled 2′-(5″-phosphoribosyl)-5′-AMP released by phosphodiesterase digestion of poly(ADP-ribose) chains removed from proteins, and thus gave an estimate of the mean length of radioactively labelled recently synthesized oligo(ADP-ribose) chains (Nishizuka *et al.*, 1969; Hayaishi & Ueda, 1977; Shall *et al.*, 1977). The mean chain length determined in this way was 2.3 for both lower crypt and upper villus cell nuclei incubated as described in the Materials and Methods section.

The second estimate of mean chain length of oligo(ADP-ribose) chains removed from nuclear proteins after incubating nuclei with NAD^+ depended upon the established linear correlation between the mean chain length of oligo(ADP-ribose) molecules and the concentration of orthophosphate buffer required to elute them from hydroxyapatite columns (Sugimura *et al.*, 1971; Tanaka *et al.*, 1977). This estimate of mean chain length was independent of any measurements of incorporation of radioactive precursors into products; both labelled and unlabelled components of mono- and oligo-(ADP-ribose) chains were included in the estimates. A small, sharp and symmetrical peak of labelled material eluted from the hydroxyapatite column before the orthophosphate concentration was increased above 1 mM (Fig. 2). The material so eluted must thus have contained fewer than 2.8 (ADP-ribose) units per oligomer chain (Tanaka *et al.*, 1977). Poly(ethyleneimine) chromatography of a sample of this material showed that it contained only mono(ADP-ribose). The remaining 80% of the radioactivity appeared in a second major peak of material (Fig. 2), the mid-point of the peak corresponding to a mean chain length of 15 (ADP-ribose) monomers per oligomer chain (Sugimura *et al.*, 1971). In these experiments over 90% of the radioactivity applied to the column was recovered in the eluted materials (Fig. 2). Experiments with lower crypt and upper villus cell nuclei yielded the same initial peak of material containing mono(ADP-ribose), and both kinds of nuclei yielded a second peak of material with a mean chain length of $(ADP\text{-}ribose)_{15}$, but crypt cell nuclei yielded material in this second peak with a much wider distribution of chain size (Fig. 2).

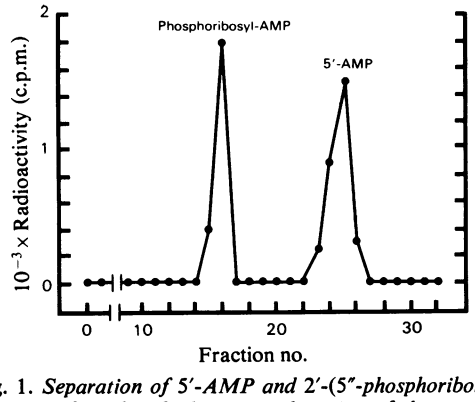

Fig. 1. *Separation of 5′-AMP and 2′-(5″-phosphoribosyl)-5′-AMP after phosphodiesterase digestion of the products formed from incubation of nuclei with [adenosine-^{14}C]NAD^+*
Nuclei from lower-crypt cells were incubated with [^{14}C]NAD^+, mono- and poly-(ADP-ribose) chains removed from total nuclear proteins, digested with snake-venom phosphodiesterase and subjected to poly(ethyleneimine) chromatography as described in the Materials and Methods section. An authentic marker was used to identify the expected position of 5′-AMP on the chromatogram; ADP-ribose was used as the marker to identify the expected position of phosphoribosyl-AMP (Lehman *et al.*, 1974).

The overall mean length of the mono- and oligo-(ADP-ribose) eluted from the hydroxyapatite column (Fig. 2) was calculated (from the information given above) to be 12.2 (ADP-ribose) units per oligomer. This value applies to the products obtained from either the crypt or villus cell nuclei and is much in excess of the 2.3 (ADP-ribose) units per oligomer estimated by the isotope incorporation and phosphodiesterase digestion procedure described above. The conclusion must be that mono(ADP-ribosylated) nuclear proteins account for only 20% of the (ADP-ribose) transferred from [^{14}C]NAD^+ during incubation of intestinal epithelial cell nuclei incubated *in vitro* under the conditions described; and that 80% of the (ADP-ribose) units transferred from NAD^+ to nuclear proteins in the same experiments must have been transferred to pre-existing oligo(ADP-ribose) chains on the proteins to give the observed mean chain length of 15 (ADP-ribose) units per oligomer (Fig. 2).

The results obtained after incubating crypt cell nuclei (Fig. 2) were most probably unaffected by the small number of contaminating villus cell nuclei, which in any case possess low synthetase activity (Table 1; see also the Appendix to this paper). The results obtained from the villus cell nuclei could reflect (*a*) the low synthetase specific activity of the numerically dominant villus nuclei obtained from this population of isolated cells (Table 1) or could result from (*b*) the high synthetase specific activity of the small number of crypt cell nuclei derived from the contaminating crypt cells in the isolated population of villus cells (Table 1). It is not possible to discriminate between (*a*) and (*b*) on the basis of the

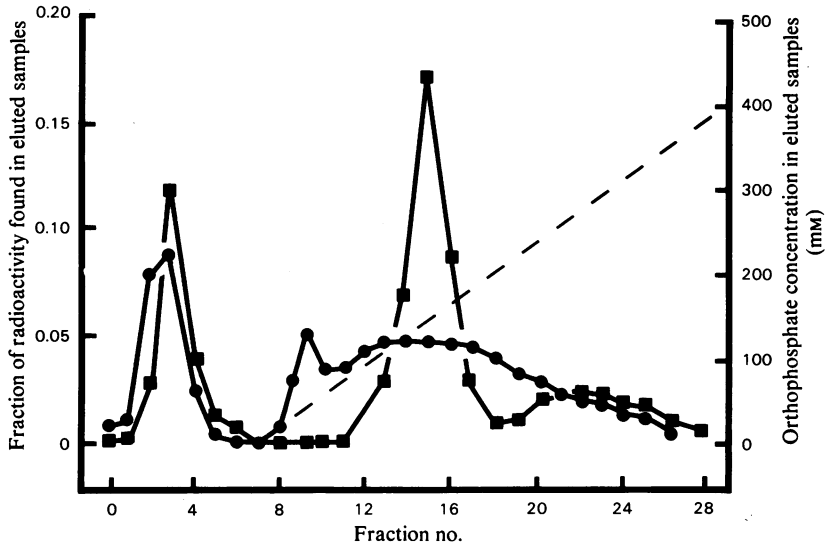

Fig. 2. *Separation of mono(ADP-ribose) and oligo(ADP-ribose) removed from nuclear proteins of lower crypt and upper villus-cell nuclei*

Nuclei were prepared from isolated upper villus cells (corresponding to cell population 1 in Table 1) and from lower crypt cells (corresponding to cell population 4 in Table 1) as described in the Materials and Methods section. Nuclei were then incubated with [*adenosine*-^{14}C]NAD$^+$ at 25°C for 15min, and the reaction was terminated; mono- and oligo-(ADP-ribose) were removed from nuclear proteins, applied to an hydroxyapatite column in phosphate buffer (1mM) and eluted with a linear gradient of phosphate buffer as described in the Materials and Methods section. The total radioactivity applied to the column was 10800c.p.m. (villus cell nuclei, ■) and 33800c.p.m. (crypt cell nuclei, ●); more than 90% of the radioactivity was recovered in the eluted materials. The radioactivity in each eluted sample collected from the column has been plotted as a fraction of the total radioactivity applied to the column. The orthophosphate gradient indicated by the broken line refers to the concentrations of orthophosphate found in the samples collected from the hydroxyapatite column.

identical oligomer mean chain lengths determined in either the phosphodiesterase or the hydroxyapatite procedures described above. But possibility (*b*) seems to be eliminated and possibility (*a*) favoured by the observed difference in the distribution of chain lengths of oligo(ADP-ribose) about the same mean chain length in the two functionally different nuclei, namely those obtained from an isolated population of cells that were predominantly crypt cells and those obtained from an isolated population of cells that were predominantly villus cells.

It then seems necessary to conclude that the ratio of mono(ADP-ribose) to oligo(ADP-ribose) attached covalently to nuclear proteins remains constant as intestinal epithelial cells migrate from the crypts on to the villi; that lower crypt cell nuclei synthesize a wide range of lengths of oligo(ADP-ribose) chains attached to nuclear proteins but that this range of sizes of oligo(ADP-ribose) chains is markedly decreased as dividing cells of the lower crypts cease to divide, emerge from the crypts, differentiate and migrate along the villi (Fig. 2). These deductions are consistent with the possibility that ADP-ribose oligomers become redistributed on the same or amongst different nuclear proteins during cell migration from intestinal crypts on to the villi but proof is as yet lacking.

Separate experiments showed that of the total ADP-ribose incorporated from [^{14}C]NAD$^+$ by isolated crypt or villus cell nuclei, 60% appeared on acid-soluble proteins (including histones) and 40% on acid-insoluble (non-histone) proteins.

Conclusions

In sum, our results show that poly(ADP-ribose) synthetase activity was present in nuclei isolated from those intestinal epithelial cells known to synthesize DNA and to undergo mitotic division; the enzyme activity was almost absent from those differentiating upper crypt cells and villus cells known not to participate in the cell-division cycle in normal intestinal epithelium *in vivo*. The overall mean size of poly(ADP-ribose) chains attached to nuclear proteins in cells at the bottom of intestinal crypts does not alter during subsequent differentiation and migration of the cells on to the intestinal villi, but a narrower range of sizes of poly(ADP-ribose) chains is synthesized in villus cell nuclei. Evidence is provided for

the ability of both crypt and villus cell nuclei to initiate the ADP-ribosylation of nuclear proteins *in vitro*, but ADP-ribosyl units were incorporated into these proteins from NAD^+ predominantly by elongation *in vitro* of oligo(ADP-ribose) chains which pre-existed on nuclear proteins *in vivo*. Both histone and non-histone proteins were modified *in vitro*.

Evidence for the further ADP-ribosylation *in vitro* of ADP-ribosylated proteins that already existed in *Xenopus laevis* nuclei has been presented (Farzaneh & Pearson, 1978); the extension *in vitro* of existing ADP-ribose oligomers in this system varied with the stage of development of the embryos from which the nuclei were prepared. Increases in poly(ADP-ribose) synthetase activity have been demonstrated in lymphocytes stimulated to proliferate (Lehman *et al.*, 1974), in oviducts of quail treated with oestrogen (Müller *et al.*, 1974) and in regenerating liver (Leiber *et al.*, 1973).

To our knowledge, the present experiments are the first to demonstrate the marked diminution of the synthetase activity in mitotically inactive daughter cells and the presence of the enzyme activity in mitotically active parent cells of a higher eukaryote that had not been subjected to experimental perturbation of the normal cell cycle. The concomitant diminution in the range of sizes of ADP-ribose oligomers attached covalently to nuclear proteins in the same differentiating daughter cells appears also to be a novel observation.

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