

## Changes in the Synthesis of Ribosomal Ribonucleic Acid and of Poly(A)-Containing Ribonucleic Acid During the Differentiation of Intestinal Epithelial Cells in the Rat and in the Chick

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Epithelial cells were isolated from rat and chick small intestine by techniques which separated subpopulations of differentiating villus and upper crypt cells from each other and from populations of mitotically dividing lower crypt cells. Incorporation of precursors into epithelial-cell DNA, cytoplasmic rRNA and cytoplasmic poly(A)-containing RNA occurred in the lower crypt cells *in vivo* when precursor was supplied from the vascular system of the intestine. Incorporation of precursor into 28S and 18S rRNA continued in the upper crypt cells, but occurred to only a very slight extent (if at all) in villus cells, whereas incorporation into poly(A)-containing RNA continued (at a diminishing rate) as the differentiating cells migrated along the villi. When precursor was supplied from the intestinal lumen, its incorporation into DNA and into rRNA of crypt cells was not very different from that observed with the other mode of precursor administration, but incorporation into villus-cell poly(A)-containing RNA then occurred at essentially the same rate in all intestinal epithelial cells *in vivo*. Cytoplasmic poly(A)-containing RNA appeared to turn over in rat crypt cells with a half-life not exceeding 24 h; crypt-cell rRNA showed no turnover and no evidence could be found for the presence of 'metabolic DNA'.

Descriptions of the proliferation, migration and differentiation of the epithelial cells which line the lumen of the small intestine have been given elsewhere (Porteous, 1978, 1979; Porteous *et al.*, 1979). Briefly, histological evidence has shown that epithelial cells divide mitotically only in the lower half of the crypts of Lieberkühn of small intestine (Lipkin, 1973); the progeny migrate as an ordered sheet of cells out of the crypts on to the villi, which project into the lumen of the intestine. In the upper region of the crypts, migrating cells begin a gradual morphological differentiation and, at the crypt–villus junctions, assume the characteristics of functional absorptive cells (Cheng & Leblond, 1974; van Dongen *et al.*, 1976); at the end of their migration, fully differentiated cells are discarded from the tips of the villi into the intestinal lumen (Quastler & Sherman, 1959; Cheng & Leblond, 1974). Provided that the system is not perturbed, cell proliferation matches cell desquamation; the total cell population remains constant in size.

The starting point for the present work was the repeated radioautographic observation that DNA synthesis is confined to the epithelial cells in the

lower half of the intestinal crypts *in vivo* (Quastler *et al.*, 1959; Cairnie *et al.*, 1965; Cheng & Leblond, 1974; van Dongen *et al.*, 1976). Radioautographic evidence alone obtained after administration of [<sup>3</sup>H]uridine to mice *in vivo* has been interpreted to mean that total RNA synthesis in the upper villus cells is less than that in lower villus cells (Shorter & Creamer, 1962). An alternative explanation would be that the precursor had restricted access from the circulation to the upper villus cells, or that total RNA was more rapidly degraded in these cells.

We wished to attempt to remove these uncertainties and to investigate the synthesis and turnover of different species of RNA. We have chosen to administer precursors of nucleic acid synthesis to the intact animal, rather than to the isolated cells, in order to preserve the normal spatial relationships of intestinal epithelial cells, and to maintain the normal kinetics of cell division, migration and differentiation in this epithelium (Potten, 1978; Wright, 1978; Porteous *et al.*, 1979) during incorporation of precursors into intracellular nucleic acids. Precursors have been administered alternatively from the lumen of the intestine or from the

circulating plasma in order to test the possibility that results were biased by the route of access of precursor to the epithelium.

Results are presented which show: that synthesis of 18S and 28S rRNA and poly(A)-containing RNA occurs, like DNA synthesis, in the lower crypt cells *in vivo*; that rRNA synthesis continues, after cessation of DNA synthesis, when cells migrate from the lower to the upper crypts, but then almost ceases when cells leave the crypts; further, that incorporation of precursor into poly(A)-containing RNA continues as cells migrate out of the crypts, on to and along the villi; incorporation of precursor into poly(A)-containing RNA (but not into the other species of nucleic acid) differs markedly with the route of access of the precursor to the intestinal epithelial cells.

## Materials and Methods

### Chemicals

Hyaluronidase (type II) and bovine serum albumin (fraction V) were from Sigma, Poole, Dorset BH17 7NH, U.K.; [*methyl*-<sup>3</sup>H]thymidine (50 Ci/mmol) and [<sup>32</sup>P]P<sub>i</sub> (10 Ci/ml) were supplied by The Radiochemical Centre, Amersham, Bucks., U.K. Oligo(dT)-cellulose (grade T3) was from Collaborative Research, Waltham, MA 02154, U.S.A.

### Animals

Male Hooded Lister rats were maintained on stock diets with water *ad libitum*; they were used at about 300 g live weight. Female Ross 1 chicks (2–4 weeks old, 500–600 g live weight) were supplied for immediate use by the Rowett Research Institute, Bucksburn, Aberdeenshire, U.K., where they were maintained on stock diets containing terramycin, with water *ad libitum*.

### Administration of isotopically labelled precursors

Animals were lightly anaesthetized with diethyl ether, then injected (via the tail vein in rats, the breast muscle in chicks) with 100 μCi of [<sup>3</sup>H]thymidine or 5–10 mCi of [<sup>32</sup>P]P<sub>i</sub> so that precursors of nucleic acid synthesis reached the intestinal epithelium mainly via the vascular bed of the intestine; alternatively rats were given the isotope from a pipette, so that precursors entered intestinal cells predominantly from the lumen of the intestine. [<sup>32</sup>P]P<sub>i</sub> was preferred to more specific precursors of RNA synthesis because the latter gave very small incorporation rates when administered to intact animals. Animals were killed by neck fracture at timed intervals after administration of isotopically labelled precursor.

### Isolation of epithelial cells from the villi and crypts of small intestine

Cell-isolation procedures were based on those described by Towler *et al.* (1978) and were similar to those used by Porteous *et al.* (1979). The small intestine was excised rapidly and the lumen washed out with 0.9% NaCl at room temperature; the jejunum was then filled with cell-isolation medium and suspended in 0.9% NaCl at 37°C. After 5 min, the intestine was removed from the incubation bath and cells were removed as described by Towler *et al.* (1978); the intestine was refilled with fresh cell-isolation medium and re-incubated for 5 min. This process was repeated until all cells had been removed. Rat villus cells were removed by two or three successive 5 min incubations with 96 mM-NaCl/8 mM-KH<sub>2</sub>PO<sub>4</sub>/50 mM-trisodium citrate/5.6 mM-Na<sub>2</sub>HPO<sub>4</sub>/1.5 mM-KCl/77 mM-mannitol containing bovine serum albumin (2.5 mg/ml) and hyaluronidase (1.5 mg/ml). Rat crypt cells were released by two further incubations with 96 mM-NaCl/8 mM - KH<sub>2</sub>PO<sub>4</sub>/5.6 mM - Na<sub>2</sub>HPO<sub>4</sub>/1.5 mM - KCl/10 mM-EDTA/185 mM-mannitol containing bovine serum albumin (2.5 mg/ml). Chick villus and crypt cells were removed by using a single medium [96 mM-NaCl/8 mM-KH<sub>2</sub>PO<sub>4</sub>/5.6 mM-Na<sub>2</sub>HPO<sub>4</sub>/1.5 mM - KCl/10 mM - EDTA/0.5 mM - dithiothreitol/185 mM-mannitol, containing bovine serum albumin (2.5 mg/ml)]; up to four 5 min incubations were used to isolate villus cells and a further two or three 5 min incubations to remove crypt cells. The first 5 min incubation with chick intestine removed very few cells; this first suspension was routinely discarded.

After isolation, each batch of rat or chick cells was suspended in 0.2 M-sucrose/76 mM-Na<sub>2</sub>HPO<sub>4</sub>/19 mM-KH<sub>2</sub>PO<sub>4</sub> (pH 7.4) at 0°C (Evans *et al.*, 1971). One rat jejunum typically yielded 2 g wet wt. of crypt and villus cells containing a total of 2.5 mg of DNA. One chick jejunum yielded 3–4 g wet wt. of cells, containing a total of 4–6 mg of DNA. In both instances, villus cells accounted for 75% and crypt cells for 25% of the cell mass isolated.

### Analyses

**Phosphatase assays.** Alkaline phosphatase activity was determined as described by Weiser (1973).

**Radioactivity measurements.** Radioactivities of <sup>32</sup>P-labelled material that had been isolated by density-gradient centrifugation were determined by Cerenkov counting (Clausen, 1968); in other instances, radioactivities of <sup>32</sup>P-labelled and <sup>3</sup>H-labelled materials were determined by scintillation counting of samples added to xylene (2 vol.)+ Triton X-100 (1 vol.)+0.8% 2,5-diphenyloxazole + 0.003% 1,4-bis-(5-phenyloxazol-2-yl)benzene.

Specific radioactivities are expressed relative to RNA and DNA determined by chemical analyses or by direct spectrophotometric determination of RNA and DNA at 260 nm.

**Isolation and determination of nucleic acids.** Total RNA and DNA of rat and chick intestinal epithelial cells were extracted and fractionated by the Schmidt–Thannhauser procedure as modified by Munro & Fleck (1966). After the alkaline-hydrolysis step,  $\text{HClO}_4$ -precipitated material was hydrolysed in 0.5 M- $\text{HClO}_4$  at 70°C for 30 min; the DNA content of rat cells was determined by analysis of the final supernatant solution (Burton, 1956). The specific radioactivity of rat cell DNA based on this determination was not distorted by the presence of any extraneous  $^{32}\text{P}$ -labelled material; thus similar results were obtained (Table 1) whether  $[^{32}\text{P}]\text{P}_i$  or  $[^3\text{H}]\text{thymidine}$  served as precursor for DNA synthesis. When this check was not available because only  $[^{32}\text{P}]\text{P}_i$  was used as precursor, rat cells were extracted with phenol/chloroform (Penman, 1969), before subjection to the Munro & Fleck (1966) extraction procedure, in order to eliminate any possible interference by contaminating phospholipids. The chick cell DNA fraction isolated by the Schmidt–Thannhauser procedure (Munro & Fleck, 1966) contained extraneous  $^{32}\text{P}$ -labelled material which distorted measurements of the specific radioactivity of  $[^{32}\text{P}]\text{DNA}$ . Chick cell DNA was therefore purified directly from nuclei by centrifugation (Monahan & Hall, 1974) through  $\text{Cs}_2\text{SO}_4$  gradients (the nuclei were isolated from a cell homogenate as described below); DNA-containing peaks from the  $\text{Cs}_2\text{SO}_4$  gradients were dialysed against 2 mM-EDTA/0.1% sodium dodecyl sulphate (pH 7.0).

**Fractionation of cytoplasmic RNA.** Cells were suspended in 10 mM-Tris/HCl/10 mM-NaCl/1.5 mM- $\text{MgSO}_4$ /0.1% diethyl pyrocarbonate (pH 7.4) and disrupted in a Dounce homogenizer; nuclear and cytoplasmic fractions were isolated and the cytoplasmic fraction was extracted with phenol/chloroform (Penman, 1969). The aqueous phase was treated with 2 vol. of ethanol overnight at -20°C, the precipitate collected by centrifugation (8000g for 20 min), redissolved in 10 mM-Tris/HCl/10 mM-EDTA/10 mM-NaCl/0.1% (v/v) sarkosyl (pH 7.4) and reprecipitated with an equal volume of 4 M-LiCl overnight at 4°C (Penman *et al.*, 1968). Poly(A)-containing RNA in the redissolved precipitate was purified by oligo (dT)-cellulose chromatography (Groner & Phillips, 1975; Craig *et al.*, 1976); the RNA fraction that did not bind to oligo (dT)-cellulose was centrifuged through sucrose density gradients (Johnson *et al.*, 1974), and material in the 18S and 28S peaks was precipitated with ethanol. The purity of 18S and 28S material was verified by gel electrophoresis (200 V for 120 min) in 2% acrylamide/0.5% agarose/0.1% sodium dodecyl sulphate

prepared in electrophoresis buffer (36 mM-Tris/30 mM- $\text{NaH}_2\text{PO}_4$ /1 mM-EDTA, pH 7.7).

## Results

### Preliminary experiments

Before deductions could be made from the results presented below, it was necessary to establish that RNA and DNA were being synthesized at steady rates *in vivo* during those periods in which intestinal epithelial cells were subsequently isolated from the intestine for analysis;  $[^{32}\text{P}]\text{P}_i$  was shown to be incorporated linearly with time into both DNA and RNA for the first 6 h after injection of the precursor. It was further shown that total  $^{32}\text{P}$ -labelled  $\text{HClO}_4$ -soluble material in both villus and crypt cells of rat and chick intestine increased linearly with time up to 1 h after injection of  $[^{32}\text{P}]\text{P}_i$  into the rat *in vivo* and then remained essentially steady for the following 3 h. It was also necessary to show that the cell-isolation procedure was distinguishing effectively between crypt and villus cells. A suitable test was to ask whether the cell-isolation procedure detected the migration along the villi of cells

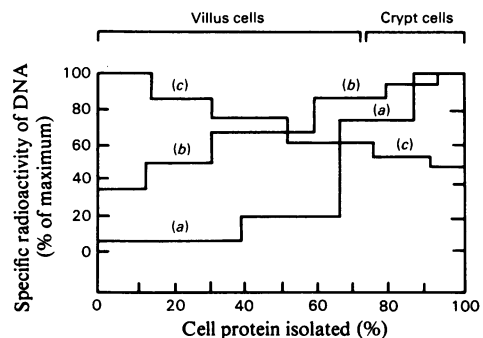


Fig. 1. Demonstration of the adequacy of the method for separating villus-cell populations from crypt-cell populations

Rats were injected intraperitoneally with  $[^3\text{H}]\text{thymidine}$ ; at intervals thereafter intestinal villus and crypt epithelial cells were isolated sequentially and subfractionated as described in the Materials and Methods section. In these experiments four or six sequential populations of cells were isolated; each vertical step in the plots represents the boundary between adjacent cell populations. The horizontal axis represents the cumulative protein content of sequentially isolated cell populations. The vertical axis gives the specific radioactivity of the DNA of each cell population, expressed as per cent of maximum specific radioactivity (c.p.m./ $\mu\text{g}$  of DNA). Three typical experiments are illustrated in which cells were isolated (a) 3 h, (b) 26 h and (c) 50 h after injection of the radioisotope.

originally labelled in their DNA while they were in the lower half of the crypts (Cheng & Leblond, 1974). Fig. 1 presents the required evidence. It will be argued below, from results shown in Tables 1 and 2, that crypt cells synthesize 28S and 18S rRNA, but that villus cells do not; it follows that newly synthesized rRNA species should appear in villus cells only when sufficient time has elapsed for crypt cells (carrying labelled 28S and 18S rRNA) to have migrated on to the villi. Results shown in Fig. 2 support this expectation and thus corroborate the evidence of Fig. 1 that the cell-isolation procedure does distinguish adequately between crypt and villus

cells. Results similar to those shown in Fig. 2 were obtained with chick villus and crypt cells. All quantitative determinations of the extent of incorporation of  $[^{32}\text{P}]\text{P}_i$  into 18S and 28S rRNA (Tables 1 and 2) involved isolation of rRNA species, as illustrated in Fig. 2, from each population of isolated cells.

*Synthesis of cytoplasmic rRNA and poly(A)-containing RNA in sequential populations of intestinal epithelial cells in vivo*

*Primary observations.* The extent of incorporation of radioactively labelled precursors into the

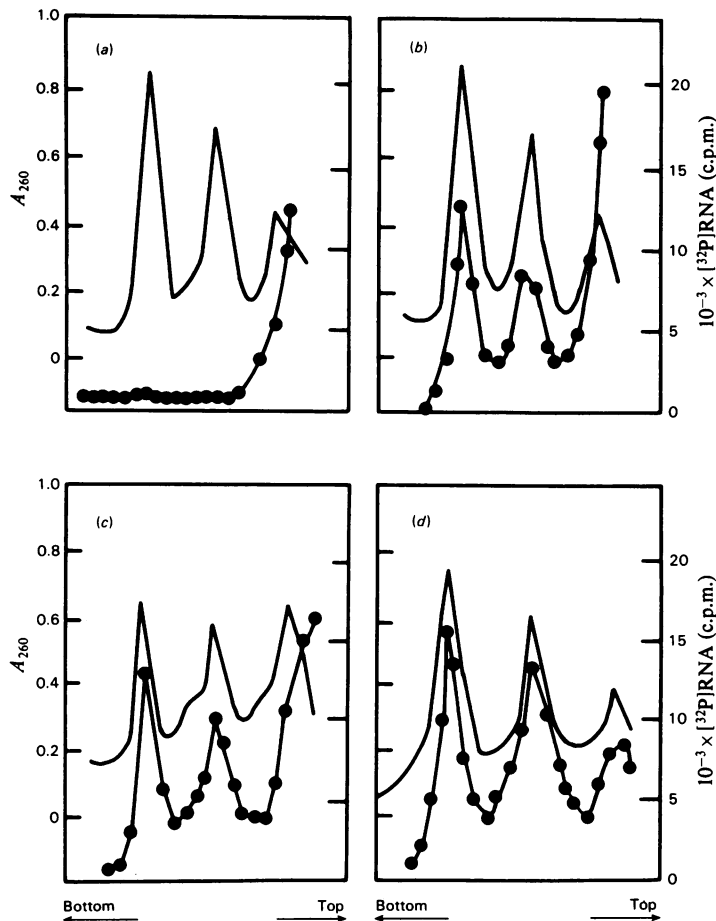


Fig. 2. Isolation of intact 28S and 18S rRNA from crypt and villus intestinal-epithelial cells. Rats were injected with  $[^{32}\text{P}]\text{P}_i$ , villus and crypt cells isolated from the jejunum, the cells subfractionated as described in the Materials and Methods section and the components of the cytoplasmic fraction that did not bind to oligo(dT)-cellulose were then subjected to sucrose-density-gradient centrifugation. Continuous plot represents  $A_{260}$  profile; ●, radioactivity; (a) and (c), villus cells, (b) and (d), crypt cells. Cells were isolated 3 h (a and b) or 45 h (c and d) after injection of  $[^{32}\text{P}]\text{P}_i$  into two rats.

Table 1. Incorporation of radioactivity into DNA and into cytoplasmic rRNA and poly(A)-containing RNA of sequential populations of intestinal epithelial cells after intravenous injection of [<sup>32</sup>P]P<sub>i</sub> or [<sup>3</sup>H]thymidine into rats

Intestinal epithelial cells were isolated from rat jejunum 3 h after injection of [<sup>3</sup>H]thymidine or [<sup>32</sup>P]P<sub>i</sub>. Alkaline phosphatase activity (column i) was determined in homogenates of each cell population isolated from six rats and expressed as a percentage of the activity of the first (upper villus) cell population to be isolated (100% activity corresponds to 0.2 μmol of P<sub>i</sub> phosphate released/min per mg of protein). The radioactivities of [<sup>3</sup>H]DNA, [<sup>32</sup>P]DNA and [<sup>32</sup>P]RNA are each expressed as a percentage of the corresponding value for the final (lower crypt) cell population to be isolated. Results for the incorporation of [<sup>3</sup>H]thymidine into DNA are from experiments with five rats: 100% values in individual experiments corresponded to 600–800 c.p.m./μg of DNA per mCi of [<sup>3</sup>H]thymidine injected (column ii). The results for [<sup>32</sup>P]DNA, [<sup>32</sup>P]rRNA and [<sup>32</sup>P]poly(A)-containing RNA are from experiments with three rats (columns iii, iv, v). In a typical experiment, 100% values corresponded to 2360 c.p.m./A<sub>260</sub> unit (DNA), 4410 c.p.m./A<sub>260</sub> unit (rRNA) and 9740 c.p.m./A<sub>260</sub> unit [poly(A)-containing RNA] each per mCi of [<sup>32</sup>P]P<sub>i</sub> injected. Total HClO<sub>4</sub>-soluble <sup>32</sup>P-labelled material (column vi) was measured in experiments with four rats killed 1–4 h after injection of [<sup>32</sup>P]P<sub>i</sub>; the radioactivity of this acid-soluble material reached plateau value (220–240 c.p.m./μg of cell RNA per mCi of [<sup>32</sup>P]P<sub>i</sub> injected) 1 h after injection. All results are given as mean values ± S.D., with the exceptions noted (\* individual results; † single experimental result).

Cell population	[ <sup>3</sup> H]Thymidine or [ <sup>32</sup> P]P <sub>i</sub> incorporated					
	(i) Alkaline phosphatase activity (%)	(c.p.m./μg of individual nucleic acid species)				(c.p.m./μg of total cell RNA)
		(ii) [ <sup>3</sup> H]DNA (%)	(iii) [ <sup>32</sup> P]DNA (%)	(iv) [ <sup>32</sup> P]rRNA (28S) (%)	(v) [ <sup>32</sup> P]Poly(A)-containing RNA (%)	(vi) Acid-soluble [ <sup>32</sup> P]-labelled material (%)
(A) Upper villus	100	7.2 ± 1.9	8.1 ± 1.8	6.8 ± 2.3	12.6, 15.0*	47.6 ± 11.6
(B) Lower villus	84 ± 16	14.5 ± 7.7	15.4 ± 0.9	14.8 ± 1.5	22.5 ± 3.0	48.6 ± 5.2
(C) Villus-crypt	31 ± 13	75.6 ± 5.5	77.0 ± 4.2	83.0 ± 3.0	84.5†	84.6 ± 10.8
(D) Lower crypt	12 ± 6	100	100	100	100	100

DNA, rRNA and poly(A)-containing RNA of each of a series of sequentially isolated populations of rat intestinal epithelial cells is shown in Table 1. Given that the [<sup>3</sup>H]DNA or [<sup>32</sup>P]DNA in cell populations (A), (B) and (C) is a quantitative measure of the contamination of these cells with population (D), which occurred during the cell-isolation procedure (Table 1, column iii), it would be reasonable to conclude that labelling of 28S rRNA in the isolated cell populations (A) and (B) is entirely accounted for by contamination of these cells with cells (D) from the lower crypt region, but that labelling of 28S rRNA in cell population (C) might not be completely accounted for in this way (Table 1, column iv). Thus these primary results suggest that rRNA probably continues to be synthesized in the upper crypt region after cells have left the lower crypts, but is no longer synthesized when the same cells subsequently migrate out of the crypts into the villi *in vivo*.

In contrast, incorporation of radioactivity into the poly(A)-containing RNA of isolated villus cells (A) and (B) could not be fully accounted for by contamination of these cells with cells from the lower crypts (Table 1, column v); synthesis of this species of RNA continues in villus cells *in vivo*, though

apparently at a rapidly diminishing rate as they migrate along the villi.

*Correction of primary results for cross-contamination among prelabelled populations of isolated cells.* The conclusions drawn above from the primary results shown in Table 1 are subject to the objection that they have not been corrected for the inevitable cross-contamination which occurs among isolated cells; nor do they take account of the possibility that the [<sup>32</sup>P]P<sub>i</sub> might have readier access to some of the intestinal epithelial cells when it is supplied to the luminal rather than to the vascular pole of these cells *in vivo*. It was also possible that any results obtained were peculiar to one animal species. The same correction procedure (Kerridge, 1979; Porteous *et al.*, 1979) has therefore been applied to primary results from three different sets of experiments, namely those in which precursor was administered to (a) rat jejunal epithelium by the vascular route, (b) rat jejunal epithelium by the luminal route and (c) chick jejunal epithelium by the vascular route.

Some general conclusions are then possible. Thus the capacity of villus cells to synthesize rRNA is very limited in both rat and chick intestine (Table 2, parts a and c; column i) and is little affected by the

Table 2. *Synthesis of cytoplasmic species of RNA in sequential populations of crypt and villus epithelial cells of rat and chick jejunum in vivo after administration of [<sup>32</sup>P]P<sub>i</sub> by two alternative routes*

Mean values shown in Table 1 for the incorporation *in vivo* of [<sup>32</sup>P]P<sub>i</sub> into 28S rRNA and into poly(A)-containing RNA have each been corrected for cross-contamination among the isolated populations of cells, (A), (B), (C) and (D), by the method of Kerridge (1979). Columns (i) and (ii) give the net incorporation of radioactivity into ribonucleic acids in each of three populations of cells as a fraction of the incorporation observed in the lower crypt-cell population (D) *in vitro*: column (iii) refers to the cold-HClO<sub>4</sub>-soluble radioactively labelled material in each population, after correction in the same manner. Primary results obtained after administration of [<sup>32</sup>P]P<sub>i</sub> (1 mCi) orally rather than by the vascular route have similarly been corrected [part (b) of the Table]. In this section, 100% values corresponded to 1125 c.p.m./A<sub>260</sub> unit (DNA), to 1050 c.p.m./A<sub>260</sub> unit (28S rRNA), to 5060 c.p.m./A<sub>260</sub> unit [poly(A)-containing RNA] and to 890 c.p.m./μg of total cell RNA (cold-HClO<sub>4</sub>-soluble material), per mCi of [<sup>32</sup>P]P<sub>i</sub> administered. Apparent incorporation of precursor into DNA as observed in the isolated cell populations (A), (B) and (C) was 11.3, 17.2 and 86.5% of the incorporation into the DNA of population (D) (cf. Table 1, column iii). The mean alkaline phosphatase activities of cell populations (A)–(D) were the same as those shown in Table 1. Primary results obtained after injection of [<sup>32</sup>P]P<sub>i</sub> into chicks have also been corrected in the same way [part (c) of the Table]. In this section, 100% values corresponded to 6230 c.p.m./A<sub>260</sub> unit (DNA), to 7510 c.p.m./A<sub>260</sub> unit (rRNA) and to 17700 c.p.m./A<sub>260</sub> unit [poly(A)-containing RNA], per mCi of [<sup>32</sup>P]P<sub>i</sub> injected. Apparent incorporation of precursor into the DNA of isolated cell populations (A), (B) and (C) was 23.2, 25.6 and 43.9% of the incorporation into the DNA of population (D) (cf. Table 1, column iii). The distribution of the mean alkaline phosphatase activity among the same isolated cell populations was 100, 82, 40 and 12% respectively (100% corresponded to 1.7 μmol of P<sub>i</sub> released/min per mg of protein) (cf Table 1, column i). Columns (iv), (v) and (vi) of the Table show the results of columns (i), (ii) and (iii) normalized to values that would be expected had it been possible to keep the specific radioactivity of the cold-HClO<sub>4</sub>-soluble material in all cells equal to that in the lower-crypt-cell population (D) *in vivo*. Further details of the correction and normalization procedures are given in the text.

		Results corrected for cross-contamination of isolated populations of cells			Results normalized after correction for cross-contamination of isolated cells		
		(c.p.m./μg of individual nucleic acid)		(c.p.m./μg of total RNA)	(c.p.m./μg of individual nucleic acid)		(c.p.m./μg of total RNA)
		(i)	(ii)	(iii)	(iv)	(v)	(vi)
Experiment	Cell population	rRNA	Poly (A)-con- taining RNA	HClO <sub>4</sub> -soluble material	rRNA	Poly (A)-con- taining RNA	HClO <sub>4</sub> -soluble material
(a) Rat jejunal cell populations (precursor by vascular route)	(A) Upper villus	0	4.4	40.0	0	11.0	100
	(B) Lower villus	0	6.4	35.8	0	17.9	100
	(C) Upper crypt	23.6	29.7	30.1	78.4	98.6	100
	(D) Lower crypt	100	100	100	100	100	100
(b) Rat jejunal cell populations (precursor by luminal route)	(A) Upper villus	6.5	116	621	1.0	18.6	100
	(B) Lower villus	6.3	108	346	1.8	31.2	100
	(C) Upper crypt	7.6	74.8	617	1.2	12.1	100
	(D) Lower crypt	100	100	100	100	100	100
(c) Chick jejunal cell populations (precursor by vascular route)	(A) Upper villus	0	14.6	92.9	0	15.7	100
	(B) Lower villus	7.2	16.4	84.4	8.5	19.4	100
	(C) Upper crypt	22.4	34.0	100	22.4	34.0	100
	(D) Lower crypt	100	100	100	100	100	100

route of administration of precursor to rat jejunum (Table 2, parts *a* and *b*; column i); lower and upper crypt cells do synthesize rRNA in all three experimental situations (Table 2, parts *a*, *b*, *c*; column i). In contrast, rat and chick jejunal villus cells continue to incorporate the same precursor into poly(A)-containing RNA to a significantly greater extent than they do into rRNA (Table 2, parts *a*, *b*, *c*; cf columns i and ii). It might be supposed that the diminishing incorporation of precursor into the poly(A)-containing RNA of rat and chick villus cell

populations B and A illustrated in Table 2 (parts *a* and *c*; column ii) was the result of preferential removal of the poly(A) tract from this species of RNA as cells migrated out of the crypts on to the villi; less of this species of RNA would then be isolated from the villus cells by the procedures used. This supposition is overruled by the observation (Table 2, part *b*, column ii) that rat villus cells can synthesize poly(A)-containing RNA as effectively as do rat crypt cells, provided that the P<sub>i</sub> precursor is supplied from the intestinal lumen. One implication

of this observation is that  $P_i$  enters rRNA and poly(A)-containing RNA from different nucleotide pools within the same villus epithelial cells. Feeding the precursor (Table 2, part *b*) thus exaggerates those differences between the capacities of crypt and villus cells to synthesize rRNA and poly(A)-containing RNA that were observed when the precursor entered the epithelium from the circulation (Table 2, parts *a* and *c*).

**Normalized results.** Given these last two results, it has to be asked whether any of the observed differences in incorporation of  $[^{32}\text{P}]P_i$  into two different RNA species in four different cell populations under three different experimental circumstances could be related to the observed differences in the distribution of acid-soluble  $^{32}\text{P}$ -labelled material amongst the cell populations (Table 2, column iii). Columns (iv), (v), (vi) of Table 2 show the results of columns (i), (ii), (iii) normalized on the explicit assumptions that (a) the acid-soluble  $^{32}\text{P}$ -labelled material in the cells reflects the radioactivity of the immediate precursors of RNA synthesis in those cells, (b) the rate of incorporation of labelled precursors into RNA would change in direct proportion to any changes in the specific radioactivity of these precursors and (c) it would be possible to maintain the acid-soluble  $^{32}\text{P}$ -labelled material at the same specific radioactivity in all cells *in vivo*. These normalized values (Table 2, columns iv, v, vi) then reveal a remarkable quantitative uniformity in the potential capacities of crypt and villus intestinal epithelial cells to incorporate  $P_i$  into rRNA and into poly(A)-containing RNA (probably mRNA) in the three experimental situations so far explored; rRNA synthesis is confined to the lower crypt-cell population (D) or to the lower and upper crypt-cells populations (D) and (C), but poly(A)-containing RNA synthesis/turnover persists after cells have migrated out of the crypts to become villus-cell populations (B) and (A). The normalized results (Table 2, columns iv, v, vi) must be regarded with some reserve until the assumptions on which they are based are validated. The corrected results (Table 2, columns i, ii, iii) are not subject to any such reservations; the conclusions already drawn from them are consistent with those drawn from the normalized results.

A key observation in making all the above deductions was that the pattern of incorporation of  $[^{32}\text{P}]P_i$  into the DNA of the cell populations (A), (B), (C) and (D) was not affected by the route of administration of the precursor or by the markedly different distribution of acid-soluble radioactivity among the cell populations in different experiments (Tables 1 and 2). It was also shown that the specific radioactivities of the 18S and 28S rRNA (Fig. 2) were always equal in each isolated population of chick and rat jejunal epithelial cells. Calculations

and conclusions (Tables 1 and 2) on one species of rRNA thus apply equally to the other. The rRNA fraction of the total cytoplasmic RNA was prepared in such a way as to eliminate tRNA. Cytoplasmic poly(A)-containing RNA isolated in these same experiments was heterogeneous when electrophoresed in 2.5% polyacrylamide/dodecyl sulphate gels, the bulk of the material migrating between 18S and 28S markers; it has not been further characterized except to assess its turnover rate.

#### *Stability of DNA; turnover of labelled RNA in intestinal epithelium*

Repeated experiments of the kind illustrated in Fig. 1 consistently demonstrated that there was no change in the specific radioactivity of labelled DNA (c.p.m./ $\mu\text{g}$  of DNA) as cells migrated from the crypts along the villi; DNA, once synthesized, was stable within crypt and villus cells, and we could find no evidence for the 'metabolic DNA' proposed for intestinal epithelium by Appleton *et al.* (1969) [see Wright *et al.* (1972), Schultze *et al.* (1972) and Burholt *et al.* (1973) for different evidence against the existence of 'metabolic DNA' in this cell system].

Attempts to measure turnover of RNA species were complicated by (i) the continuous migration of cells out of the crypts on to the villi and into the lumen, a process sustained by a mean cell-cycle time in the crypts of about 12h (Cairnie *et al.*, 1965; Schultze *et al.*, 1972; Wright *et al.*, 1972; Al-Dewachi *et al.*, 1974); (ii) by the persistence of acid-soluble  $^{32}\text{P}$ -labelled material in the circulation *in vivo*; and (iii) by re-utilization of degradation products of labelled nucleic acids, especially from cells desquamated into the intestinal lumen. Thus the steady exponential rate of decay of labelling of DNA, RNA and acid-soluble material in the cells, and that of the acid-soluble material in the plasma, was shown to decrease markedly about 30–50h after administration of the  $[^{32}\text{P}]P_i$  and to assume a slower, steady rate of exponential decay thereafter. The time required for crypt cells to traverse the length of the villi (Cheng & Leblond, 1974), desquamate and release degradation products for re-utilization is about 40h. Similarly, the half-life of decay of labelling of DNA in crypt cells should have equalled the cell-cycle time; in fact it was closer to 24 than to 12h. These difficulties precluded accurate measurements of turnover times, but approximate measurements were possible. The complication arising from cell migration was eliminated by determining the specific radioactivity of  $[^{32}\text{P}]$ RNA relative to the specific radioactivity of a stable molecule (DNA) labelled at the same time in the same cells with the same precursor. The ratio specific radioactivity of rRNA/specific radioactivity of DNA was then shown to be unchanged in crypt cells when tested between 20 and 48h after injection

Table 3. Ratio of total RNA/total DNA for sequential populations of intestinal epithelial cells or rat and chick small intestine

Values shown are means  $\pm$  s.d. for the numbers of determinations given in parentheses.

Cell population	Total RNA Total DNA	
	Rat (11)	Chick (3)
(A) Upper villus	1.40 $\pm$ 0.28	1.91 $\pm$ 0.26
(B) Lower villus	1.32 $\pm$ 0.28	1.62 $\pm$ 0.10
(C) Villus-crypt	1.30 $\pm$ 0.22	1.42 $\pm$ 0.21
(D) Lower crypt	1.50 $\pm$ 0.26	1.69 $\pm$ 0.26

of the [ $^{32}$ P]P<sub>i</sub>. In the same way, poly(A)-containing RNA was shown to have a relatively rapid turnover (half-life  $t_{1/2}$   $\approx$  24 h). Because of the experimental difficulties outlined above, it is most probable that the turnover rate for the poly(A)-containing RNA ( $t_{1/2}$   $\approx$  24 h) is an overestimate; similarly, the techniques used may have failed to detect slow turnover of rRNA.

#### Relative proportions of poly(A)-containing RNA, total RNA and DNA in intestinal epithelial cells

Webster & Harrison (1969) claimed that the (unlabelled) total RNA/DNA ratio increased as cells migrated out of the crypts, but decreased again as cells moved along the villi. These conclusions appear to be based on results from two intestines, one yielding the DNA content of each cell population, the other giving the RNA content of another set of cell populations. Our determinations of the DNA and RNA contents of intestinal epithelial cells (Table 3) were performed on each batch of cells from several individual animals and show no significant change in the mean total RNA/DNA ratio between the bases of the crypts and the tips of the villi in either rat or chick intestine. In the rat, poly(A)-containing RNA accounted for 2.2–2.6% of total cytoplasmic RNA in cell populations (A)–(D), and in the chick, for between 3.8 and 6.3% of the total cytoplasmic RNA in the corresponding cell populations. We have no evidence that this poly(A)-containing RNA is functional mRNA, but the presence of a poly(A) tract, its relatively rapid turnover and its small contribution to the total cytoplasmic RNA together make it likely that the poly(A)-containing RNA of Tables 1 and 2 is mRNA.

#### Discussion

The kinetics of cell proliferation and migration in the small intestine have been described in detail (e.g. Potten, 1978; Wright, 1978). The morphological

changes, and the changes in various enzyme activities, which characterize epithelial cells as they emerge from intestinal crypts and migrate along the villi have also been described in detail (de Both *et al.*, 1974, 1975; Peterson, 1976; van Dongen *et al.*, 1976; Sassier & Bergeron, 1978). But we lack any knowledge of the changes in transcriptional activity that might occur as dividing cells in the bottom half of intestinal crypts migrate, cease mitotic division and differentiate, first in the upper crypts and then on the villi *in vivo*. The present work was designed to provide such information.

Wieggers *et al.* (1976) demonstrated that different pools of nucleotides were used for the synthesis of rRNA and mRNA in HeLa cells. The less elaborate approach used in the present work has led to the same conclusion after supply of [ $^{32}$ P]P<sub>i</sub> first predominantly to one then mainly to the opposite pole of intestinal epithelial cells *in vivo* (see Table 2 and the accompanying text). Our observation that poly(A)-containing RNA (probably mRNA) in villus cells is more readily labelled by P<sub>i</sub> supplied predominantly to the luminal pole of the cell than it is by P<sub>i</sub> supplied mainly to the vascular pole suggests two fundamentally different explanations. First, it is possible that the nucleotide pool that sustains synthesis of poly(A)-containing RNA is associated with the luminal pole of the cell and has only limited access to P<sub>i</sub> entering the cell across the contraluminal membrane of the cell. Alternatively, it is possible that the microcirculation within the intestinal villi delivers a limited amount of P<sub>i</sub> to the adjacent epithelial cells. In this event, there need be no unique association of either of the two nucleotide pools with a particular pole of the cell; the limited extracellular supply of [ $^{32}$ P]P<sub>i</sub> alone could be sufficient to deprive one intracellular nucleotide pool of precursor at the expense of the other pool when the precursor was delivered by the vascular rather than by the luminal route *in vivo* (see Table 2 and the accompanying text). The first explanation places the rate-limiting step (in the pathway between extracellular phosphate and intracellular nucleotides of the postulated 'mRNA pool') on the contraluminal cell-surface membrane; the second places it within the cell, possibly on the enzymes catalysing incorporation of P<sub>i</sub> into nucleotides. Whichever explanation is the true one, the implications for the continued regeneration and differentiation of intestinal epithelium *in vivo* are obvious; it may be necessary to ensure an adequate supply of phosphate to intestinal epithelium by the luminal route, particularly in order to sustain the synthesis of those species of RNA which are characterized by a relatively high turnover rate and which are essential to the expression of the special functions of the differentiating villus cells. The same may be true of substrates other than P<sub>i</sub>. The subtotal villus atrophy



observed in the small intestine of dogs (Feldman *et al.*, 1976) and of rats (Baird *et al.*, 1979) might be the inevitable consequence of a failure to maintain mRNA synthesis in the differentiating villus epithelial cells when the animals were maintained solely by parenteral nutrition.

This proposition requires experimental testing, but it is consistent with the view of nucleic acid metabolism in intestinal epithelium which emerges from the present study and which is summarized as follows. The incorporation of [<sup>3</sup>H]thymidine or of [<sup>32</sup>P]P<sub>i</sub> into DNA was always greatest in the last cell population to be isolated (see Tables 1 and 2 and the accompanying text). This is consistent with the earlier histological and radioautographic observations that DNA synthesis is confined to the lower half of the crypts of normal small intestine *in vivo* (Quastler *et al.*, 1959; Cairnie *et al.*, 1965; Cheng & Leblond, 1974; van Dongen *et al.*, 1976). We then conclude that DNA, once it is synthesized as a prerequisite of cell division in the lower crypt cells, is stable during subsequent migration of the progeny cells into the upper crypts, on to and along the villi (Fig. 1). Both cytoplasmic species of rRNA (28S and 18S), and poly(A)-containing RNA (probably mRNA), are also synthesized in the lower crypt cells (Table 2), presumably as a necessary concomitant of cell proliferation. These syntheses continue in the upper crypt cells (Table 2), where DNA synthesis has ceased, presumably as a necessary concomitant of the marked morphological developments in these cells reported by de Both *et al.* (1974, 1975) and by van Dongen *et al.* (1976). rRNA appears to be stable, at least in crypt cells, whereas poly(A)-containing RNA (probably mRNA species) turns over during cell proliferation and differentiation with a half-life not greater than 24 h. de Both *et al.* (1974, 1975) and van Dongen *et al.* (1976) showed that the morphological differentiation of epithelial cells, which starts in the upper crypts and continues on the villi, is also characterized by the appearance or disappearance of selected enzyme activities as cells migrate from the crypts along the villi (see also Nordström *et al.*, 1968; Imondi *et al.*, 1969; Grey & LeCount, 1970; Fortin-Magana *et al.*, 1970; Peterson, 1976). It is then remarkable that the present results show immediate and almost complete cessation of rRNA synthesis as cells migrate out of the crypts on to the villi (Table 2). rRNA synthesized in crypt cells must thus suffice to sustain whatever protein synthesis occurs when these cells migrate out of the crypts and become differentiating villus cells (Fig. 2). Poly(A)-containing RNA (probably mRNA), on the other hand, exhibits continued synthesis and/or turnover in the villus cells (Table 2), presumably to support synthesis of those selected proteins that first appear as these functionally distinct cells emerge from the crypts and migrate

along the villi. It is incorporation of P<sub>i</sub> into this poly(A)-containing RNA in these particular cells that is peculiarly sensitive to the route by which P<sub>i</sub> enters the cells.

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