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Exogenous nucleic acids and nucleotides are efficiently hydrolysed and taken up as nucleosides by intestinal explants from suckling piglets

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Human milk is a rich source of RNA, free nucleotides (NT) and nucleosides (NS). To determine the uptake of different NS sources by the intestinal epithelium, jejunal explants from suckling piglets were cultured in a medium supplemented with a mixture of NS (adenosine, cytidine, guanosine, inosine, uridine; 10 mg/l each), a mixture of five NT (AMP, CMP, GMP, IMP, UMP; 7 mg/l each) or RNA (60 mg/l), respectively. Aliquots from the media were taken at different times (0·5, 2, 5, 15, 30, 60, 180 min). NS and NT concentrations were analysed in the different supernatants at those periods using solid-phase extraction followed by HPLC. When explants were cultured in the presence of NS the concentration of these compounds, excepting cytidine, rapidly decreased, suggesting that they are efficiently taken up. When explants were incubated in the presence of NT, the total concentration of these compounds decreased while the total concentration of NS increased, suggesting that enterocytes efficiently hydrolyse NT into NS. Likewise, when explants were incubated in the presence of RNA, the total concentration of both NT and NS increased, indicating that intestinal explants are able to hydrolyse RNA to NT and then to NS in the absence of luminal enzymes. In conclusion, the jejunum of piglets at weaning is able to hydrolyse RNA and free NT to NS, and NS, excepting cytidine, are efficiently taken up by the small intestine. These results suggest that the current concentration of NT used to supplement infant formulas should be reconsidered.

Intestine: Nucleic acids: Nucleosides: Nucleotides: Pig explants

Nucleotides (NT) are low molecular weight intracellular compounds, which play key roles in nearly all biochemical processes (Stryer, 1995). Nucleosides (NS) and NT are naturally present in all foods of animal and vegetable origin as free NS and NT, and as nucleic acids; soluble NS and NT, and RNA and DNA are present in milk from various mammals, contributing up to 20 % of the non-protein nitrogen (Gil & Uauy, 1989, 1995; Leach *et al.* 1995; Gil, 2001). Although NT deficiencies have not been related to any particular disease, dietary NT have been reported as beneficial for infants because they positively influence lipoprotein and PUFA metabolism, cellular and humoral immunity, and intestinal growth, development and repair (Carver & Walker, 1995; Gil & Uauy, 1996; Cosgrove, 1998; Sánchez-Pozo *et al.* 1998; Gil, 2002; Aggett *et al.* 2003; Buck *et al.* 2004; Schaller *et al.* 2004; Hawkes *et al.* 2006). However, there is a lack of information about the bioavailability of dietary NT and derivatives in infants.

Digestion of nucleic acids and NT clearly occur in man and animals. Most dietary NT are ingested in the form of nucleoproteins from which the nucleic acids are liberated in the intestinal tract by the action of proteolytic enzymes. Pancreatic ribonuclease and deoxyribonuclease degrade RNA and DNA into a mixture of mono-, di-, tri- and polynucleotides. In response to gastrointestinal hormones, intestinal Brunner's and Lieberkuhn's glands secrete phosphodiesterases, which supplement the action of pancreatic nucleases in producing mononucleotides (Gil & Uauy, 1996; Gil, 2001). Hydrolysis of NT to NS is carried out by intestinal alkaline phosphatase and ecto-5′-nucleotidase, which have a broad substrate selectivity (Anderson & Parkinson, 1997). The capacity for intestinal nucleic acid digestion and absorption varies between species, being higher in pigs compared with rabbits, dogs and cats (Barnard, 1969; Roth & Kirchgessner, 1978; Greife, 1984, 1986). Using an intestinal homogenate from a 22-week-old human fetus, researchers qualitatively demonstrated that luminal and mucosal enzymes can digest RNA from human milk to cytidine, uridine and uric acid (Thorell *et al.* 1996). The efficiency of these enzymes to catabolize RNA and NT to absorbable products on a quantitative basis needs to be further investigated. A previous study from our group showed that cells from the intestinal epithelium are capable of hydrolysing RNA (Rueda *et al.* 2002).

Because *de novo* synthesis of NT is an energy-intensive process, cells fulfil their NT requirements by NS salvage from both inside and outside the cell (Carver & Walker, 1995; Griffith & Jarvis, 1996). Enterocytes have a limited capacity for *de novo* NT synthesis. Therefore, they rely heavily on NS transporters to salvage NS from their environment to meet their metabolic

Abbreviations: NS, nucleosides; NT, nucleotides.

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demands. To date, five Na^+-dependent concentrative (N1, N2, N3, N4 and *csg*) transporters and two Na^+-independent equilibrative (*es* and *ei*) transporters have been identified (Griffith & Jarvis, 1996). Only the purine-specific (N1; hCNT2) and the pyrimidine-specific (N2; hCNT1) Na^+-dependent NS transporters have recently been found along the entire length of the fetal and adult small intestines (Ngo *et al.* 2001).

Evidence demonstrating the presence of concentrative and equilibrative transporters has come from a wide variety of experimental preparations from several species, including perfused tissues, isolated intact cells and membrane vesicles (Gil & Uauy, 1996; Griffith & Jarvis, 1996; Scharrer *et al.* 2002). However, there is no information about the potential capacity of the neonatal intestine to absorb NS. Thus, the aim of the present study was to evaluate to what extent the suckling piglet intestine can hydrolyse RNA and NT to NS and absorb extracellular NS using cultivated intestinal explants.

Experimental methods

Preparation of intestinal explants

The protocol for the present study was approved by the University of Granada (Spain) and piglets received humane treatment according to European Union regulations. A 20-d-old piglet supplied by the laboratory animal facility of the University of Granada was separated from the mother and fasted for 6 h. The animal was anaesthetized and killed by terminal bleeding of the jugular vein. The entire small intestine was removed quickly. A 60 cm-long segment of the small intestine from the ligament of Treitz was selected and considered as the proximal jejunum. The intestine segment was rinsed thoroughly with ice-cold saline solution, opened lengthwise and blotted dry. Explants (1 cm long) were obtained from the jejunum segment and put on Netwell inserts (Costar, Cambridge, MA, USA), one explant per insert.

Study of nucleoside utilization by intestinal explants

Intestinal explants were deposited on six-well cell culture plates (Costar), one insert per well. The explants were cultured in Williams' medium E (Sigma, St Louis, MO, USA) supplemented with 43·6 pmol/l (6 mU/ml) insulin (Sigma), 2 mmol/l glutamine (Sigma) and 100 IU penicillin, 0·1 mg streptomicin and 0·25 μg amphotericin B per ml (Sigma). The medium was not supplemented with fetal calf serum since in previous experiments it showed nuclease activity. The experimental design is shown in Fig. 1. RNA (60 mg/l) from calf liver (Sigma), a mixture of five NT (AMP, CMP, GMP, IMP, UMP; 7 mg/l each; Yamasa Shoyu Co., Choshi, Japan) and a mixture of NS (adenosine, cytidine, guanosine, inosine, uridine; 10 mg/l each; Sigma) were used as substrates, respectively. Four replicates of explants for each one of the substrates (RNA, NT and NS) and four replicates in the absence of any substrate were cultivated for 3 h. In the case

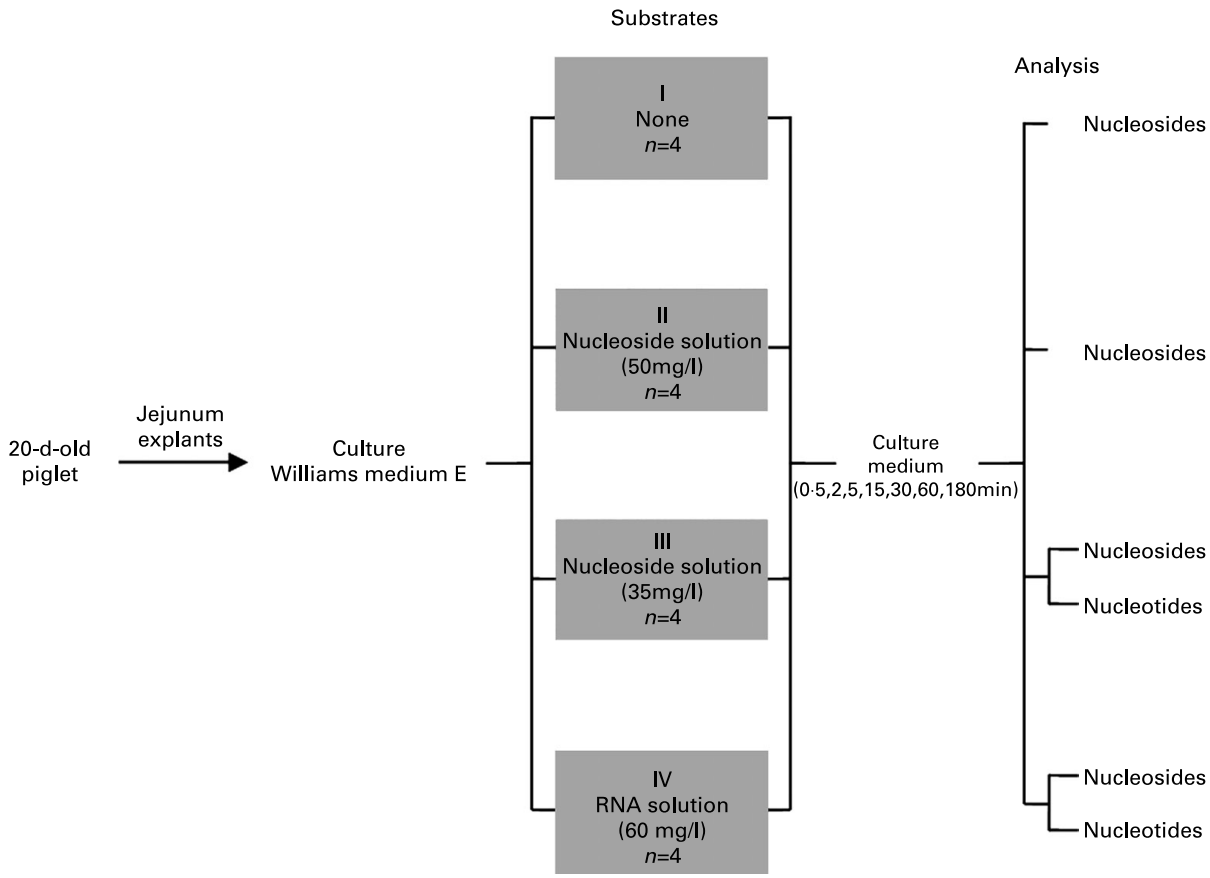

Fig. 1. Experimental design.

of explants cultured in the absence of substrate, a negative control, which contained the medium without explant, was also cultured. When the medium was supplemented with RNA a positive control containing nuclease P1 (Sigma), and a negative control, without explant and nuclease, were cultured. When the medium was supplemented with the NT mixture we also cultured a positive control containing bacterial alkaline phosphatase (Sigma), and a negative control, without explant and enzyme. When the medium was supplemented with NS, a negative control, which contained the medium with NS but without any explant, was cultured.

Aliquots of the medium were taken at different times (0·5, 2, 5, 15, 30, 60, 180 min) and the nucleoside content was determined for explants cultured in the absence of substrate and for those cultured in the presence of the NS mixture. NS and NT contents were determined in the medium at those same periods when explants were cultured in the presence of RNA and the NT mixture. Nucleotide and nucleoside contents in the different culture supernatants were analysed by HPLC according to the method of Leach *et al.* (1995).

Statistical analysis

The four replicates of transplants for each one of the substrates (RNA, NT and NS) and the four replicates in the absence of any substrate were used for statistical analysis. A general linear model of variance for repeated measures was used to assess the effects of time on free NS and NT concentrations. When significant effects ($P<0·05$) were found, *post hoc* comparisons of means were done using the adjusted Bonferroni *t* test. All statistical analyses were done with the Statistical Package for Social Sciences software (SPSS Inc., Chicago, IL, USA).

Results

The total concentration of NS in the medium increased during the period of culture when explants were incubated in the absence of NS, indicating that the explants are liberating NS (Fig. 2). However, when explants were cultured in the presence of NS, the concentration of NS in the medium rapidly decreased, suggesting that NS are efficiently absorbed by the explants as well (Fig. 2).

The total concentration of NT decreased while the total concentration of NS increased, when explants were incubated in the presence of NT, suggesting the hydrolysis of NT to NS (Fig. 3). The total concentration of both NT and NS increased during the period of culture when explants were incubated in the presence of RNA, indicating that RNA was hydrolysed to NT and then to NS (Fig. 3).

Table 1 shows the concentration of individual NS when explants were cultured in the absence or presence of NS and Table 2 shows the concentration of individual NT and NS when explants were cultured in the presence of NT or RNA. Adenosine rapidly disappeared from the medium with a parallel increase in inosine but after 30 min of explant incubation inosine levels started to decrease. These changes were especially evident when explants were cultured in the presence of NS (Table 1). Guanosine and uridine also disappeared from the medium at similar rates. On the contrary, cytidine increased over time both in the absence or presence of any of the assayed substrates (Tables 1 and 2). Uric acid was

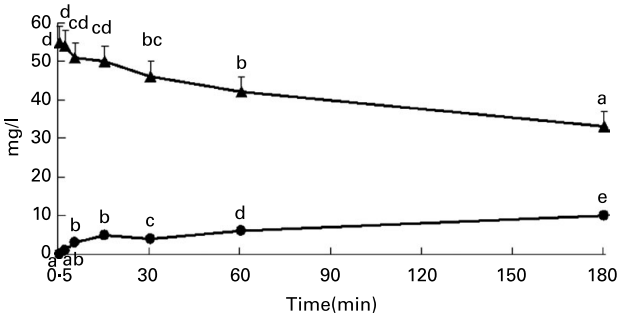

Fig. 2. Total free nucleosides (NS; mg/l) in the culture medium of suckling piglet intestinal explants cultured in the absence (●) or presence (▲) of NS for 3 h. Explants were cultured in Williams' medium E supplemented with 43·6 pmol/l insulin, 2 mmol/l glutamine and 100 IU penicillin, 0·1 mg streptomycin and 0·25 μg amphotericin B per ml. A mixture of five NS (adenosine, cytidine, guanosine, inosine and uridine; 10 mg/l each) were used as substrates with four replicates of explants for each one of the substrates. Values are means with their standard deviations depicted by vertical bars. ^{a,b,c,d,e} Mean values with unlike superscript letters were significantly different ($P<0·05$) (a < b < c < d < e).

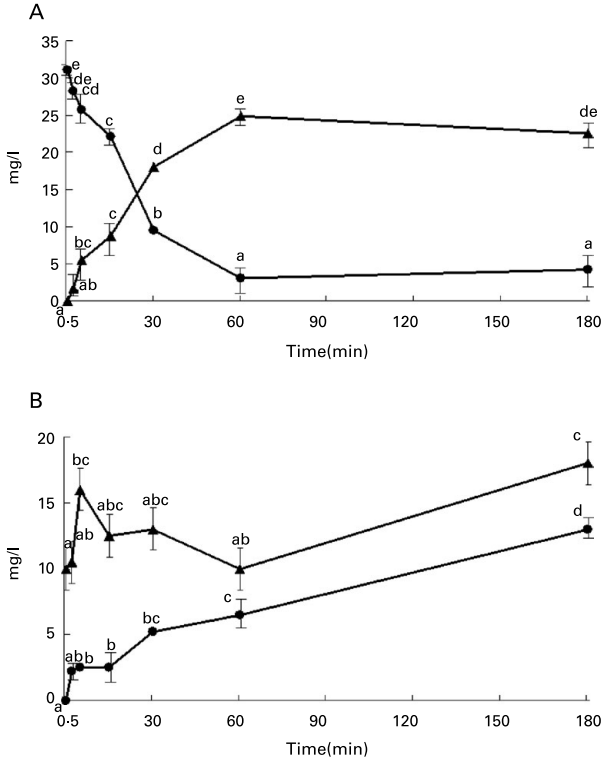

Fig. 3. Total free nucleotides (NT, ●; mg/l) and total free nucleosides (NS, ▲; mg/l) in the culture medium of suckling piglet intestinal explants cultured with NT (A) or RNA (B) as substrates for 3 h. Explants were cultured in Williams' medium E supplemented with 43·6 pmol/l insulin, 2 mmol/l glutamine and 100 IU penicillin, 0·1 mg streptomycin and 0·25 μg amphotericin B per ml. RNA (60 mg/l) or a mixture of five NT (AMP, CMP, GMP, IMP and UMP; 7 mg/l each) were used as substrates with four replicates of explants for each one of the substrates. Values are means with their standard deviations depicted by vertical bars. ^{a,b,c,d,e} Mean values with unlike superscript letters were significantly different ($P<0·05$; a < b < c < d < e).

Table 1. Individual free nucleosides (NS, mg/l) in the culture medium of suckling piglet intestinal explants incubated in the absence or presence of NS for 3 h*

(Mean values and standard deviations)

	Time (min)													
	0·5		2		5		15		30		60		180	
Substrate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
None														
U	0·00^{a}	0·0	0·69^{ab}	0·59	1·23^{ab}	0·31	2·43^{b}	1·25	1·16^{ab}	0·45	1·18^{ab}	1·16	1·13^{ab}	0·27
C	0·00^{a}	0·0	0·00^{a}	0·0	0·00^{a}	0·0	0·15^{a}	0·17	0·75^{ab}	0·21	2·16^{b}	1·17	4·70^{c}	1·65
I	0·00^{a}	0·0	0·33^{a}	0·48	0·65^{ab}	0·27	1·38^{bc}	0·16	1·47^{bc}	0·23	2·30^{c}	0·57	2·08^{c}	0·75
G	0·00	0·0	0·00	0·0	1·11	0·79	0·89	0·64	0·12	0·06	1·27	0·86	1·09	0·71
A	0·00^{a}	0·0	0·00^{a}	0·0	0·00^{a}	0·0	0·00^{a}	0·0	0·00^{a}	0·0	0·14^{ab}	0·22	0·86^{b}	0·88
NS														
U	9·50^{d}	1·41	9·08^{d}	0·73	8·61^{d}	0·75	8·12^{cd}	0·40	5·88^{bc}	0·56	4·26^{ab}	1·02	3·03^{a}	1·37
C	12·17^{a}	0·84	12·29^{a}	0·37	11·7^{a}	0·64	12·02^{a}	0·68	12·50^{a}	0·41	12·98^{a}	0·88	17·15^{b}	0·38
I	11·58^{b}	0·52	16·04^{c}	0·60	17·06^{c}	0·81	21·08^{e}	1·33	19·52de	0·58	17·88^{cd}	0·93	9·36^{a}	0·33
G	10·09^{c}	0·82	9·69^{c}	0·41	9·61^{c}	0·30	8·88^{bc}	0·10	7·94^{bc}	0·90	6·62^{b}	1·62	3·72^{a}	1·20
A	11·73^{d}	0·65	7·18^{c}	0·47	3·64^{b}	1·00	0·75^{a}	0·35	0·05^{a}	0·09	0·06^{a}	0·07	0·21^{a}	0·33

A, adenosine; C, cytidine; G, guanosine; I, inosine; U, uridine.

^{a,b,c,d,e} Mean values with unlike superscript letters were significantly different ($P<0·05$; a < b < c < d < e).

* Explants were cultured in Williams' medium E supplemented with 43·6 pmol/l insulin, 2 mmol/l glutamine and 100 IU penicillin, 0·1 mg streptomycin and 0·25 μg amphotericin B per ml. A mixture of five NS (A, C, G, I, U; 10 mg/l each) were used as substrates with four replicates of explants for each one of the substrates.

Table 2. Individual free nucleotides (NT, mg/l) and nucleosides (NS, mg/l) in the culture medium after culturing suckling piglet intestinal explants with NT or RNA for 3 h*

(Mean values and standard deviations)

	Time (min)													
	0·5		2		5		15		30		60		180	
Substrate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
NT†														
UMP	7·64^{c}	0·51	5·62^{bc}	0·95	5·97^{bc}	0·44	5·67^{bc}	2·89	2·93^{ab}	0·65	1·92^{a}	1·17	0·12^{a}	0·24
CMP	6·59^{c}	0·24	6·38^{c}	0·17	5·74^{c}	0·57	5·12^{c}	0·88	2·91^{b}	0·39	0·11^{a}	0·18	1·23^{ab}	1·45
IMP	5·16^{c}	0·13	5·12^{c}	0·39	3·90^{bc}	1·15	4·94^{bc}	1·40	2·92^{b}	0·79	0·00^{a}	0·0	0·07^{a}	0·08
GMP	5·52^{c}	0·04	5·28^{c}	0·11	4·74^{c}	0·59	3·55^{bc}	1·75	0·05^{a}	0·08	0·00^{a}	0·0	1·62^{ab}	1·30
AMP	6·14^{c}	0·08	5·83^{c}	0·05	5·33^{c}	0·46	2·91^{b}	0·60	0·70^{a}	0·29	1·02^{a}	0·49	1·19^{a}	0·68
NT‡														
U	0·00^{a}	0·0	1·16^{a}	1·39	1·10^{a}	0·30	2·00^{ab}	1·45	2·34^{ab}	0·17	2·38^{ab}	0·77	4·91^{b}	2·49
C	0·00^{a}	0·0	0·00^{a}	0·0	0·84^{a}	0·54	1·83^{a}	0·87	4·98^{b}	0·79	7·16^{b}	0·51	10·74^{c}	2·58
I	0·00^{a}	0·0	0·37^{ab}	0·27	2·20^{bc}	1·25	3·15^{c}	1·75	6·41^{d}	0·34	10·33^{e}	0·18	6·59^{d}	0·50
G	0·00^{a}	0·0	0·12^{a}	0·08	0·76^{a}	0·57	1·49^{ab}	1·94	4·41^{bc}	0·11	4·97^{c}	0·45	1·96^{ab}	1·87
A	0·00	0·0	0·02	0·03	0·22	0·32	0·24	0·21	0·28	0·10	0·02	0·03	0·12	0·15
RNA‡														
UMP	2·72	1·89	1·14	0·50	2·41	0·85	1·60	0·82	1·68	0·63	0·98	0·19	1·57	0·72
CMP	2·64^{a}	0·59	3·43^{ab}	0·06	4·20^{b}	0·38	3·49^{ab}	0·63	4·22^{b}	0·39	3·09^{ab}	0·64	7·98^{c}	1·02
IMP	0·05^{a}	0·09	1·15^{bc}	0·30	1·96^{c}	0·40	1·44^{bc}	0·53	0·76^{ab}	0·30	1·06^{abc}	0·60	2·06^{c}	0·35
GMP	3·33	1·04	3·43	0·23	4·00	0·63	3·89	1·40	3·61	0·41	3·03	0·31	4·01	0·41
AMP	1·53^{a}	0·49	2·52^{bc}	0·12	3·44^{d}	0·49	2·33^{abc}	0·43	2·81^{cd}	0·35	1·80^{ab}	0·16	2·93^{cd}	0·16
RNA‡														
U	0·00^{a}	0·0	1·51^{ab}	0·58	1·06^{ab}	0·29	1·32^{ab}	1·01	1·41^{ab}	0·40	3·11^{b}	1·93	2·98^{b}	1·06
C	0·00^{a}	0·0	0·00^{a}	0·0	0·00^{a}	0·0	0·21^{a}	0·33	0·58^{ab}	0·18	1·07^{b}	0·36	4·76^{c}	0·45
I	0·00^{a}	0·0	0·13^{ab}	0·25	0·50^{ab}	0·23	0·77^{b}	0·36	1·66^{c}	0·22	1·98^{cd}	0·22	2·61^{d}	0·47
G	0·00^{a}	0·0	1·03^{bcd}	0·14	1·27^{bcd}	0·15	0·56^{abc}	0·65	1·52^{cd}	0·33	0·32^{ab}	0·03	1·94^{d}	0·89
A	0·00	0·0	0·00	0·0	0·00	0·0	0·00	0·0	0·00	0·0	0·00	0·0	0·84	1·46

A, adenosine; C, cytidine; G, guanosine; I, inosine; U, uridine.

^{a,b,c,d,e} Mean values with unlike superscript letters were significantly different ($P<0·05$; a < b < c < d < e).

* Explants were cultured in Williams' medium E supplemented with 43·6 pmol/l insulin, 2 mmol/l glutamine and 100 IU penicillin, 0·1 mg streptomycin and 0·25 μg amphotericin B per ml. RNA (60 mg/l) or a mixture of five NT (AMP, CMP, GMP, IMP and UMP; 7 mg/l each) were used as substrates with four replicates of explants for each one of the substrates.

† Free NT measured.

‡ Free NS measured.

not detected or it was at a very low concentration in the culture media (results not shown).

When RNA was used as substrate (Table 2) most NT concentrations remained constant during the period of study; only CMP increased during this period. However, when explants were cultured with NT (Table 2), the concentration of individual NT rapidly decreased while that of NS increased. Figure 4 shows the percentage of total NT disappearing in the course of the experiment, which appear as NS. After 180 min of explant incubation, about 12·1 % remained as NT, 64·4 % appeared as NS and a net percentage of 23·5 % of NT was taken up. Likewise, the percentages of UMP, IMP, GMP and AMP disappearing, and appearing as uridine, inosine, guanosine and adenosine, were 71·4, 95·1, 36·4 and 2·1, respectively, while cytidine increased 164·1 % from CMP.

Discussion

The results found in the present study demonstrate that the intestine of suckling piglets is able to hydrolyse both RNA and free NT to NS in the absence of luminal content, and that uridine, inosine, guanosine and adenosine are efficiently taken up. Only cytidine is not absorbed at similar rates.

Since the piglet intestinal explants were washed before culture, it appears the hydrolysis of RNA can be achieved in the absence of pancreatic ribonuclease and that enterocyte phosphodiesterase should be the main enzyme responsible for the hydrolysis. The present result confirms previous findings reported by our group (Rueda *et al.* 2002). In addition, Kuchan *et al.* (2000) studied the influence of purine intake on uric acid excretion in infants fed on soya infant formulas suggesting that healthy infants can digest RNA and subsequently absorb the ribonucleotides liberated.

Phosphodiesterase I has been found in the intestinal mucosa of animals and man (Hawley *et al.* 1983; Morley *et al.* 1987). Thorell *et al.* (1996) showed that RNA from human milk was hydrolysed by intestinal homogenates from a 22-week gestation human fetus. However, they could not ascertain to what extent the hydrolysis was due to luminal ribonucleases (pancreatic ribonuclease and biliary phosphodiesterase) or to endogenously secreted enterocyte phosphodieserases, because the homogenates were contaminated with the luminal content. Moreover, they could not exclude that part of the hydrolysis of RNA might be due to the presence of ribonucleases, 5′-nucleotidase and alkaline phosphatase from the mother's milk. These and other enzymes involved in NT metabolism have been identified in human milk. RNase activity has been associated with lactoferrin and RNase II and 5′- nucleotidase have been found in human milk (Hamosh, 1995). Since both alkaline phosphatase and ecto-5′-nucleotidase are present in the enterocyte membrane, we hypothesize that the hydrolysis of free NT, liberated from RNA or added to the culture medium, is mediated by both enzymes in the intestine of suckling piglets. However, in contrast to other studies related to NS transport, which used luminal vesicles, in the experimental set-up employed in the present study (explants put on Newel inserts with the mucosa facing the solution containing the substrate) we cannot exclude that both surfaces of the jejunum (mucosal and serosal) were exposed to substrates and this may complicate the interpretation of results.

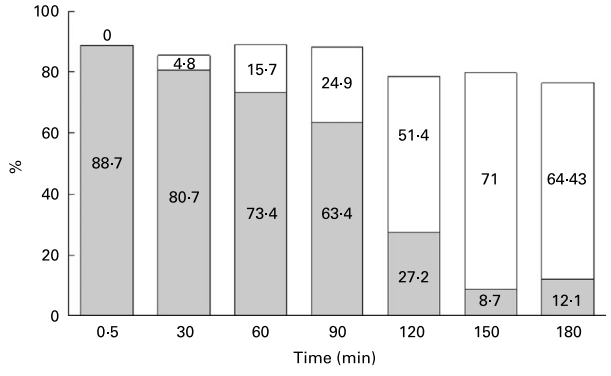

Fig. 4. Percentage of total nucleotides (NT, ▨) disappearing in the course of 180 min incubation of suckling piglet intestinal explants, which appear as nucleosides (NS, □). Intestinal explants were cultured in Williams' medium E supplemented with 43·6 pmol/l insulin, 2 mmol/l glutamine and 100 IU penicillin, 0·1 mg streptomycin and 0·25 μg amphotericin B per ml. A mixture of five NT (AMP, CMP, GMP, IMP and UMP; 7 mg/l each) were used as substrates with four replicates of explants for each one of the substrates. Percentages are expressed as mean of total NT disappearing, which appear as NS.

The concentrations of RNA and NT used in the present study are within the range of those found in human and sow's milk. RNA in human milk has been reported to range from 100 to 5600 mg RNA/l, reflecting differences attributed to both stages of lactation and socioeconomic groups (Gil & Uauy, 1995). More recently, total potentially available NS, including free NS, monophosphate and diphosphate NT, NT adducts of purine and pyrimidine, and RNA have also been reported in human milk with values being highest at earlier stages of lactation. The mean of the total potentially available NS ranged from 49 mg/l in colostrum to 87 mg/l in mature milk, with a grand mean of 67 mg/l (Leach *et al.* 1995). Cytidine and uridine NT comprise the first and second largest free-nucleotide fraction. CMP, CDP-choline, UMP, and UDP adduct of glucose, galactose, *N*-acetyl-glucosamine and *N*-acetyl-galactosamine remain high throughout lactation in human milk (Gil & Uauy, 1995). On the other hand, sow's milk contains about 450 mg/l of free NT, uridine derivatives being the predominant NT forms (Gil & Uauy, 1995).

The hydrolysis of RNA renders a mixture of free NT and NS and that of NT a mixture of NS. The hydrolysis of both substrates was almost completed after 180 min of incubation, taking into account that liberated NS are efficiently absorbed. Although there is a small liberation of NS to the medium by intestinal explants, the rapid disappearance of exogenous NS contributes to a net absorption of these compounds by piglet gut.

Adenosine rapidly disappeared from the medium with a parallel increase in inosine. Thus adenosine is likely converted to inosine by the action of adenosine deaminase, which is known to be present in the intestinal mucosa (Witte *et al.* 1991). Inosine levels decreased after 30 min of explant incubation. Guanosine and uridine also disappeared from the medium at similar rates. On the contrary, cytidine levels increased over time.

The true RNA digestibility in pigs has been reported to be about 99 % when an oral dose of 3 % RNA is used (Roth & Kirchgessner, 1978) and large oral loads of purine and pyrimidine NT are readily absorbed by the small intestine in pigs (Greife & Molnar, 1984*a*,*b*). The rapid digestion of nucleic

acids and NT by the pig intestine has been associated with higher concentrations of pancreatic ribonuclease compared with other species (Barnard, 1969), and the rapid absorption appears to be due to the presence of transmembrane Na^+-dependent NS contransporters with overlapping substrate specificity, one for purine NS (N1) and one for pyrimidine NS (N2) (Scharrer *et al.* 2002). In contrast to pigs, in rats a considerable amount of purine derivatives seems to reach the hind gut (Greife, 1986) and NS, particularly purine NS and bases, are intensively metabolized in the enterocytes (Bronk & Hastewell 1989*a*,*b*; Stow & Bronk, 1993). In contrast to previous reports in rats, in the present study we were not able to detect uric acid in the culture media, thus suggesting that NS derived from purine NT hydrolysis are efficiently taken up by pig enterocytes and incorporated into the NT cell pools. Nevertheless, we cannot exclude that at least in part purine NT are catabolized to uric acid and later converted to allantoin by uric acid oxidase which is active in pigs (Wu *et al.* 1989).

The human adult intestine expresses two Na^+-dependent NS transporters, N1 and N2, and two Na^+-independent transporters, *es* and *ei*, which exhibit differential sensitivity to *S*-(*p*-nitrobenzyl)-6-thioinosine (nitrobenzylmercapto-purine riboside) (Chandrasena *et al.* 1997). These N1 (hCNT2) and N2 (hCNT1) selectively transport purine and pyrimidine NS, respectively, and are found on the apical surface of adult jejunal enterocytes. The Na^+-independent transporters, *es* and *ei*, are not present in fetus or adult enterocytes (Ngo *et al.* 2001). The apparent K_m values for hCNT1-mediated transport of uridine and cytidine are 59 and 140 μM, respectively (Graham *et al.* 2000). This suggests that uridine is preferentially taken up by intestinal cells. The bioavailability of uridine is particularly crucial to the formation of pyrimidine nucleotide–lipid conjugates used to synthesize RNA and biomembranes (Connolly & Duley, 1999). NS transporters in pigs appear to be similar to those of man, the K_m for the N1 (guanosine) and N2 (thymidine) transport being 58 and 91 μM, respectively (Scharrer *et al.* 2002). However, pig NS transporters have higher K_m and V_{max} than man and other monogastic species (Griffith & Jarvis, 1996), which may be related to the high capacity of nucleic acid digestion observed in pigs (Barnard, 1969; Roth & Kirchgessner, 1978; Greife, 1984). In man, the K_m of the N1 (hCNT2) transporter for guanosine ranges from 10 to 12 μM, which explains the high affinity for this NS (Graham *et al.* 2000), whereas that of pigs is about 5-fold (Scharrer *et al.* 2002). We suggest that the suckling piglet intestine will express the N1 transporters as in adult pigs and man, and will take up exogenous guanosine, explaining the rapid disappearance of this NS from the medium. This is conceivable since uric acid was at a very low concentration 3 h after the intestinal explant incubation.

Under the conditions of the present study cytidine seems to be poorly absorbed by the piglet intestine in the presence of uridine. There is an efflux of cytidine from the intestinal explant to the culture medium, which might be due to intracellular catabolization of cytidine derivatives formed from absorbed uridine. It is well known that uridine is salvaged by uridine kinase to form UMP and later UDP and UTP which is used in part to form CTP and CDP derivatives (Sutle *et al.* 1995).

We cannot exclude the presence of equilibrative nucleoside transporters 1 and 2 (*es*, ENT1 and *ei*, ENT2) in piglet intestine. Although both transporters are broadly selective in man, ENT2 is a generally low-affinity NS transporter with 2·6-, 2·8-, 7·7- and 19·3-fold lower affinity than ENT1 for thymidine, adenosine, cytidine and guanosine, respectively. In contrast, the affinity of ENT2 for inosine is 4-fold higher than ENT1, which suggests that ENT2 might be important in transporting inosine (Ward *et al.* 2000).

In conclusion, the intestine of suckling piglets is able to hydrolyse RNA and free NT to NS, which are efficiently absorbed by the enterocytes, except cytidine.

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