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Dietary nucleotides and gut mucosal defence

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Abstract

The informational aspects of nucleic acid synthesis have attracted much more attention than the quantitative significance of DNA, rRNA, tRNA, and nucleotide synthesis. Animal and human studies suggest that in energetic terms, 5–10% of the energy used in synthesising tissue protein is expended in manufacturing an appropriate amount of synthetic machinery, that is the ribosome and tRNA. The two sources for synthesis of nucleotides are salvage of nucleotides released by intracellular degradation or derived from the diet, and nucleotides synthesised de novo from amino acids (for example, glutamine) and sugars (glucose). The comparative importance of these two processes is not well defined, but rRNA production requires a high de novo input in cell types with the capacity for rapid division (for example, lymphocytes). The gut is unusual in requiring a ready arterial supply of nucleotides synthesised by hepatic de novo pathways. Animal studies show that an exogenous supply of nucleotides (salvage) can improve liver regrowth, immune responsiveness to a microbial challenge, and gut morphology in diarrhoea models. Humans adapt to dietary nucleotide intake by downregulating de novo pathways. All total parental nutrition regimens, and most enteral regimens lack nucleotides, which may predispose to an inadequate supply of preformed nucleotides to gut and immune cells in the critically ill, artificially fed patient. Unfortunately, there are no clinical studies that answer this point at present.

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Nucleic acids are not thought to be essential nutrients as pathways exist for synthesis of purines and pyrimidines, de novo. Most recent basic nucleic acid research has concentrated on the 'informational' aspects, rather than 'metabolic' aspects of their metabolism. Examples would include modulation of cytokine mRNA concentrations[1] or muscle ribosome concentrations as mediators of post-traumatic changes in protein synthesis.[2]

This paper will define several factors related to dietary provision of nucleotides (Table). How are synthetic pathways for purine and pyrimidines affected by the dietary intake of purines and pyrimidines? What is the nature of purine and pyrimidine requirements and can endogenous purine and pyrimidine supply ever be exceeded by demand in the acutely ill patient? Will organ function be impaired by no intake of purines and pyrimidines as occurs for most patients fed with most current enteral and parenteral formulas?

Pathways of purine and pyrimidine biosynthesis

Endogenous purines and pyrimidines are synthesised de novo, from amino acids and other small molecules. The two pathways differ in that the ribose moiety of purine nucleosides is incorporated as 5-phosphoribosyl-1-phosphate (PRPP) in the first reaction of purine synthesis, but for pyrimidines is incorporated at the end stage after the pyrimidine ring has been fully formed, as reviewed elsewhere.[3–5]

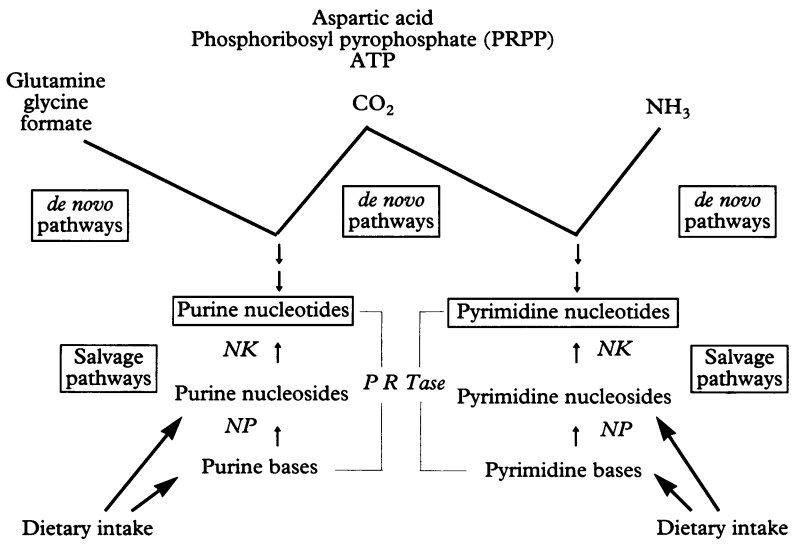


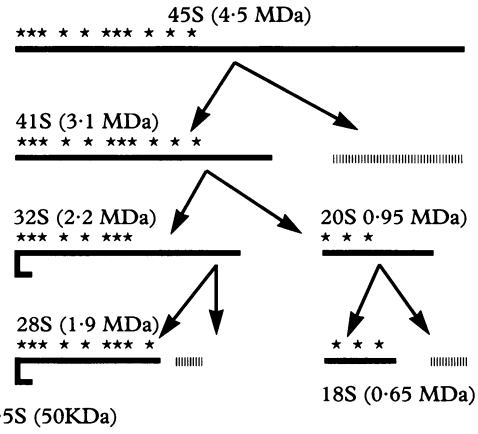
Figure 1: Pathways of purine and pyrimidine metabolism. The intracellular nucleotide precursor pool is maintained by de novo synthesis and salvage of purines and pyrimidines from dietary sources and from intracellular degradation of RNA and DNA. PRTase=phosphoribosyltransferase, NP=nucleoside phosphorylase, NK=nucleoside kinase.

SYNTHESIS

Endogenous purines and pyrimidines are synthesised de novo, from amino acids and other small molecules (Fig 1). Pyrimidines are synthesised from NH_3, CO_2, and aspartate, the

Key aspects of dietary nucleotide metabolism in relation to gut mucosa

1 What are the pathways of synthesis of purines and pyrimidines?
2 Are they affected by dietary intake of purines and pyrimidines?
3 What is the nature of purine and pyrimidine requirements?
4 Is endogenous purine and pyrimidine supply ever exceeded by demand (for example, in acutely ill patients)?
5 Is organ function impaired by no intake of nucleotides (for example, all total parenteral nutrition and most enteral nutrition)?



*=*methylnucleosides conserved during processing.*

Figure 2: Nucleolar rRNA processing in mammalian cells.

latter being synthesised from glutamine in lymphocytes.[6] The ribose and phosphate moieties are derived from glucose and ATP respectively. In contrast, purines incorporate the γ-NH_2 group of glutamine (2), nitrogen from glycine and aspartate (1 or 2), and carbon from CO_2 formate, glycine, and aspartate. The sugar and phosphate moieties are as for pyrimidines.

SALVAGE AND CATABOLISM

Degradation of the 5′ mono, di, and triphosphates occurs through the stepwise removal of phosphate and ribose (or 2′ deoxyribose) to form the nucleobases, uracil, cytosine (pyrimidines) or hypoxanthine (purines). Salvage of the nucleobases occurs by a single step addition of PRPP to form the 5′ monophosphate (hypoxanthine-guanosine phosphoribosyl transferase (HGPRTase)) (see Fig 1).

URINARY EXCRETION OF BREAKDOWN PRODUCTS

Degradation products of orotic acid, uracil, and cytosine include β-alanine and β-aminoisobutyric acid. Purine catabolism seems to be controlled by three factors, namely intracellular PRPP concentrations, the activity of the phosphoribosylamidotransferase (PRPP+ gln−>5′ P ribosylamine+glu), and the intracellular concentrations of IMP, AMP, and GMP.[5] An adequate intracellular concentration of nucleotide monophosphates will inhibit the first step in purine synthesis, an adequate supply of the precursor PRPP, will activate it.

Relation between salvage and de novo synthesis of purines and pyrimidines

EVIDENCE FROM WHOLE ANIMAL STUDIES

There is some incorporation of dietary purines and pyrimidines into tissue ribonucleic acid (RNA), DNA, and nucleotide pools[3] and this represents the exogenous counterpart of the intracellular salvage pathways. The route of administration considerably affects urinary excretion of purines, the same oral dose being poorly incorporated in tissue nucleic acids, compared with intravenous administration.[7] This may relate to the low capacity of intestinal transport mechanisms that exist for all four purine/pyrimidine bases[8–10] as excess intake is fermented by colonic bacteria.

Savaiano *et al*[7] saw that those tissues most actively participating in nucleic acid synthesis had the highest incorporation ratio (^{14}C intravenous:^{14}C oral) and included salivary, adrenal, thyroid, thymus and pituitary glands, and lymph tissue (ratio 25–59), whereas it was only three for liver. Similar results have been obtained in rats infused with nucleoside/nucleotide mixtures.[11] Adenine could be taken up intact by the gut, whereas guanine, hypoxanthine, and xanthine were extensively catabolised in their passage from mucosa to serosa, implying that the gut has low capacity for de novo adenine synthesis.[12] This would explain the different fate of oral or intravenous purines on the basis of the gut mucosa's high requirement for exogenous purines, which results in preferential extraction of luminal nucleotides during first pass through gut and liver.[13]

A reciprocal relation between purine intake and de novo synthesis exists in the gut[14 15] as urinary excretion of catabolites was reduced during dietary purine restriction.[16 17] Dietary supplementation with pyrimidines suppressed de novo synthesis in gut mucosa, suggesting that under normal circumstances, this pathway was comparatively inactive and could only be stimulated by omission of purines and pyrimidines from the diet.[14 15] This relation, however, relies on an adequate dietary protein intake to supply substrate for de novo synthesis.[18 19]

EVIDENCE FROM CELL CULTURE STUDIES

The technique of measuring RNA synthesis in cultured cells is complicated by intracellular compartmentation of nucleotide pools. These can be labelled by [^{14}C]-uridine or [^{14}C]-orotic acid, which enter the salvage and de novo pathways, respectively. In addition, there are different precursor pools for mRNA and rRNA synthesis (small/non-expandable and large/expandable) in Ehrlich ascites cells,[20] HeLa S3 cells,[21] regenerating rat liver,[22] and rat hepatoma cells.[23] The small pool is nucleolar (rRNA synthesis) and supplied mainly by de novo synthesis.[21 24 25] The large pool is under metabolic control in the sense that de novo synthesis is suppressed by exogenous supply.[26]

Precursor labelling problems can be circumvented by using [^{14}C-*methyl*]-methionine to label intracellular S-adenosylmethionine (SAM), the donor for methylation of rRNA during synthesis.[27 28]

MECHANICSMS OF CONTROL OF RIBOSOME SYNTHESIS

Several studies have noted that the time course of changes in cellular ribosome content usually comes before those of protein, often by a

considerable margin. Ribosomal RNA is the most abundant species and accounts for the largest requirement for de novo and salvage of purines and pyrimidines.

It is synthesised in the nucleolus by RNA polymerase I, which is separately regulated from the other nuclear polymerases. The primary transcript, 45 S pre-rRNA, is cleaved to 18S and 28S rRNA species, which exit the nucleus as mature 40S and 60S ribosomal subunits (Fig 2). A significant proportion of unmethylated 'spacer' region of pre-rRNA is degraded during processing. As such their nucleotides can enter the salvage pathway for subsequent reutilisation in RNA synthesis (see reviews by Warner[29 30]). In non-growing tissues, a further proportion (up to 50%) of the 45S pre-RNA is degraded completely, termed 'wastage'.

The supply of rRNA and ribosomal proteins is coordinated because if rRNA synthesis is inhibited, degradation of nucleolar rProteins increases[31 32] whereas if rProtein synthesis is inhibited, there is increased degradation of 45S pre-rRNA.[24 33 34]

This apparently expensive process ('wastage') that is loosely coupled to rProtein synthesis, gives exquisite control of cytoplasmic ribosome appearance rates. Warner[29–31] has aptly called the inhibitory modulation of excess rRNA and rProtein concentrations (through degradation), 'fine tuning' of ribosome supply. The level of 'coarse tuning' would be supplied by gross coordinate changes in rProtein and rRNA synthesis in response to growth or nutritional depletion.

RIBOSOME PRODUCTION AND GROWTH STIMULI

Liver

The two phases of liver regeneration in the rat can be described as 'proliferative' (days 1–5) and 'post-proliferative' (days 6–12). Proliferative growth is accompanied by appearance of new cytoplasmic ribosomes (fivefold increase) and a twofold increase in rates of nucleolar 45S pre-rRNA synthesis with a doubling of rates of 45S rRNA elongation.[35] In contrast, degradation of cytoplasmic ribosomes is unchanged.[36–38] This implies that in the resting liver, nearly 60% of 45S pre-rRNA was 'wasted' and because processing results in the loss of nearly 50% of 45S pre-rRNA, of nucleotides incorporated into 45S pre-rRNA, only 20% ever reaches the cytoplasm.

An acute phase response to turpentine injection increases cytokine production and the export of acute phase proteins by liver. This response is mediated by increased ribosome production and their transport from the nucleolus within five hours of treatment which comes before the activation of the acute phase protein response.[39 40]

Kidney

Unilateral nephrectomy causes compensatory hypetrophic growth of the contralateral kidney, which will reach about 75% of the weight of both kidneys within two weeks. Within 48 hours, however, rRNA content/cell increases by 40%.[41] Two mechanisms are responsible for this, an increase in the efficiency of processing of 45S pre-rRNA[41 42] and an increase in rDNA transcription and 45S pre-rRNA synthesis within a few hours of surgery.[43]

Growing muscle – normal and rapid growth

During 'catch up' growth of protein depleted animals, the number of ribosomes in skeletal muscle doubles, as does the efficiency of ribosome use in protein synthesis.[44] Severe protein/energy malnutrition results in a coordinated loss of cellular ribosomes and cell muscle protein[45] whereas energy deficiency reduces efficiency of ribosome use in protein synthesis.[46 47] There are thus two mechanisms for modulating muscle protein synthesis, through changes in the number of ribosomes and their efficiency of use. During accelerated growth, this relation is perturbed as was shown during weight induced hypertrophy of the anterior and posterior latissimus dorsi muscles of chicken.[48] Although muscle protein increased steadily over 50 days, RNA concentrations doubled within three days. The important factor that mediated increased muscle protein synthesis was therefore the number of ribosomes, not their efficiency of use – as also occurs during hypertrophy of the heart after surgical aortic stenosis.[49–51]

NORMAL/TRANSFORMED CELL LINES

Normal cells are responsive to exogenous growth signals in a way not shared with transformed cell lines. This is especially true in relation to the amount of every cellular component a normal or tumour cell must accumulate, before passing into the replicative phases of the cell cycle.

A 'competence signal' initiates recruitment into the cell cycle, while a 'progression signal' is essential for effective expression of growth.[52 53] Normal 3T3 cells do not enter the cell cycle until their ribosome content reaches a certain threshold, in contrast with transformed 3T6.[54] Thus, during stimulated cell growth, ribosome content should double before recruitment.[55] A proliferative signal for cells of the immune system must therefore be accompanied by increased ribosome synthesis before division and immunoglobulin synthesis can occur.

RESTING AND GROWING CELLS IN CULTURE

The growth stimulus to lymphocytes, provided by the mitogen phytohaemagglutinin (PHA) is mediated in large part by increased ribosome production (10 to 50-fold), which comes before DNA replication and cell division.[56] This model thus represents an extreme anabolic stress and as in hypertrophying kidney, the increase in ribosome production is achieved through the end of the 'wastage' of 45S pre-rRNA in resting cells. As described, this mechanism permits rapid increases in ribosome production at the least energetic

cost. De novo synthesis provides most of the nucleotide flux for increased RNA and DNA synthesis, as suggested by the limited ability of exogenous nucleobases and nucleosides (salvage pathway) to relieve inhibition of growth by limiting amounts of glutamine in the culture medium (de novo pathway).[57]

RESPONSES OF PURINE, PYRIMIDINE, AND RIBOSOME METABOLISM TO GROWTH STIMULI

Where protein intake is adequate, de novo synthesis provides the main source of nucleotides for nucleic acid synthesis. Although it can be suppressed by dietary purine and pyrimidine supply, the degree to which this occurs depends on the tissue type. Liver and gut behave differently. In the liver dietary purines increase the activity of salvage and catabolism pathways simultaneously, while the gut, which has only a low capacity for de novo synthesis is dependent on the liver for the supply of nucleotides. Where protein intake is reduced, the salvage pathway assumes a greater importance as a means of recycling purines and pyrimidines released by nucleic acid catabolism. Lack of dietary purines and pyrimidines will switch on de novo synthesis in the gut, to a limited extent.

Within the cell itself, the split between do novo and salvage pathways differs for each species of RNA. Ribosomal RNA production seems to rely more heavily on de novo synthesis than does mRNA or tRNA synthesis. In this sense, an important component of protein synthesis (the ribosome) can be seen to be under the same general nutritional controls as protein synthesis itself, that is the dietary amino acid supply.

The importance of an adequate increase in cellular ribosome synthesis during enterocyte production and maturation can be inferred from the known effects of malnutrition on mucosal architecture and cell kinetics. This relation holds true for many other cell types and there is no reason to believe that the enterocyte is an exception. Its reliance on de novo pathways, and the low levels of these pathways, may make this cell type particularly sensitive to any limitation of purine and pyrimidine supply. This situation will occur in the malnourished patient, or one receiving nutrition support without added purines or pyrimidines.

Evidence for a positive role for purines and pyrimidines in clinical nutrition

Dietary supplementation with nucleotides, nucleosides or nucleobases can improve growth rates and nitrogen retention in young animals.[10 58] Furthermore, four lines of evidence suggest that supplementation with dietary or parenteral nucleotides/nucleosides may be of clinical significance.

INFECTION AND IMMUNE FUNCTION

The ability of mice to survive intravenous injections of *Candida albicans* was shown to be considerably enhanced by the addition of yeast RNA or individual purines and pyrimidines to an otherwise nucleotide free diet.[59 60] As described above, this protective effect is dependent on the route of administration because oral nucleotide intake does not result in such avid uptake by the spleen. The most recent study has confirmed that intraperitoneal administration of nucleosides can reduce death, after *Staphylococcus aureus* innoculation, from 71 to 21% in mice.[61] In addition, Kulkarni *et al* investigated the effects of nucleotide free diets in impairing fatal graft *v* host reactions in irradiated mice. The mirror to these experiments was to determine the proliferative potential of transplanted lymphocytes in animals receiving nucleotide free and nucleotide supplemented diets.[62] Surprisingly, nucleotide free diets were immunosuppressive in that mortality graft *v* host reactions was significantly reduced. Conversely, nucleotide free diets reduced the responsiveness of lymphocytes to PHA, to a very noticeable extent. These results are similar to those seen in malnourished animals but should be considered with some care. Thus, lymphocyte responsiveness is a measurable aspect of the ability of a cell type to mount its appropriate response but its clinical significance is not clear. Although it has proved difficult to show clinically significant effects of the nutritional state on immune function,[63] the finding that death rates were significantly reduced by nucleotide supplementation is highly suggestive.

LIVER REGENERATION

Using the 70% hepatectomy model in rats, supplementation of total parenteral nutrition regimens with nucleotides and nucleosides (as 10% of amino acid nitrogen) significantly increased post-operative nitrogen balance (became positive) and whole body protein turnover and synthesis.[64 65] This treatment also reduced the extent of galactosamine induced liver injury, as judged by histological tests and circulating concentrations of the liver enzymes aspartate transaminase and alanine transaminase.[11] These data suggest that repair and growth in an important organ of purine and pyrimidine biosynthesis can be improved by providing an external supply of preformed nucleotides and nucleoside.

INTESTINAL REPAIR

The effect of nucleotides present in white cells in milk on gut maturation has never been quite clear, but has interested paediatric gastroenterologists nevertheless. As a model of mucosal damage during infective diarrhoea, Nunez *et al*[66] induced chronic diarrhoea in weaning rats by substituting the maltodextrins in the enteral diet with lactose. Nucleotide supplementation partially restored the biochemical atrophy of the small intestine at proximal and distal sites. There were significant increases in protein content and brush border saccharidases, although the changes were not large. Nucleoside supplementation

increased the rate of maturation and growth in the young rat, as assessed by mass, RNA, DNA, and protein concentrations and activity of brush border enzymes.[67]

As argued above, a limitation in de novo synthesis may be relieved by input into the salvage pathway. If true, it would reflect an interesting phenomenon because less than 1% of the flux of glutamine seems to go into de novo synthesis.[68] If there is a limitation then nucleotide and not glutamine supplementation should relieve any de novo limitation, which can be detected biologically. A recent study[69] investigated the effect of total parenteral nutrition supplementation with nucleosides (see above[64 65]) or glutamine on reversal of total parenteral nutrition induced gut atrophy in the rat. Nucleosides alone, or in combination with glutamine significantly increased villus height, total and mucosal jejunal wet weight, protein and RNA and brush border maltase and sucrase. The effects of nucleosides were more potent than those of glutamine alone.

In conclusion, because the gut has a high rate of protein turnover and is uniquely dependent on salvage and exogenous supplies of purines and pyrimidines, limitation in supply may impair maintenance of the barrier function in the malnourished and stressed patient. Normal diets probably provide sufficient purines and pyrimidines to allow for any such limitation, indeed 'requirements' are probably quite modest given the tight metabolic control of purine synthesis and degradation and low utilisation of dietary sources. Total parenteral nutrition or enteral nutrition is unusual in that no purine/pyrimidine intake is given.

The few, relevant studies cited above are certainly persuasive and can only serve to promote further studies. It may be particularly fruitful to take animal models of stress in which it is known that the gut (barrier function) or immune system, is affected.

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