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GUEST EDITORIAL

Why are dietary nucleotides essential nutrients?

A Nutrition Society Symposium examined this question 14 years ago and concluded that dietary nucleotides were 'small beer' in terms of nitrogen economy (D'Mello, 1982; Giesecke *et al.* 1982; Zollner, 1982). This was understandable, as it was known that orally ingested ^{14}C-labelled purines and pyrimidines were incorporated into tissue RNA and DNA to a minor extent (D'Mello, 1982) and had little bioavailability in children (Golden *et al.* 1981). Pathways for *de novo* purine and pyrimidine synthesis, active in most tissues, were thought to predominate over salvage of nucleotides released by RNA and DNA degradation or from dietary sources. Thus, incorporation of ^{14}C-label in the medium into RNA of 6C3HED lymphoma cells was faster from ^{14}C-glutamine than from ^{14}C-uridine. The large intracellular nucleotide pool was demonstrated to be under metabolic control in the sense that *de novo* synthesis could be suppressed by exogenous nucleotide supply (Goody *et al.* 1975). More specifically, rRNA synthesis was shown to be supplied from a pool of pyrimidines, synthesized *de novo* (Wiegers *et al.* 1976). These data did not suggest that purines and pyrimidines were essential nutrients, indeed excess intake had negative health implications in relation to gout and inborn errors of purine metabolism.

This view has now changed and the paper by López-Navarro and colleagues (López-Navarro *et al.* 1996) provides a further strong clue about the nature of apparent essentiality for dietary nucleotides (Grimble, 1993). There is now highly suggestive evidence for a view that dietary nucleotides are extremely important. In three animal studies, a nucleotide-free diet was shown to reduce survival after intravenous infection with *Candida albicans* or *Staphylococcus aureus*, whilst the proliferation of peripheral lymphocytes after lectin challenge was significantly reduced (see Grimble, 1994). Net protein catabolism following 75% hepatectomy was significantly reduced during total parenteral nutrition (TPN) supplemented with a mixture of nucleosides and nucleotides (Ogoshi *et al.* 1985). Finally, enterocytes, which have a very limited capacity to synthesize purines *de novo*, may rely on dietary sources or on hepatic *de novo* synthesis.

This peculiarity may be reflected in the specific effects of dietary nucleotides on intestinal function (Grimble, 1994). Thus, first incidences of diarrhoea in children from low socioeconomic backgrounds in Santiago, Chile, were reduced from 68% to 52% by supplementing formula milk with nucleotides (Brunser *et al.* 1994). Nucleotide supplementation inhibits endotoxin-induced intestinal translocation of bacteria in protein-deficient mice (Adjei *et al.* 1995), and has been shown to reduce pathological changes to the mucosa which result from chronic lactose feeding which induces diarrhoea (Bueno *et al.* 1994). The basis of mucosal protection by nucleotides is unclear but several mechanisms have been proposed (Grimble, 1994). The most persuasive is that a 'normal' diet contains nucleotides and this therefore directs the split between salvage and *de novo* pathways for RNA and DNA precursors. It is known that dietary nucleotides partially suppress *de novo* synthesis in the intestinal mucosa (Leleiko *et al.* 1983) and that, conversely, a nucleotide-free diet leads to profound loss of mucosal RNA and the mRNA for two of the salvage pathway enzymes in the small bowel and colon, but not in the liver (Leleiko *et al.* 1987). As López-Navarro and colleagues have shown (López-Navarro *et al.* 1996), after dietary

nucleotide removal it takes time to develop a new metabolic set-point in which *de novo* pathways can accommodate this loss. If this is so, then it will have definable consequences. The first is that there may be temporary imbalances in the nucleotide pools of the major organs of *de novo* synthesis and supply. Certainly, the same group (López-Navarro *et al.* 1995) have shown that nucleotide deprivation led to a transient loss of approximately 50% of liver RNA (but not DNA) within 7 d, which recovered by 21 d. Addition of nucleotides to the diet maintained the amount of RNA in the liver. Since most liver RNA (85%) consists of ribosomal RNA (rRNA), the machinery of protein synthesis, this result must be significant. Furthermore, there was loss of a significant proportion of the intracellular liver purine nucleotide pool (minimum at 14 d, recovery by 21 d) although this did not affect the intracellular energy charge, i.e.

$$\frac{\mathrm{ATP}+\frac{\mathrm{ADP}}{2}}{\mathrm{ATP}+\mathrm{ADP}+\mathrm{AMP}}.$$

The present study by López-Navarro and colleagues (Lopez-Navarro *et al.* 1996) examined another aspect of nucleotide deprivation. Histological analysis showed loss of nucleolar volume, suggesting reduced ribosome synthesis. Rough endoplasmic reticulum was disrupted and between 25%–45% of the ribosomes (depending on polysome size) present in each microscopic field of liver taken from control animals was missing from similar sections from nucleotide-deprived rats. Furthermore, deposition of fat droplets within the liver was greatly increased. The transient nature of these changes should not allow their fundamental significance to be obscured. It is clear that nucleotide deprivation leads to temporary loss of half of the hepatic protein synthetic machinery in the rat, although it is not known if there are compensatory changes in the efficiency of ribosome use. The question may not, however, be entirely academic to human nutrition. It is easy to imagine a formerly healthy, dietary nucleotide-adapted (ie. salvage-adapted) citizen being admitted to the local Intensive Care Unit after a road traffic accident. All diets used in nutrition support of patients (bar one) are deficient in nucleotides. If the animal studies of López-Navarro and colleagues can be extended to our imaginary situation, the nucleotide-free period during which the liver of the wounded citizen is attempting to mount an adequate inflammatory response is also a period of maximum adaptation. This takes the form of hepatic reorganization of purine and pyrimidine metabolism which is accompanied by loss of protein synthetic capacity in the gut and liver. If true, then addition of nucleotides to enteral and parenteral diets should be considered quite urgently. In two prospective, randomized, controlled clinical trials, those patients receiving a nucleotide-supplemented diet had significantly fewer complications and a shorter hospital stay after major upper intestinal surgery (Daly *et al.* 1992) or after admission to an Intensive Care Unit because of major trauma, surgical complications or sepsis (Bower *et al.* 1995). Alas, the effect of nucleotide supplementation alone could not be determined because they were added together with supplemental fish-oil and arginine.

I predict that 'nucleotides' will attract intense clinical nutrition research activity in the future. They are pro-inflammatory in most animal models and provide a useful counterpoint to the anti-inflammatory nutritional components such as fish-oils. My only plea is that some respect is given to nomenclature because dietary supplements are variously called 'nucleotides', 'nucleoside-nucleotide mixtures', 'RNA', 'polynucleotides' and 'purines and pyrimidines'. The metabolic fate of any exogenously administered component of the nucleotide/nucleoside/nucleobase axis depends on its entry level on the axis (Fig. 1), as was illustrated brilliantly by Henderson (Henderson *et al.* 1977).

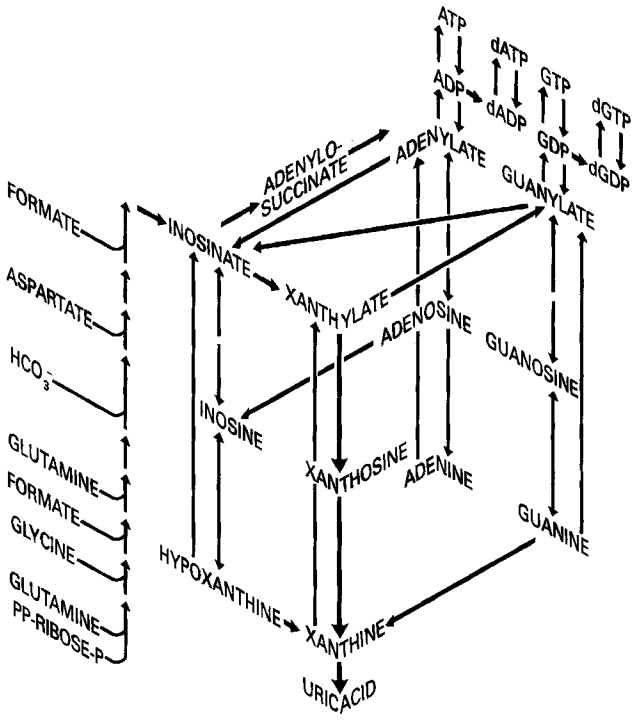

Fig. 1. Pathways of purine metabolism (from Zöllner, 1982; reproduced from Henderson *et al.* 1977, courtesy of the Ciba Foundation and Elsevier).

For parenteral nutrition, purified nucleotides may be more than adequate, whereas for enteral diets a crude RNA extract may suffice.

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REFERENCES

Adjei, A. A. & Yamamoto, S. (1995). A dietary nucleoside–nucleotide mixture inhibits endotoxin-induced bacterial translocation in mice fed protein-free diet. *Journal of Nutrition* **125**, 42–48.

Bower, R. H., Cerra, F. B., Bershadsky, B., Licari, J. J., Hoyt, D. B., Jensen, G. L., van Buren, C. T., Rothkopf, M. M., Daly, J. M. & Adelsberg, B. R. (1995). Early enteral administration of a formula (Impact®) supplemented with arginine, nucleotides, and fish-oil in intensive care unit patients: results of a multicenter, prospective, randomized, clinical trial. *Critical Care Medicine* **23**, 436–449.

Brunser, O., Espinoza, J., Araya, M., Cruchet, S. & Gil, A. (1994). Effect of dietary nucleotide supplementation on diarrhoeal disease in infants. *Acta Paediatrica* **83**, 188–191.

Bueno, J., Torres, M., Almendros, A., Carmona, R., Nuñez, M. C., Rios, A. & Gil, A. (1994). Effect of dietary nucleotides on small intestinal repair after diarrhoea. Histological and ultrastructural changes. *Gut* **35**, 926–933.

D'Mello, J. P. F. (1982). Utilization of dietary purines and pyrimidines by non-ruminant animals. *Proceedings of the Nutrition Society* **41**, 301–308.

Daly, J. M., Lieberman, M. D., Goldfine, J., Shou, J., Weintraub, F,, Rosato, E. F. & Lavin, P. (1992). Enteral nutrition with supplemental arginine, RNA and omega-3 fatty acids in patients after operation: immunologic, metabolic, and clinical outcome. *Surgery* **112**, 56–67.

Giesecke, D. & Tiemeyer, W. (1982). Availability and metabolism of purines of single-cell proteins in monogastric animals. *Proceedings of the Nutrition Society* **41**, 319–327.

Golden, M. H. N., Waterlow, J. C. & Picou, D. (1981). Metabolism in ^{15}N nucleic acids in children. In *Nitrogen Metabolism in Man*, pp. 269–273 [J. C. Waterlow and J. M. L. Stephen, editors]. London: Applied Science Publishers.

Goody, H. E. & Ellem, K. A. O. (1975). Nutritional effects on precursor uptake and compartmentalization of intracellular pools in relation to RNA synthesis. *Biochimica et Biophysica Acta* **383**, 30–39.

Grimble, G. K. (1993). Essential and conditionally-essential nutrients in clinical nutrition. *Nutrition Research Reviews* **6**, 97–119.

Grimble, G. K. (1994). Dietary nucleotides and gut mucosal defence. *Gut* **35** (Suppl.), S46–S51.

Henderson, J. F., Lowe, J. K. & Barankiewicz, J. (1977). In *Purine and Pyrimidine Metabolism. Ciba Foundation Symposium* 48. Amsterdam: Elsevier.

Leleiko, N. S., Martin, B. A., Walsh, M., Kazlow, P., Rabinowitz, S. & Sterling, K. (1987). Tissue-specific gene expression results from a purine- and pyrimidine-free diet and 6-mercaptopurine in the rat small intestine and colon. *Gastroenterology* **93**, 1014–1020.

Leleiko, N. S., Bronstein, A. D., Baliga, B. S. & Munro, H. N. (1983). De novo purine nucleotide synthesis in the rat small and large intestine: effect of dietary protein and purines. *Journal of Pediatric Gastroenterology and Nutrition* **2**, 313–319.

López-Navarro, A. T., Gil, A. & Sánchez-Pozo, A. (1995). Deprivation of dietary nucleotides results in a transient decrease in acid soluble nucleotides and RNA concentration in rat liver. *Journal of Nutrition* **125**, 2090–2095.

López-Navarro, A. T., Bueno, J. D., Gil, A. & Sánchez-Pozo, A. (1996). Morphological changes in hepatocytes of rats deprived of dietary nucleotides. *British Journal of Nutrition* **76**, 579–589.

Ogoshi, S., Iwasa, M., Tonegarua, T. & Tamiya, T. (1985). Effect of nucleotide and nucleoside mixture on rats given total parenteral nutrition after 70% hepatectomy. *Journal of Parenteral and Enteral Nutrition* **9**, 339–342.

Wiegers, U., Kramer, G., Klapproth, K. & Hilz, H. (1976). Separate pyrimidine-nucleotide pools for messenger-RNA and ribosomal-RNA synthesis in HeLa S3 cells. *European Journal of Biochemistry* **64**, 535–540.

Zollner, N. (1982). Purine and pyrimidine metabolism. *Proceedings of the Nutrition Society* **41**, 329–342.

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