ON THE EXISTENCE OF A GUANINE NUCLEOTIDE TRAP, THE ROLE OF ADENOSINE KINASE AND A POSSIBLE CAUSE OF EXCESSIVE PURINE PRODUCTION IN MAMMALIAN CELLS

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SUMMARY

It is usually assumed, in part from studies of bacteria, that there is free interconversion of adenosine 5'-monophosphate (AMP) and guanosine 5'-monophosphate (GMP), and that the balance between the 2 nucleotides is maintained exactly by allosteric controls on the activity of the enzymes involved in the interconversion. However, there are good reasons for believing that in most mammalian cells the conversion of GMP to AMP is not an effective process. Furthermore, the activity of certain other enzymes of the purine scavenger pathways will tend to induce imbalance between AMP and GMP. This imbalance will be compensated, but the compensation mechanism will involve increased production and excretion of purines. It is suggested that the operation of this mechanism in the human would result in gout.

In the course of experiments on hybrid mammalian cells for which the purine scavenger pathways have been used to develop selective systems (Szybalski, Szybalska & Ragni, 1962; Littlefield, 1964; Kusano, Long & Green, 1971), our attention has been directed to the as yet undefined roles of some of the enzymes of these pathways. The reactions are generally similar to those carried out by micro-organisms (Hoffmeyer & Neuhard, 1971) but differ in important respects (Murray, Elliott & Atkinson, 1970). A scheme of those known to occur in mammalian cells is shown in Fig. 1.

Inosine monophosphate (IMP) is the endogenous source of both AMP and GMP. The scavenger pathways are not symmetrical, in that a kinase exists for one of the nucleosides, adenosine (Ho, Luce & Frei, 1968; Schnebli, Hill & Bennett, 1967; Lindberg, Klenow & Hansen, 1967), while with rare exception none is found for inosine or guanosine (Friedman, Seegmiller & Subak-Sharpe, 1969; Meikle, Gotta & Touster, 1967). On the other hand there is a single phosphorylase which converts guanosine and inosine to free bases (Krenitsky, 1967) but none which acts on adenosine. Only one conversion exists at the level of nucleoside or free base – the deamination of adenosine to yield inosine. Consideration of these pathways leads us to suggest that mammalian cells may be susceptible to a disturbance of the balance between intracellular GMP and AMP, and that such a disturbance in humans could be a cause of gout.
Our hypothesis is as follows:

1. The pathway leading from IMP to GMP is not freely reversible in mammalian cells. In enteric bacteria, guanine can satisfy all purine requirements; this is possible owing to the presence of a reductase (Mager & Magasanik, 1960), which converts GMP to IMP. While a similar enzyme has been detected in erythrocytes (Hershko, Wind, Razin & Mager, 1963), its activity appears to be quite weak; these cells (Lowy, Williams & London, 1961) and reticulocytes (Cook & Vibert, 1966) convert very little labelled guanine to adenine nucleotides, even when the other enzymes required for this conversion are present. Furthermore, while it is well known that cultured mammalian cells can grow in the presence of aminopterin using hypoxanthine as a source of all purines, we have found that neither guanine nor guanosine is able to substitute for hypoxanthine in cultures of a number of different cell types. Most lines (WI-38, 3T6, and L5178y): are killed in GAT medium (guanine + aminopterin + thymidine) while a few, such as HeLa, are able to survive but can sustain very little growth. There therefore does not seem to be a very effective route from guanine or guanosine to AMP in any mammalian cells, though at least in some the pathway is not completely closed.

2. 5'-nucleotidase is continuously degrading AMP, IMP and GMP to the nucleosides. This enzyme has been purified from mouse cells (Murray & Friedrichs, 1969) and is very active in human fibroblast extracts (Fujimoto & Seegmiller, 1970). The inosine and guanosine produced can only be further degraded by nucleoside phosphorylase to the free bases, from which the nucleotides may be resynthesized by the action of the enzyme hypoxanthine-guanine phosphoribosyl transferase (Hpt). In human males bearing a mutation in this enzyme (Lesch-Nyhan Disease) there is an increased rate of endogenous purine synthesis, presumably to compensate for the loss of hypoxanthine and guanine, which are excreted as uric acid (for a complete account, see Seegmiller, 1969).
Excessive purine production in mammalian cells

Adenosine produced by 5′-nucleotidase has a different fate. It is not converted to adenine, as there is no phosphorylase capable of acting on it. It can undergo 2 possible reactions. One is rephosphorylation by adenosine kinase. The other is deamination to inosine; this will be followed by phosphorolysis to hypoxanthine and synthesis of IMP. In cultured fibroblasts, this is the only effective route of utilization of externally added adenosine, which can supply all the purines necessary for growth in the presence of aminopterin. The enzyme Hpt is required, since Lesch-Nyhan fibroblasts do not survive in adenosine + aminopterin. Evidently the deaminase has an overwhelming advantage over the kinase in acting on externally added adenosine, but this is probably not true for adenosine generated internally as the substrate affinity of the kinase is about 5-fold greater than that of the deaminase (Schnebli et al. 1967; Lindberg et al. 1967; LePage & Junga, 1965). Adenosine analogues are acted on by the kinase and transformed to toxic nucleotides (Ho et al. 1968; Schnebli et al. 1967; Lindberg et al. 1967).

(3) To the extent that the kinase does not recapture the adenosine produced by the nucleotidase, a cycle exists

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\text{adenosine} \rightarrow \text{inosine} \rightarrow \text{hypoxanthine} \rightarrow \text{IMP} \rightarrow \text{AMP} \rightarrow \text{adenosine (Balis, 1968)}.
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In order to prevent diversion of the IMP generated by this cycle to GMP, allosteric controls on the activity of the enzymes leading from IMP to GMP and from IMP to AMP would tend to reduce GMP synthesis and activate AMP synthesis. However, as these controls are not fully effective in inhibiting IMP dehydrogenase (Mager & Magasanik, 1960), some GMP will be made, and effectively trapped, as it is not easily returned to the other half of the pathway. It should therefore be the function of adenosine kinase to prevent this cycle from operating at too high a rate relative to endogenous purine synthesis, and diminution in the effectiveness of the enzyme should lead to acceleration of the adenosine cycle and to imbalance between AMP and GMP. An increase in the activity of 5′-nucleotidase or adenosine deaminase would have a similar effect. Apart from the adenosine cycle, an imbalance might also arise as a result of an excessive rate of deamination of AMP to IMP (Lowenstein & Tornheim, 1971). Though this reaction has been thought to be of doubtful importance in intact cells (Murray et al. 1970), we have found that it proceeds at a sufficient rate so that, in the presence of aminopterin, all purine requirements can be met by adenine alone in both normal and Lesch-Nyhan fibroblasts. Any further decrease in GMP reductase would work toward imbalance by increasing the effectiveness of the guanine nucleotide trap.

Control over the fate of endogenously generated IMP in the absence of perturbation by the salvage pathways would require only slight adjustments through the action of allosteric effectors, since the demand for AMP and GMP should be of similar magnitude; but a high rate of functioning of the adenosine cycle (or AMP deaminase) might make close control by this means impossible, especially in non-growing cells requiring no net synthesis of GMP. In the absence of an effective GMP reductase, the only way of restoring the balance between AMP and GMP would be by deamina-
tion of excess guanine to xanthine and its excretion as uric acid, together with an increased rate of endogeneous purine synthesis to make up the deficit in AMP. We suggest that the operation of this compensation mechanism in the human could result in gout. Study of the GMP/AMP ratio and of the activity of the enzymes which affect it should provide the necessary information.

It is worth noting that studies of purine excretion in normal humans (Sørensen, 1970) and in cases of xanthinuria (Seegmiller, 1969) have shown that most xanthine originates from guanine rather than from hypoxanthine, a point in accord with the hypothesis outlined here.

REFERENCES

Excessive purine production in mammalian cells


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