Purine and Pyrimidine Metabolism
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Chairman's opening remarks

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The programme of this symposium has been so constructed that emphasis is mainly on three aspects: the regulation of purine and pyrimidine biosynthesis; the relationship between immunology and the biochemistry of purine metabolism; and the interface between neurology and biochemistry of purine metabolism.

As we are a heterogeneous group I hope and expect that our discussions will chiefly cover the fertile areas between the identifiable disciplines or, to put it more figuratively, the marshy areas between the well-trodden pathways, whether they be metabolic or otherwise. The work that we shall discuss justifies the argument that clinical and fundamental research cannot be separated and that multidisciplinary research ultimately pays off. These ideas are not original, as can be seen from the following quotation from Harvey (1766): 'Nature is nowhere accustomed more openly to display her secrete mysteries than in cases where she shows traces of her workings apart from the beaten path nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of Nature by careful investigation of cases of rarer forms of disease. For it has been found, in almost all things, that what they contain of useful or applicable nature is hardly perceived unless they are deprived of them, or they become deranged in some way'.

The following quotation from Heberden (1816) is also appropriate at the start of this symposium: 'The gout affords a striking proof of the long experience and wary attention necessary to find out the nature of diseases and their remedies'. Although we shall probably hear little about gout as such, it can be justly claimed that the 'wary attention necessary to find out the nature' of that disease has stimulated research in the diverse areas which form the three main props of our programme.
Most aspects of these subjects were barely discernible as recently as three
years ago when the first international symposium on purine metabolism was
held in Israel under the chairmanship of Professor de Vries, whose foresight
led to this present symposium.

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Heberden, W. (1816) *Commentaries on the History and Cure of Diseases*, 4th edn., p. 39,
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Purine and pyrimidine metabolism: pathways, pitfalls and perturbations

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Abstract The conceptual framework which underlies many studies of purine and pyrimidine metabolism in intact cells has been critically evaluated. The model that is implicit in many such studies is the single, partially purified enzyme. This paper gives examples both of instances in which the extrapolation of results of enzyme studies to intact cells has been successful and of instances in which enzymes behave differently in the intact cell than in cell extracts. Pitfalls in the extrapolation of results of enzyme studies to intact cells concern (a) metabolic pathways, (b) intracellular enzyme activities, (c) enzyme regulation, and (d) intracellular metabolite concentrations. Examples are also given of situations in which perturbations in one aspect of purine or pyrimidine metabolism lead to changes in other aspects, often distant in the network of reactions.

It seems appropriate to begin this symposium on purine and pyrimidine metabolism with an examination of the conceptual framework which underlies much of the experimental work both in this field and on other aspects of intermediary metabolism as well. In order to do research in this or any other area, it is necessary to construct models and to make assumptions, generalizations and hypotheses. Often these are not stated or recognized explicitly but instead remain unspoken and implicit; however they are no less important or influential for remaining in the background. In this paper we shall try to make explicit some of the assumptions, generalizations and hypotheses which seem to be important and influential in current research on purine and pyrimidine metabolism, and to examine them critically. We shall attempt to assess the extent to which they are justified and the extent to which they are contradicted by experimental results.

The thinking and experimental approaches of most investigators who are studying intermediary metabolism today seem to be heavily influenced and perhaps even dominated by the results and the methods of enzymological
studies: studies with broken cells or partially or highly purified enzyme preparations. And yet the major concern of an increasing number of investigators is a different type of system, the metabolism of intact cells, tissues and animals.

Because enzyme studies, for the most part, chronologically preceded detailed studies of intermediary metabolism, because they are often more specific, precise and quantitative, and because they rest on a well developed theoretical base, there is a great tendency to extrapolate the results of test-tube studies to the operation of intact cells and tissues and to use the principles and methods of enzyme studies as bases for thinking about the metabolism of intact cells.

So long as extrapolations from enzymes to cells concern qualitative matters, for example, the identity of enzymes and metabolites that are present, they seem justified. However, it is quite another matter to extrapolate quantitative results from enzyme studies to the intact cell. After all, the conditions in which enzyme studies are done—low enzyme and total protein concentration, few or no alternative reactions of substrates and products, artificial ionic environments, use of specific metabolites at concentrations that usually are arbitrary, etc.—are not at all like those that exist in intact cells.

This is not say that enzyme studies are not important or that they are irrelevant to intracellular metabolism; far from it—they form an essential groundwork on which the study of intracellular metabolism rests. Nevertheless, the two types of systems are different, and the limitations inherent in enzyme studies should be recognized.

Although it seems unjustified to extrapolate results of many enzyme studies to the metabolism of intact cells, except as hypotheses, this is the trap into which, at one time or another, most investigators seem to fall. In addition to reasons already given, this results from the ways in which investigators are trained and in which textbooks and reviews are written, from the obvious success achieved by extrapolating qualitative results of enzyme studies to cells, and from the influence of enzymologists in the scientific community at large. In addition, most investigators have done or are doing enzymological studies themselves, and they naturally are motivated by a desire and expectation that their enzymological data be applicable to intact cells.

Here we shall examine several ways in which enzyme studies seem to influence thinking about the metabolism of intact cells and consider experimental results relating to what we shall call 'pitfalls' and 'perturbations'. The examples given will be selective, will pertain only to mammalian cells, and will stress purine metabolism simply because of our familiarity with this area.
Identification of individual enzymes and metabolites in cell extracts (and other types of studies) has led to the construction of metabolic maps of purine and pyrimidine metabolism. Fig. 1 shows the major pathways by which purine ribonucleotides and deoxyribonucleotides can be synthesized and catabolized in mammalian cells, and Fig. 2 depicts the corresponding pathways of pyrimidine metabolism.

Pitfalls concerning metabolic pathways

Although metabolic maps such as those shown in Figs. 1 and 2 are useful, they have limitations. Thus although they are correct, they are not complete; the pathways of synthesis and metabolism of the nucleotide coenzymes are omitted, for example. It must also be recognized that new pathways of purine and pyrimidine metabolism are still being discovered. In as much as metabolic
maps are generalizations, they may not be completely applicable to all experimental systems, and they often include reactions whose general occurrence or significance may be uncertain. Finally, metabolic maps depict processes that can be catalysed by more than one enzyme, and this is not always recognized. Space does not allow further discussion of these topics.

PITFALLS CONCERNING INTRACELLULAR ENZYME ACTIVITIES

Simply because particular enzyme activities can be identified in cell extracts, it is ordinarily expected not only that these activities be expressed in intact cells but also that intracellular activities be proportional if not equal to their activities in extracts. However, it cannot be emphasized too strongly that the rates of enzyme activities in intact cells are not necessarily equal to the total activities assayed in extracts. In addition, it must be pointed out that changes in enzyme activities as assayed in extracts do not necessarily mean that the metabolism of the cells has changed.

Differences between total enzyme activity in extracts and the activity that is
expressed in intact cells may be due to various factors, including metabolism of substrates by alternative catabolic or anabolic pathways, and low intracellular substrate concentrations. Other factors may also be important but are more difficult to evaluate: intracellular compartmentation of enzymes or substrates, presence of known or unidentified inhibitory or stimulatory metabolites and their possible compartmentation, sub-optimal ionic environments etc.

Numerous cases are known of the importance of alternative pathways of catabolism of substrates, both within the cells and in the incubation or growth medium. Thus guanine phosphoribosyltransferase (EC 2.4.2.8) can be demonstrated in extracts of mouse brain, with high concentrations of guanine and PP-ribose-P, but this reaction cannot be demonstrated in slices of mouse brain incubated \textit{in vitro} (Wong & Henderson 1972). Apparently guanine is so rapidly deaminated to xanthine by tissue guanine deaminase (EC 3.5.4.3) that almost none is left to be converted into nucleotide. Guanine deaminase is also present in some sera used in tissue culture media.

The same complication affects nucleotide synthesis from hypoxanthine in cells that have appreciable xanthine oxidase (EC 1.2.3.2) activity, nucleotide synthesis from adenosine in cells or tissue culture media that have appreciable adenosine deaminase (EC 3.5.4.4) activity, and nucleotide synthesis from thymidine in cells possessing thymidine phosphorylase (EC 2.4.2.4) activity.

Studies that attempt to compare rates of nucleotide formation from radioactive precursors are enormously complicated by this problem of metabolism of the precursor by alternative pathways. What may appear to be variation in one or another enzyme of nucleotide synthesis may instead be variation in the alternative pathway.

Anabolism of substrate by alternative pathways may also be a basis for differences between enzyme activities in intact cells and in cell extracts. Thus the deamination of adenylate may proceed in cells at only a fraction of its potential maximum rate when the energy metabolism of the cells is good. The phosphorylation of adenylate to ADP is the favoured route of metabolism in these conditions and proceeds at 70 times the rate of deamination (Snyder & Henderson 1973a). However, when energy metabolism is disrupted by addition of 2-deoxyglucose or 2,4-dinitrophenol, phosphorylation of adenylate is greatly reduced and its deamination to inosinate increases about 200-fold (Lomax et al. 1975).

Intracellular substrate concentrations may also limit intracellular enzyme activities. Table 1 shows that the actual rates of nucleotide formation with adenine phosphoribosyltransferase (EC 2.4.2.7) and hypoxanthine–guanine phosphoribosyltransferase (EC 2.4.2.8) in intact cells are only 22% of their total activities assayed in cell extracts (Henderson \textit{et al.} 1975a). That these
TABLE 1
Purine phosphoribosyltransferase activities in cells and extracts

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Phosphate concentration (mmol/l)</th>
<th>Ratio of activities in cells / in extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>5</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.24</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>25</td>
<td>0.22</td>
</tr>
<tr>
<td>Guanine</td>
<td>25</td>
<td>0.21</td>
</tr>
</tbody>
</table>

differences may be due at least in part to low concentrations or limited availability of PP-ribose-P is suggested not only by the low concentrations of this substrate that are usually found in cells but also by the fact that raising the extracellular concentration of phosphate stimulates both PP-ribose-P synthesis and nucleotide synthesis from purine bases (Henderson et al. 1975a).

Finally, it must be admitted that the activities of some enzymes are the same in extracts and in intact cells; this has, for example, been demonstrated in the case of deoxycytidine deaminase (EC 3.5.4.14) (Henderson et al. 1975b).

PITFALLS REGARDING ENZYME REGULATION

It is a common contemporary practice to extrapolate the results of studies of enzyme regulation with cell extracts or partially purified enzymes to the operation of intact cells or tissues. Thus on the basis of results of studies of the effects of metabolites on isolated enzymes, predictions and hypotheses are made about the operation of the same enzymes in intact cells. To make such hypotheses is entirely appropriate, of course, but unless and until appropriate experiments are done with intact cells they remain merely hypotheses; they should not be considered as proven with respect to intact cells. Experience has shown that some such hypotheses will be upheld when they are tested with intact cells, whereas others will be shown to be invalid.

Thus PP-ribose-P synthetase (EC 2.7.6.1) seems to be regulated by nucleotide concentrations in intact cells as well as in extracts. Fig. 3 shows that when the concentration of ATP (and similarly for GTP or both) is increased, PP-ribose-P synthesis in cells is inhibited (Bagnara et al. 1974a). In addition, we have recently shown that the rate of PP-ribose-P synthesis increases when ATP and GTP concentrations in cells are lowered. Another example of an enzyme whose regulation in intact cells has been predicted, at least in part, by test-tube studies,
is IMP dehydrogenase (EC 1.2.1.14); this enzyme is considerably inhibited in cells containing elevated GTP concentrations (Snyder & Henderson 1973b).

Demonstrating that enzymes in intact cells are regulated in the same general manner as predicted by enzyme studies does not necessarily prove that the mechanism of regulation is the same in both cases. This still requires experimental verification.

Among enzymes which do not appear to be regulated in intact cells in the same way as in extracts is amidophosphoribosyltransferase (EC 2.4.2.14). In intact cells its activity is not inhibited by raised concentrations of ATP or GTP or both together (Bagnara et al. 1974a), nor is its activity increased when the concentration of these nucleotides is lowered. Other enzymes for which no evidence of regulation by nucleotides was found are adenylate deaminase (EC 3.5.4.5) and adenylosuccinate synthetase (EC 6.3.4.4) (Snyder & Henderson 1973b; Lomax et al. 1975). Among enzymes of pyrimidine metabolism, we have recently found that uridine kinase (EC 2.7.1.48) was not inhibited in cells which contained elevated concentrations of UTP or CTP, nor was CTP synthetase (EC 6.3.4.3) inhibited when concentrations of GTP were lowered.

PITFALLS CONCERNING INTRACELLULAR METABOLITE CONCENTRATIONS

It is usually assumed that the relationship between enzyme activity and substrate concentration is the same in intact cells as in studies with purified
TABLE 2
Nucleotide concentrations in lymphoma L5178Y cells

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Concentration (nmol/10⁹ cells)</th>
<th>Nucleotide</th>
<th>Concentration (nmol/10⁹ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>3175</td>
<td>dCTP</td>
<td>53</td>
</tr>
<tr>
<td>UTP</td>
<td>1465</td>
<td>dTTP</td>
<td>36</td>
</tr>
<tr>
<td>UDP-glucose</td>
<td>1450</td>
<td>IMP</td>
<td>29</td>
</tr>
<tr>
<td>GTP</td>
<td>595</td>
<td>dATP</td>
<td>11</td>
</tr>
<tr>
<td>CTP</td>
<td>350</td>
<td>GMP</td>
<td>7</td>
</tr>
<tr>
<td>ADP</td>
<td>402</td>
<td>XMP</td>
<td>4</td>
</tr>
<tr>
<td>GDP</td>
<td>86</td>
<td>Succinyl-AMP</td>
<td>4</td>
</tr>
<tr>
<td>AMP</td>
<td>62</td>
<td>dGTP</td>
<td>4</td>
</tr>
</tbody>
</table>

enzymes. If this be the case, then it is important to know the actual intracellular concentrations of the purine and pyrimidine bases, nucleosides and nucleotides, and to relate them to the Michaelis constants of the enzymes for which they are substrates. It must be recognized that we do not know the intracellular concentrations of the purine and pyrimidine bases and nucleosides; presumably they are very low. Table 2 gives the concentrations of some of the ribonucleotides and deoxyribonucleotides; these span an 800-fold range (Snyder et al. 1973; L. W. Brox, personal communication).

The relationship between intracellular substrate concentrations and Michaelis

![Graph](image_url)

FIG. 4. Relationship of amidophosphoribosyltransferase activity in intact cells to intracellular concentrations of PP-ribose-P.
Fig. 5. Relationship of rate of nucleotide synthesis to adenine concentration.

constants can be seen in two examples involving PP-ribose-P. Fig. 4 shows the results of a study of the kinetic properties of amidophosphoribosyltransferase in intact cells (Bagnara et al. 1974b). The apparent Michaelis constant for PP-ribose-P, 2.5 mmol/l, is much higher than the concentrations of PP-ribose-P found in cells even in the most favourable conditions. This enzyme, therefore, normally operates at only a few percent of its potential maximum activity.

Mutants of hypoxanthine-guanine phosphoribosyltransferase are known in man in which the Michaelis constants are elevated 10- to 15-fold (Henderson et al. 1976); both clinical and biochemical evidence indicates that this elevation leads to marked reductions in the activity of this enzyme in intact cells at normal intracellular substrate concentrations.

However, there are other instances in which the relationship between substrate concentration and enzyme velocity is not the same as that predicted by enzyme theory. Thus, in intact cells, the rate at which a particular substrate is synthesized or supplied from outside the cell may be as important or even more important than its steady-state concentration in determining the rate of the reaction or reactions that use it.

Fig. 5 shows that the rate of use of PP-ribose-P for nucleotide synthesis in cells increases with increasing concentrations of exogenous adenine, even though the concentrations of PP-ribose-P are low at the higher concentrations of adenine (Henderson & Khoo 1965). The cell's increased need for PP-ribose-P in these conditions leads to an increase in the rate of synthesis of PP-ribose-P from glucose 6-phosphate (Bagnara et al. 1974a).
In addition, rates of deamination of adenylate and of dephosphorylation of inosinate increase markedly when cells are incubated with 2-deoxyglucose; however, the rates of these processes are not at all proportional to the concentrations of adenylate or inosinate (Lomax et al. 1975). It is the rate of synthesis of the substrate that determines the rate of substrate metabolism in these conditions.

It is also often assumed that the intracellular concentrations of purine and pyrimidine nucleotides are tightly regulated and that deviations from 'normal' concentrations are detrimental to cells. Certainly the second assumption is not true.

Treatment of cultured cells with certain concentrations of 6-methylthiopurine ribonucleoside, for example, does not lead to any inhibition of growth but does reduce the concentrations of purine ribonucleotides to about 40% of normal and trebles the concentrations of pyrimidine ribonucleotides (Warnick & Paterson 1973). Thus excessive pyrimidine ribonucleotides are not detrimental and there seems to be a considerable surplus of purine ribonucleotides. Furthermore we have recently shown that intracellular concentrations of guanine ribonucleotides can be doubled without any effect on growth rate.

INTRACELLULAR PERTURBATIONS AFFECTING PURINE AND PYRIMIDINE METABOLISM

In most enzymological investigations, one enzyme is studied at a time or, at most, a few are studied as a group. However, in intact cells the pathways of purine metabolism and of pyrimidine metabolism are not isolated entities but interact with each other and with those of carbohydrate, amino acid and energy metabolism. As a result of the justified contemporary interest in the regulation of metabolism, the interrelation of different aspects of intermediary metabolism sometimes is overlooked. Because of limitations of space, effects of perturbing intracellular carbohydrate, amino acid and energy metabolism on the metabolism of purines and pyrimidines cannot be illustrated here.

However, within the area of nucleotide metabolism itself, there is ample evidence that perturbations at various specific sites are reflected throughout the networks of purine and pyrimidine metabolism. Best known is the acceleration of purine biosyntheses de novo that is consequent on hypoxanthine–guanine phosphoribosyltransferase deficiency. Table 3 shows that inhibition of purine biosynthesis de novo by methotrexate leads to numerous changes in concentrations of purine and pyrimidine ribonucleotides and deoxyribonucleotides, and ultimately affects DNA synthesis, at least 13 steps away from the primary site of drug action (Hryniuk et al. 1975; L. W. Brox, personal communication).
TABLE 3

Effects of methotrexate and mycophenolic acid on purine and pyrimidine nucleotide concentrations

<table>
<thead>
<tr>
<th>Drug</th>
<th>ATP</th>
<th>GTP</th>
<th>UTP</th>
<th>CTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate</td>
<td>26</td>
<td>11</td>
<td>230</td>
<td></td>
</tr>
<tr>
<td>Mycophenolic acid</td>
<td>78</td>
<td>22</td>
<td>171</td>
<td>166</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug</th>
<th>dATP</th>
<th>dGTP</th>
<th>dTTP</th>
<th>dCTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate</td>
<td>56</td>
<td>31</td>
<td>32</td>
<td>37</td>
</tr>
<tr>
<td>Mycophenolic acid</td>
<td>110</td>
<td>38</td>
<td>250</td>
<td>74</td>
</tr>
</tbody>
</table>

Similarly, we have found that, when IMP dehydrogenase is inhibited by mycophenolic acid, not only do GTP concentrations decrease and UTP concentrations increase but dGTP and dCTP concentrations decrease whereas the concentration of dTTP increases.

CONCLUSIONS

It is our contention that the basic conceptual model that is implicit in many, if not most, contemporary studies of purine and pyrimidine metabolism in intact cells is the single, partially purified enzyme in the test tube. In a way this is not a bad starting place, and the extrapolation of purely qualitative information from test tube to cell can be made with considerable justification. In addition, there are not many other models to choose from. However, we contend that to attempt to extrapolate more quantitative results of enzyme studies to the operation of the intact cell cannot, or at least should not, be done without serious qualification. Thus one should ask, 'To what extent does this result of an enzyme study pertain to the metabolism of the intact cell?' or one should say, 'As a working hypothesis I will tentatively suppose that this result of an enzyme study holds in the intact cell, but this hypothesis still has to be tested experimentally'.

Simply to assume that a particular result of an enzyme study will inevitably apply to intact cells is to gamble; in some cases this will be true, but in other cases it will not be. This is no way to do research, and it certainly ignores an increasing body of experimental evidence that shows that in many cases enzymes do behave differently in intact cells and in cell extracts.

Principles for thinking first of enzymes in the context of intracellular metabolism and only secondly about them as isolated, partially purified entities still
remain to be developed, and we need to develop conceptual models that recognize that cells operate somewhat differently than their individual parts. At present these principles and concepts are not available; in their absence we should be cautious and should recognize the limitations both of our experimental systems and our implicit working assumptions.

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References

Discussion

Kelley: Those of us who work with both purified enzymes and intact cells realize that neither system is perfect. As you said, we do not know whether the conditions in which we study intact cells would exist in those cells were they not in the test tube; for example, conditions in the test tube might alter the intracellular concentrations of various substrates. In addition, the intracellular concentrations of substrates that we determine for intact cells may not be correct; for instance, we really do not know what the PP-ribose-P concentrations are.

A further point is that amidophosphoribosyltransferase probably has the property of hysteresis (E. W. Holmes, unpublished results); changes in enzyme activity and conformation need a finite period of time. This, too, might be missed in the study of intact cells. It is vital that we have both enzymological data and data on intact cells, for only then can we put the information together and come up with a reasonable notion about what is going on. It may be misleading to focus completely on what goes on in the intact cell, thereby disregarding the basic enzyme data that also exist.

Henderson: I agree; it is important in all these cases to recognize our assumptions and, if we are making hypotheses, to say so. Also, we must test our hypotheses, not just talk about them.

Nyhan: In working with human inborn errors I try to remind myself that, if we had done all our studies on the fibroblast, we would never have recognized the inborn error in phenylketonuria, for instance. I am still struggling to make fibroblasts do anything in the metabolism of glycine.

Adenine arabinoside, which was first synthesized as an anti-tumour agent, was until recently thought to be completely inactive. The problem is relevant to the activity of adenosine deaminase. The levels of activity in cell culture are considerably greater than they are in vivo, because of the enormous amount of adenosine deaminase in the fetal calf serum that we add. Connor et al. (1974) demonstrated good anti-viral activity in tests for cytotoxicity of adenosine arabinoside in cell cultures to which an inhibitor of the deaminase has been added. We and others are now using adenine arabinoside to treat diseases such as herpes infection in humans, in whom the deaminase is not so active in vivo as in cell culture.

Seegmiller: At this point in our knowledge, we should be able to use the physical constants derived from the study of pure enzyme systems in other situations to define the limits and perhaps the relative priorities to be expected for the use of certain substrates by various enzymes competing for a given substrate within a cell. The competition for PP-ribose-P is a good example of
this: adenine phosphoribosyltransferase has the highest affinity for PP-ribose-P; hypoxanthine–guanine phosphoribosyltransferase has the next highest and glutamine PP-ribose-P amidotransferase, the presumed rate-limiting enzyme, has the lowest activity. Together, these enzymes provide a nice system, which has been confirmed in cell cultures, for giving the re-use of purine bases by the phosphoribosyltransferases priority over the de novo synthetic pathway initiated by the PP-ribose-P glutamine amidotransferase. This results in a thrifty economy for use of ATP within the cell. Since six ATP molecules are consumed in the de novo synthetic pathway in producing one purine nucleotide but only one ATP molecule is needed to make a nucleotide from the free purine base, this built-in priority system saves substantial amounts of ATP within the cell.

Henderson: Regulation of phosphoribosyltransferase activity by PP-ribose-P can be demonstrated in some systems. However, we do not know the concentration in cells of the other substrates—adenine, hypoxanthine and guanine—nor do we know the rates at which they are synthesized in cells. To me, this is the greater area of ignorance about phosphoribosyltransferase function. We have tried to study this point in Ehrlich ascites tumour cells in vivo (Smith & Henderson 1976) and have found that the cells contain enough hypoxanthine (as it is formed sufficiently rapidly) to support reasonably active hypoxanthine phosphoribosyltransferase activity. However, there is little guanine phosphoribosyltransferase activity in the same conditions. These results may vary considerably from one biological system to another, however, and I should expect to find considerable guanine phosphoribosyltransferase activity in brain, for example. In considering the neurological aspects of the Lesch–Nyhan syndrome, I am struck by the inapplicability of the fibroblast for an understanding of the brain. We have begun to study purine metabolism in mouse brain; but that may not be a good model for the study of hypoxanthine–guanine phosphoribosyltransferase in the brain of primates. For example, the activities of adenine phosphoribosyltransferase and hypoxanthine–guanine phosphoribosyltransferase are almost equal in mouse brain, whereas the latter enzyme activity is much higher than the former in human brain (see Henderson & Paterson 1973).

Raivio: Mammalian metabolism has been studied with tissue slice preparations and organ perfusion systems and, for the evaluation of purine metabolism (especially that of nucleotides), adequate oxygenation and intact energy metabolism are essential. In our studies on the role of the liver in the body economy of purines, we found that slice preparations were unsatisfactory in many respects and perfusion methods were limited in their application. Isolated hepatocytes seemed to offer many advantages over the other approaches