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Plenary Lecture

Metabolism 2000: the emperor needs new clothes

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Metabolism is one of the corner stones of nutritional science. As biology enters the post-genomic era and with functional genomics beginning to take off, we anticipate that the study of metabolism will play an increasingly important role in helping to link advances made via the reductionist paradigm, that has been so successful in molecular and cellular biology, with those emerging from observational studies in animals and human subjects. A reconstructive metabolically-focused approach offers a timely paradigm for enhancing the elegance of nutritional science. Here we give particular attention to the use of tracers as phenotyping tools and discuss the application of our metaprobe concepts with respect to some novel features of metabolism, including 'underground metabolism', 'metabolic hijacking', 'catalytic promiscuity' and 'moonlighting proteins'. The opportunities for enhancing the study of metabolism by new and emerging technologies, and the importance of the interdisciplinary research enterprise are also touched upon. We conclude that: (1) the metaprobe concepts and approach, discussed herein, potentially yield a quantitative physiological (metabolic) phenotype against which to elaborate partial or focused genotypes; (2) physiological (metabolic) phenotypes which have a whole-body or kinetically-discernible inter-organ tissue-directed metabolic signature are an ideal target for this directed tracer-based definition of the 'functional' genotype; (3) metabolism, probed with tracer tool kits suitable for measuring rates of turnover, change and conversion, becomes in the current sociology of the 'Net', like AOL, Yahoo, Alta Vista, Lycos or Ask Jeeves, the portal for an exploration of the metabolic characteristics of the 'Genomics Internet'.

Metabolism: Metaprobe: Tracers: Physiological (metabolic) phenotypes

'"*But he hasn't got anything on" a little child said.' *The Emperor's New Clothes* (Hans Christian Anderson, 1805–1875)

Introduction: beyond reductionism and toward integration

In the present paper we will try to justify why we consider the study of metabolism, a foundation cornerstone of nutritional science, to be significant and exciting beyond its traditional boundaries. This is especially so, as we begin to contemplate the metabolic implications of the spectacular achievements in molecular and cell biology over the past decade, that have resulted recently in the complete sequences of human chromosomes 21 and 22 and culminating on 26 June 2000, the day before the Society's Summer Meeting began, with the announcements by President Clinton and Prime Minister Blair that the first rough, but still very incomplete, draft of the human genome has now been accomplished. Coupled with the earlier completion of the sequence of three other eukaryotic organisms, *Drosophila melanogaster*, *Caenorhabditis elegans* and *Saccharomyces cerevisiae*, the foundation for a more complete and rapid analysis of some of the basic processes involved in human nutrition or performance and diseases is now at hand. However, from the perspective of a nutritional scientist it may be an even more interesting fact, emerging from a comparison of the genomes of a fly, worm

Abbreviation: GSH, glutathione.

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and yeast, that the complexity of different organisms is not achieved by sheer number of genes (Rubin *et al*. 2000), but rather on new combinations of protein domains or novel interactions (Jasny, 2000). This thought might appropriately be extended to the importance of looking beyond genomics, including an emphasis on metabolism. After all, to paraphrase Vukmirovic & Tilghman (2000), 'organisms are networks of genes that make networks of proteins which function in metabolic pathways that nutrients regulate and so affect networks of signalling systems that regulate networks of genes and so on *ad infinitum*'. The genomic sequence tells us about potential, what may happen, and a great deal about the metabolic repertoire of the organism (Adams *et al*. 2000; Heidelberg *et al*. 2000). It does not tell us about capacity, precisely what gene products actually do, how cells work to maintain homeostasis in organs, what goes wrong in major nutritional disease, how we age and what contribution nutrients and nutritional state have on senescence and via what mechanism(s). This stage is where functional genomics will take the lead and, in this context, we submit that the study of metabolism will play increasingly important roles in this emerging new field of biology where metabolism will help to enhance knowledge about the functioning of organisms, in the post-genome era that we have just entered.

An additional comment, however, before turning to our emphasis on metabolism, about the reductionist paradigm. It is made in reference to a recent paper, The theory of everything (Laughlin & Pines, 2000), that we find instructive. Thus, according to these authors this theory, a term for the ultimate theory of the Universe, is a set of equations capable of describing all phenomena that have been observed or will be observed. A special case of this idea is represented by the following equations, written by and taken from Laughlin & Pines (2000), that describe, using conventional non-relativistic quantum mechanics, the everyday world of man, including rocks, fire, people and more:

$$i\hbar \frac{\partial}{\partial t} |\psi> = \mathcal{H} |\psi>, \tag{1}$$

where

$$\mathcal{H} = -\sum_{j}^{N_e} \frac{\hbar^2}{2m} \nabla_j^2 - \sum_{\alpha}^{N_i} \frac{\hbar^2}{2M_\alpha} \nabla_\alpha^2 -$$

$$\sum_{j}^{N_e} \sum_{\alpha}^{N_i} \frac{Z_\alpha e^2}{|\vec{r}_j - \vec{R}_\alpha|} + \sum_{j<<k}^{N_e} \frac{e^2}{|\vec{r}_j - \vec{r}_k|} + \sum_{\alpha<<\beta}^{N_j} \frac{Z_\alpha Z_\beta e^2}{|\vec{R}_\alpha - \vec{r}_\beta|}, \tag{2}$$

where equation 1 delimits the wave functional properties (Ψ) of an electron (i) as partial derivatives ($\partial/\partial t$) in terms of the Hamiltonian ($\mathcal{H}$) for a molecule with N atoms at varying energy levels and charges (e, i as subscripts), given in equation 2. Z_α and M_α are the atomic number and mass of the αth nucleus, R_α is the location of this nucleus, e and m are the electronic charge and r_j is the location of the j^{th} electron, β and k are the corresponding index variables for any second interacting nucleus, ∇^2 is a Laplacian operator from Schrödinger's classical three-dimensional wave equation and $\hbar$ is Planck's constant. For a detailed description of quantum chemistry nomenclature, interested readers should refer to: http://cmm.info.nih.gov/modeling/guide_documents/quantum_mechanics_document.html

We are told that this equation can be written down simply and completely by a small number of known quantities and that it is correct as proved by experiment. However, it cannot be solved accurately when the number of particles exceeds about ten, the reason being that it faces a catastrophe of dimension and no computer large enough will ever exist to solve it. This situation exists simply because the amount of computer memory required to represent the wave function of K particles is N^k. Hence, as pointed out by the authors (Laughlin & Pines, 2000), while reductionism has been fabulously successful and continues to be the cultural paradigm in physics (not to mention molecular biology), the correct Theory of Everything has revealed nothing about many things of importance to the understanding of the natural world, and we include here nutrition! Indeed, there is now an exciting shift in biology toward a more global perspective. In their essay, Journey to the center of biology, Lander & Weinberg (2000) offer the view that biology in the 21st century 'will focus increasingly on the study of entire biological systems, by attempting to understand how component parts collaborate to create a whole. For the first time in a century, reductionists have yielded ground to those trying to gain a holistic view of cells and tissues'. This view should be extended to the study of organs and their interrelationships, and how they determine the condition of the whole organism, leading to a further unravelling of the complexity of metabolism (for example, see Fig. 15-1 of Voet & Voet, 1995), that will contribute enormously to the understanding of the physiology of cells with particular reference to the intact organism. The study of metabolism, in our opinion, is now poised to enter yet another era of intense and exciting activity, with its prospect of helping to link the advances in molecular and cellular knowledge with information emerging from observational studies of animals, including human subjects, under different nutritional and environmental conditions. In so doing we will establish the basis for making more precise predictions of the way in which individuals and population groups respond to changes in homeostasis triggered by nutritional environments, therapy or any intervention which leaves a metabolic signature. A reconstructive metabolically-focused approach, therefore, offers a timely paradigm in nutritional biology and a renewed opportunity for enhancing the elegance of nutritional science. It also sets the stage for extending this form of heuristic thinking to other arenas of health science and predictive medicine.

Metabolism defined and its new opportunities

If the foregoing can be accepted for purposes of the present argument, we should now focus on metabolism, and to do this it is first necessary to say a word about what metabolism means to us, with emphasis on nutrition. Thus, nutritional metabolism might be defined as: 'the overall process through which living systems acquire and utilize (a) the free energy and substrates needed to carry out cellular functions,

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including molecular and macromolecular synthesis and turnover, movement, conduction, transport, replication and the conversion, interconversion and catabolism of metabolites and (b) the essential, conditionally-essential and dispensable nutrients needed or used to serve as building blocks (e.g. amino acids, fatty acids, Ca, P), as regulators or catalysts (Zn, leucine) and as cofactors (folic acid, riboflavin)'. As might be suggested from the illustration in Fig. 1, metabolism is an extraordinary elaborate and complex process, even the metabolism of a single amino acid such as glutamate (Young & Ajami, 2000). Metabolic studies may involve an organelle, cell, tissue, organ and/or whole-body level of enquiry and our emphasis for the present purpose will be on the last level.

Second, as Rennie (1999) indicated in his account of the use of tracers in nutrition and metabolism, the word metabolism comes from Greek roots at the dawn of medicine itself (μεταβολη, μεταβαλλειν; to turn about, change, alter). Even its use in the modern connotation is attributable jointly to the physician-philosophers Hippocrates and Heracleitus (Jones, 1931*a*,*b*), who first juxtaposed the root syllables of 'metabolism' with the concept of flux through the body. Thus, we will highlight the use of tracers (Young & Ajami, 1999*a*,*b*) that permit the study of changes, help explore metabolic routes and assist in the estimation of rates of specific metabolic reactions and/or of discreet pathways or processes. We will call upon our own experience in the study of human protein, amino acid and energy metabolism to make our case for the significance of metabolic study *in vivo*, beginning with two examples from investigations of the nutrition–aging axis.

Aging and nutrition: two examples of the importance of metabolism

First, Michikawa *et al*. (1999) identified the accumulation of a large number of point mutations in the mitochondrial DNA control region for replication, with up to 50 % of the mitochondrial DNA molecules in individuals above 65 years of age containing a T414G transversion. In a commentary to this paper, Pennisi (1999) suggests that such mutations could make mitochondria less efficient at generating ATP, and there is some evidence for this process showing altered kinetics of the mitochondrial proton leak, of ATP turnover reactions and in metabolic control in aging mice (Harper *et al*. 1998).

Second, using high-density oligonucleotide arrays (Lockhart & Winzeler, 2000), Lee *et al*. (1999) observed that aging resulted in a differential pattern of gene expression in mouse gastrocnemius muscles; of the 6347

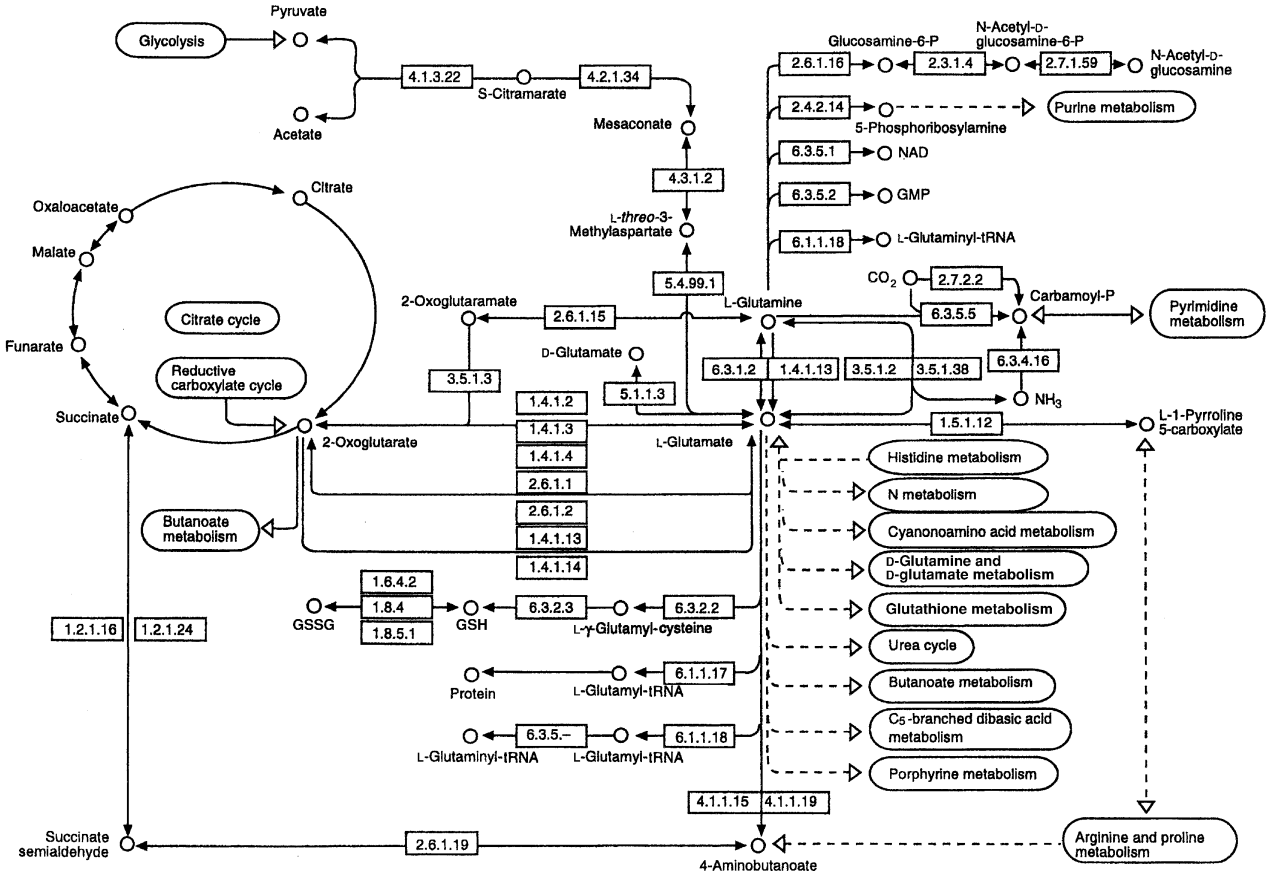

Fig. 1. A schematic map of L-glutamate metabolism in human subjects. Extracted from the GenomeNet Database Service, developed and operated jointly by the Institute for Clinical Research, Kyoto University and the Human Genome Center of the University of Tokyo (2000). The enzymes involved in the various reactions are represented by their *EC* nos. GMP, guanosine-5′-monophosphate; GSH, reduced glutathione; GSSH, oxidised glutathione; P, phosphate.

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genes surveyed fifty-five displayed a greater than twofold decrease in expression, and 13 % of these genes were involved in energy metabolism and seven other genes were related to protein synthesis and the ubiquitin-proteasome pathway of protein degradation. Most of these changes were completely or partially prevented by energy restriction, an intervention known to retard aging in mammals (Weindruch & Sohal, 1997). While this study emphasizes the powerful and versatile nature of high-density arrays, of oligonucleotides or of complementary DNA, as tools, it also underscores the desirability to integrate this methodology into metabolic studies. The array profile, although an important new technology for nutritional investigations (Harris, 2000), is only a static or current picture of gene expression. Actually, Lander (1999) predicted that in the near future researchers will be able to purchase standardized arrays containing the complete set of 100 000 human genes. While the number of human genes is still open to debate (Pennisi, 2000), ranging from a possible low of about 30 000 to a high of more than 150 000 (estimates are regularly posted; see ENSEMBL: The European Bioinformatics Institute, 2000), the profiling of gene expression patterns provides little information about the functional metabolic status of the cell or the individual from which the samples were derived. Furthermore, altered expression profiles do not necessarily infer a particular transcriptional mechanism since, for example, the 'silencing' of genes (Ogbourne & Antalis, 1998) is now known to be a widespread mechanism that occurs at the mRNA level, i.e. preventing gene expression by degrading the mRNA (Marx, 2000). Microarrays cannot identify post-transcriptional changes in protein expression or activity, despite, for example, their ability to revolutionize the study of cancer and metastasis (Ridley, 2000).

However, with respect to the nutritional and metabolic significance of changes in mitochondrial DNA, as noted earlier, a previous review of the literature (Young, 1992) did not reveal that there are major changes in BMR, expressed per unit body-cell or lean body mass, with advancing old age. Furthermore, using the experimental design shown in Fig. 2, and in collaboration with Susan Roberts (Heyman *et al*. 1992; Roberts *et al*. 1990, 1996*a*,*b*), we noted essentially similar changes in body energy deposition and loss in response to short-term periods of over- or underfeeding respectively in healthy young and older men. Our studies did not reveal marked differences in the overall processing and transfer of useful energy from the major energy-yielding substrates when given via the diet between the young and aged subjects, and this observation can now include postprandial thermogenesis (Melanson *et al*. 1998). Nevertheless, what was of great interest to us was the fact that after returning the subjects to their free-choice diets the younger subjects adjusted their intakes more promptly in response to the earlier period of restriction (Fig. 3) or excess by increasing or decreasing their *ad libitum* intakes, in comparison with a delayed and blunted response in elderly subjects (Roberts *et al*. 1994). Clearly, various mechanisms might account for this age-dependent effect on food intake behaviour, including possibly the leptin–neurohormonal–peripheral metabolic network (Lowell & Spiegelman, 2000; Schwartz *et al*. 2000), NO-dependent mechanisms (Morley, 1997), insulin–cholecystokinin–glucose interactions (Roberts, 2000), and many other possible mechanisms, as well as behavioural and social factors. Certainly, this post-dietary restriction or excess response could not have been predicted from gene expression profiles taken before and following the intervention, at least not at this stage in our knowledge of the full meaning of microarray data.

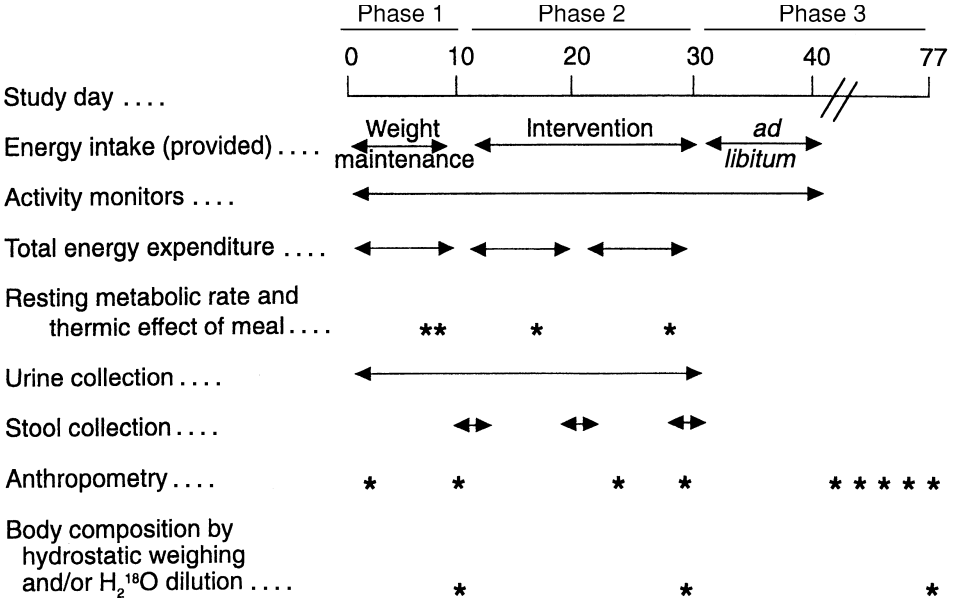

Fig. 2. Design of the experiments by Roberts *et al.* (1990, 1996*a*,*b*) and Heyman *et al.* (1992) to explore the changes in energy balance and utilization when young and elderly men were either underfed –3·3 MJ (about 800 kcal)/d or overfed + 4·2 kJ (about 1000 kcal)/d. *Days on which measurements were made.

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The twofold or greater changes in the expression of genes related to protein synthesis and turnover in mouse gastrocnemius muscle (Lee *et al*. 1999) are qualitatively in line with the age-related decline in the synthesis rates of mixed muscle proteins (Yarasheski *et al*. 1993), myofibrillar protein (Welle *et al*. 1993) and myosin heavy chain (Balagopal *et al*. 1997), as well as in mitochondrial protein synthesis (Rooyackers *et al*. 1996). Some differences in fractional rates of protein synthesis in proteins from skeletal muscle in adults of different age are summarized in Table 1, for illustrative purposes. However, muscle mass can be increased in older subjects with strength training exercise (Evans, 1998), and muscle protein synthesis in elderly subjects responds well to intravenous and oral amino acids (Volpi *et al*. 1998, 1999). Further, sarcoplasmic protein synthesis does not appear to be diminished in aged muscles (Balagopal *et al*. 1997). Again, the point we are making here is that coupling expression profiling, using microarray techniques, with studies designed to explore the metabolic implications of altered expression patterns would seem to us to offer a synergistic opportunity to mine, more completely, the nutritional significance of data obtained with this relatively new tool of microarrays. A possible example to point to might be the integration of measurements of mitochondrial substrate oxidation and protein synthesis in muscle, using stable-isotope tracers (Rennie, 1999; Young & Ajami, 1999*a*) and with ^{13}C and ^{31}P NMR (Jucker *et al*. 2000) in awake rodents together with microarray analysis, such as that used by Lee *et al*. (1999) to query the content of the genome in the aging mouse. This combined metabolic–tracer–genomic type of approach should prove useful in testing our hypothesis that changes in the activity of the Na^+-K^+-ATPase pump and in proton leakage across the mitochondrial membrane might account for a significant proportion of the rise in basal energy expenditure in patients suffering from major thermal injury (Yu *et al*. 1999).

With this mention of tracers, it is appropriate now to turn to their utility as phenotyping tools, or as a contrasting opportunity to what has been obtained by gene-expression techniques.

Tracers as phenotyping tools

Administration and delivery

We will consider tracers as phenotyping tools largely in the context of our own experience in human amino acid metabolism. Also, we will attempt to exploit a number of interesting characteristics of metabolism, that include ‘underground metabolism’, ‘metabolic hijacking’, ‘catalytic promiscuity’ and ‘moonlighting proteins’, all to be defined as our discussion continues. Two general points about tracer

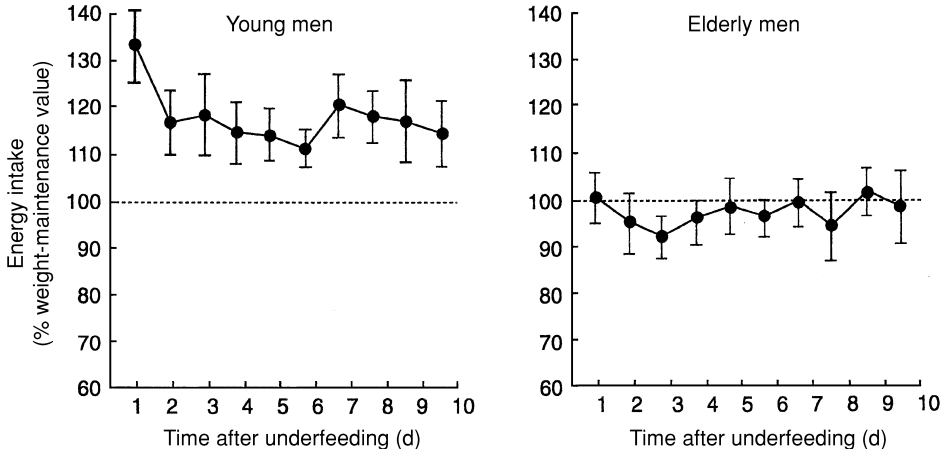

Fig. 3. Voluntary energy intake during a 10 d period (phase 3) following underfeeding (phase 2) in young and elderly men. For details of experimental design, see Fig. 2. (….), Weight-maintenance intake (phase 1 of the experiment, i.e. the period before the underfeeding intervention period). Values are means and standard deviations represented by vertical bars. (From Roberts *et al*. 1994.)

Table 1. Muscle (*vastus lateralis*) protein synthesis in relation to age in healthy adults (from Rooyockers *et al*. 1996) (Mean values with their standard errors)

	Young		Middle-aged		Old	
	Mean	SE	Mean	SE	Mean	SE
Fractional protein synthesis (%/h)						
Mixed proteins	0·043	0·002	0·038	0·003	0·038	0·003
Mitochondrial	0·081	0·004	0·046	0·005	0·051	0·004
Myosin Heavy-Chain*	0·036	0·0009	0·0247	0·034	0·0215	0·0018
Sarcoplasmic*	0·0426	0·006	0·0370	0·0068	0·049	0·0048
Cytochrome c oxidase† (μmol/min per g protein)	146	18	106	10	101	12

* From Balagopal *et al*. (1997).
† Data for men.

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use, however, should be made here. First, although there has been a worthy research effort to develop comprehensive models of metabolism *in vivo*, such as those of leucine compartmental disposal (Cobelli *et al*. 1991; France *et al*. 1999), it is our view that more parsimonious designs can be used to probe the metabolic significance of genetic data and so for phenotyping purposes. For example, a relatively-simple primed constant-tracer-infusion paradigm, using labelled urea and glutamine, has been applied recently by Lee *et al*. (2000) to distinguish between and correlate with phenotypic severity in disorders of the urea cycle.

We would tend to even further reduce the approach, and in this context we find the theory and abridged computations as outlined by Katz (1989) and applied, for example, by Said *et al*. (1989), to be attractive. This point we have presented elsewhere (Young *et al*. 2000), and we have also indicated why potentially more information can be gained about the *in vivo* system under study when using tracer protocols involving a bolus administration of a labelled amino acid rather than a constant-tracer-infusion paradigm. Thus, by numerical analysis of the decay of the enrichment of the administered tracer in plasma, for example, it is possible to estimate the replacement rate (appearance, which equals utilization at steady-state) of the compound, the area under the curve and mean residence (transit) time in the system (Wagner, 1976; McNamara *et al*. 1987; Katz, 1989; Cheng *et al*. 1991); the constant-infusion plateau enrichment can also be predicted. This simplified bolus input technology contrasts favourably with a widely-used constant-tracer-infusion approach (Waterlow, 1995), and we have presented (Young *et al*. 2000) an illustration of this bolus-type approach in studies on the rates of leucine oxidation at differing intakes of leucine, which are of interest to us in assessing leucine requirements (El-Khoury *et al*. 1994*a*,*b*). Thus, for example, healthy adults were given an L-amino acid diet supplying either 14 or 38 mg leucine kg/d for 5 d before receiving a bolus dose of L-[1-^{13}C]leucine (16·8 μmol/kg) during the post-absorptive state, with measurement of the levels of ^{13}C in expired CO_2 and plasma α-ketoisocaproic acid during the following 6 h (Young *et al*. 2000). The estimate of leucine oxidation was derived as described earlier (Young *et al*. 2000), and the results for the estimated leucine oxidation rates based on the analysis of the initial 3 h and also the entire 6 h data are given in Table 2, for the subjects receiving the 38 mg leucine intake level. Here we compare the bolus-derived values with the 12 h post-absorptive rate published previously from our 24 h constant-tracer-infusion experiments (El-Khoury *et al*. 1994*b*). As can be seen, the agreement between the estimated rates of leucine oxidation from the bolus and constant infusion approaches is excellent. Clearly, there are potential advantages to be gained from carrying out tracer studies using the bolus administration approach followed by appropriate kinetic analysis of the isotopic decay curves.

Table 2. Comparison of estimates of the rate of leucine (Leu) oxidation (Ox) based on an intravenous bolus or constant tracer infusion in five healthy adults given a diet supplying 38 mg Leu/kg per d

	Bolus		Constant infusion
Tracer dose	about 16·5 μmol/kg		about 2·8 μmol/kg per h (infusion)
Leu Ox: Derived from	3 h decay*	6 h decay*	12 h infusion‡
Rate (μmol/kg per h)			
Mean	24·6	22·3	20·9
SD	3·3	4·8	2·3

* For details of analysis, see Young *et al.* (2000).
‡ From El-Khoury *et al.* (1994*b*).

Second, in further reference to tracers as phenotyping tools, attention needs to be given to the delivery and design of tracer probes that are intended to provide quantitative dynamic information about discrete portions or steps in biochemical pathways or about the status of cellular processes. The latter might include the monitoring of the activity of proteins involved in leucocyte activation, apoptosis which involves the caspase family of cysteine proteases, the activity of the cell surface membrane-associated peptidases and cellular endopeptidases that play roles in signal transduction, immune regulation of processing of antigens and neuropeptide mediators, just to name a few. We will pursue this potential in some more detail later (p. 38).

Metaprobes are dynamic biomarkers

Here, we should re-introduce our concept of a metaprobe, which we have discussed more fully elsewhere (Young & Ajami, 1999*a*). In this context, an isotopically-labelled precursor probe is administered to a subject, with a labelled product being formed by the action of the enzyme or cellular process of interest. Rates of appearance, concentration and related kinetic variables of the product are then measured in relation to the diminishing precursor. The data and analyses can then be correlated to the genotype of interest, or metabolic or disease conditions under study. The method involves administering a defined amount of labelled metaprobe (input) at a site that provides access to a desired pool of the enzyme or process, followed by measuring the labelled conversion product (output). The presence and amount of the specific labelled product, e.g. the $^{13}CO_2$ in breath, definitively indicates that the labelled metaprobe has been metabolized by the specific enzyme in the biochemical pathway or via the step in the cellular process under consideration. From the calculated rate of substrate–product conversion by the enzyme or step (i.e. input *v*. output as a function of time), an assessment is made of the status of the pathway or cellular process. Thus, a metaprobe, in principle, allows for a real-time quantitative phenotyping of the system via a relatively non-invasive but dynamic procedure.

The usefulness of this approach depends on a careful determination of the appropriate metaprobe substrate for the selected biochemical step or process, possibly requiring a structural modification of the natural substrate of the enzyme into a surrogate substrate, whose metabolism can be measured readily *in vivo*, especially in the presence of multiple confounding biochemical and physiological processes (Young & Ajami, 1999*a*). Other desirable characteristics of such phenotyping tools or 'dynamic biomarkers' have also been discussed, including the fact that the enzymic conversion product is (1) accessible by non-invasive or minimally invasive means, permitting ready

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isolation from the biological system containing it and (2) it is amenable to rapid quantitative analysis for its isotopic content. Ideally, the metaprobe should permit clear and rapid differentiation between precursor and product so that calculations of rate of precursor conversion into product, and other rate-dependent variables, are unambiguous.

Some novel applications of the metaprobe concept

'Underground metabolism'

Now we will explore use of the metaprobe concept in relation to a number of interesting characteristics of metabolism that we mentioned earlier, beginning with what D'Ari & Casadesus (1998) have termed 'underground metabolism'. This term is meant to describe reactions catalysed by normal enzymes acting on substrate analogues, which are themselves 'endogenous' metabolites. During normal states, underground reactions are rare and the resulting products used or removed. However, under certain conditions 'underground' reactions might have a negative effect on the organism, or it may provide for alternative syntheses or structures.

For example, we have been interested in the consequences of major burn injury on the regulation of arginine metabolism. Thus, our various studies (Yu *et al*. 1995*a*,*b*, 1996, 2000) have revealed enhanced rates of arginine catabolism (oxidation) without compensatory increases in net arginine synthesis during the day. This finding indicates, therefore, the need for a sufficient exogenous supply of pre-formed arginine to adequately nourish critically-ill patients. In addition, these findings also raise the question about the availability of arginine for NO production, especially over the time of the day when feeding might be curtailed, since it is now recognized that arginase, which catalytically hydrolyses L-arginine to urea and ornithine, plays an important role in determining the rate of NO synthesis (Chang *et al*. 1998; Boucher *et al*. 1999).

In view of the pathophysiological importance of NO (Rubanyi 1999*a*,*b*), with its cytoprotective and cytotoxic actions (Liaudet *et al*. 2000), we conducted multi-tracer studies with arginine and citrulline to estimate the rate of whole-body NO synthesis by monitoring the conversion of the [^{15}N, ^{15}N]guanidino moiety of arginine to [^{15}N]ureido-moiety of citrulline. An additional measure of NO production in these studies was based on the appearance of ^{15}N in urinary nitrite–nitrate (Castillo *et al*. 1996). There was good agreement between these two approaches for estimating the rates of NO synthesis. We have not yet applied this approach in studies with burn patients, but we have used it in recent studies of NO production in patients with end-stage renal disease (Lau *et al*. 2000). As summarized in Table 3, we found markedly higher rates of NO production in patients with end-stage renal disease, in comparison with rates measured earlier in healthy adults (Castillo *et al*. 1996). Interestingly, although the kidney is an important site of arginine synthesis, we found that the net rate of arginine synthesis was not apparently compromised despite the reduced renal function. This factor seemed to be associated with a substantially higher concentration of citrulline and accelerated rate of removal in plasma in these patients with end-stage renal disease. This finding also illustrates the importance of understanding the metabolic interrelationships among organs, which can hardly be achieved using a reductionist approach.

Table 3. Arginine, citrulline and nitric oxide metabolism (μmol/kg per h) in patients with end-stage renal disease (ESRD; post-absorptive; summarised from Lau *et al*. 2000)
(Mean values and standard deviations)

	ESRD		Healthy controls	
	Mean	SD	Mean	SD
Arginine flux	98	21	55	10
Citrulline flux	87	10	18	6
NO synthesis	9·4	5·9	1·0	0·2
Net *de novo* arginine synthesis	11·7	0·8	6·3	1·4

The point we wish to make, however, is that the tracer protocol used in these studies is relatively long and complex. Hence, we propose that an 'NO synthesis' metaprobe might offer a more 'patient friendly' and clinically useful means of assessing the status of NO production *in vivo*. For this purpose, use of labelled homoarginine as a metaprobe is proposed (Fig. 4); this amino acid is present in detectable levels in fasting serum drawn from a number of mammalian species (Marescau *et al*. 1992), including human subjects (Marescau *et al*. 1995), and it appears to be produced as a minor metabolic consequence of hyperammonaemia (Kato *et al*. 1988, 1989; Kato & Sano, 1993). In addition, L-homoarginine is oxidized by both purified neuronal and macrophage NO synthase (I and II), with major formation of NO and homo-L-citrulline in a molar ratio of approximately 1 (Moali *et al*. 1998). Thus, it would seem to be highly profitable to study, in human subjects, the pharmacokinetics of homoarginine and its conversion to homocitrulline, as a surrogate index of the *in vivo* rate of NO production. Since (a) the homoarginine and homocitrulline pools are normally very small (in patients with the hyperornithinaemia–hyperammonia–homocitrullinuria syndrome urinary homocitrulline is greatly elevated in comparison with normal subjects) and (b) the homologues are not used in protein synthesis and are not diluted by the release of amino acids from protein breakdown, it can be expected that relatively short tracer protocols using labelled homoarginine (with or without a labelled infusion of homocitrulline) would be suitable for measurement of NO production. Furthermore, use of the L-homoarginine tracer potentially dispenses with the need to measure the citrulline flux, which is a necessary determination when using the L-[guanidino-^{15}N, ^{15}N]arginine–[ureido-^{15}N]citrulline tracer pair (Castillo *et al*. 1996). We are now planning to examine this abridged tracer paradigm that probes the underground world of biochemistry.

Parenthetically, we note that supplemental arginine has been investigated for its possible beneficial effects in individuals with endothelial dysfunction, but with varied results (Loscalzo, 2000). It is our opinion that a much better understanding of the pharmacokinetics of arginine

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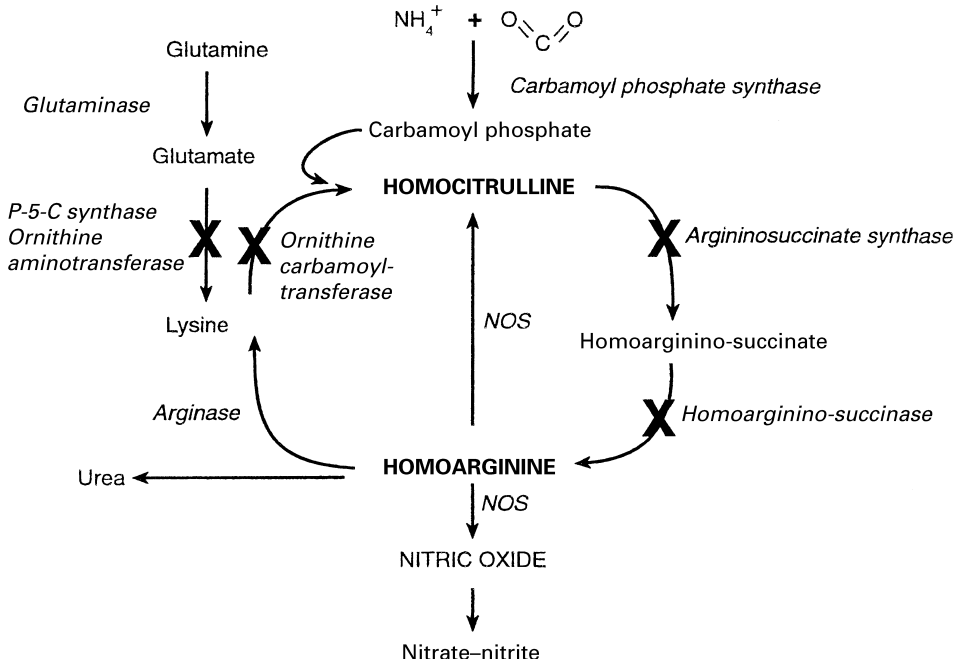

Fig. 4. A schematic outline of the metabolic relationships between the metaprobe, homoarginine and homocitrulline and nitric oxide production. Nitrate–nitrite is the stable endproduct of nitric oxide and its output in urine is used as a measure of whole-body nitric oxide production. NOS, nitric oxide synthase; P-5-C synthase, Δ′-pyrroline-5-carboxylate synthetase; X, The reaction is limited except, possibly, for rare pathophysiological states, as in the case of 'underground metabolism', discussed on p. 33.

metabolism under different clinical and therapeutic situations would help to address this problem. Again, use of suitable metaprobes of arginine with bolus administration would seem to offer a valuable tool for this purpose.

'Metabolic hijacking'

The second novel application in the context of tracers (metaprobes) as phenotyping tools exploits what D'Ari & Casadesus (1998) would regard as 'metabolic hijacking'. This characterization involves the idea that foreign analogues are first introduced by the investigator, and they are then acted upon by normal enzymes and used in cell metabolism; the study of homocysteine metabolism offers not only a timely example for our purposes, but it is linked to our interest in NO production. Thus, tetrahydrobiopterin is a critical cofactor for the endothelial-specific isoform of NO synthase (Ortiz de Montellano *et al*. 1998), and its availability is limited in atherothrombotic disease (Stroes *et al*. 1997). The reductive synthesis of tetrahydrobiopterin from dihydrobiopterin is achieved via the folate-dependent methylenetetrahydrofolate reductase (Matthews & Kaufman, 1980; Kaufman, 1991) which, as discussed later (p. 35), is intimately involved in the methylation of homocysteine. Of further interest, then, is the occurrence of endothelial dysfunction in hypercholesterolaemia which can be restored by giving patients 5-methyltetrahydrofolate (Verhaar *et al*. 1998) or folic acid (Verhaar *et al*. 1999). This restoration of function is presumably achieved via an effect on NO production (Chow *et al*. 1999; Ikeda *et al*. 1999; Boger *et al*. 2000) and/or possibly by a reduction in homocysteine-induced oxidative stress (Chambers *et al*. 1999; Kanani *et al*. 1999; Brattström & Wilcken, 2000).

Also, of course, the possible causal involvement of homocysteine in the pathogenesis of cardiovascular disease (Weir & Scott, 1998), including arteriosclerosis and thrombosis, is an active area of nutritional interest and investigation (Finkelstein, 2000; Finkelstein & Martin, 2000; Seshardi & Robinson, 2000), and the molecular genetics of hyperhomocysteinaemia have been reviewed (Fodinger *et al*. 1999, 2000). Although a number of mechanisms have been proposed (Welch & Lascalzo, 1998; Krumdieck & Prince, 2000) to account for the possible causal nature of the increased risk of CHD with a mild rise in plasma total homocysteine levels that exceed a proposed cut-off of about 15 μM, there is not firm agreement as to the underlying reasons for and consequences of high homocysteine in patients at risk of vascular disease (Dudman, 1999; Brattström & Wilcken, 2000; Scott, 2000; Ubbink & Delport, 2000; Ueland *et al*. 2000). Indeed, much remains to be learned about the regulation of homocysteine metabolism. Thus, from tracer studies with methionine, Fukagawa *et al*. (2000*b*) suggest there are differences in homocysteine remethylation between men and women, possibly accounting for differences in plasma homocysteine concentrations and, presumably, the higher risk of vascular disease in men. Clearly, this problem is ripe for a more intense application of the metaprobe concept and approach.

With further reference to homocysteine and our interests in amino acid metabolism in major trauma and other catabolic states, it is pertinent that Schindler *et al*. (2000) reported recently that there was a much higher frequency of

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hyperhomocystinaemia (>15 μM) in critically-ill patients than in control subjects. However, the determinants of the hyperhomocysteinaemia appeared to be folate and vitamin B_{12} status rather than the 5,10-methylenetetrahydrofolate reductase genotype. Furthermore, these investigators asked whether the metabolism of homocysteine is impaired in critical illness, but this question cannot be answered directly from measurements of plasma levels alone. In any case, it is not evident that the hyperhomocysteinaemia of critical illness is due to a remethylation defect (i.e. a reduced 5,10-methylenetetrahydrofolate reductase activity and/or its genotype); in this situation a single nucleotide polymorphism would not necessarily afford any useful phenotypic information, because of the complexity of factors regulating and affecting homocysteine metabolism, involving transporters, enzymes, biochemical sub-pathways cofactors etc.

In the genomic approach, clusters of genetic material are expressed and analysed. On the other hand, a tracer (metaprobe) approach provides an integrative summary of the physiological capacity of the gene cluster expression. Thus, studies of homocysteine kinetics and metabolism using suitable metaprobes, and exploiting the concept of 'metabolic hijacking', would be highly desirable. This tracer approach would be accomplished by assessing the *in vivo* activity of enzymes and steps associated with the formation and metabolic removal of homocysteine, with metaprobes used as the functional biomarkers of polymorphic capacity.

Furthermore, in our view, studies of S amino acid metabolism more generally, including the status and regulation of methionine, cyst(e)ine and glutathione (GSH) homeostasis, in addition to homocysteine, warrant increased emphasis. Dröge & Holm (1997) postulate that cystine is a physiological regulator of N balance and body cell mass. Also, Dröge (1999) suggests that the redox couple cysteine–cystine may serve as an indicator of plasma redox status, and recently the latter measure has been shown to be related to disease severity in critically-ill patients (Alonso de Vega *et al*. 2000). In addition, there is now a great deal of interest in the role of the redox state in modulating signal transduction, gene expression and apoptosis (Powis *et al* 1995; Arrigo, 1999; Voehringer, 1999). This interest has stimulated research into the biological regulation of GSH synthesis and turnover (Lu, 1999), because the major determinant of intracellular redox is GSH. Thus, Griffith (1999) and Griffith & Mulcahy (1999) have reviewed aspects of GSH biology and they have illustrated how the analysis of the γ-glutamylcysteine synthetase gene sequences provides insight into how oxidative stress increases γ-glutamylcysteine synthase (the key rate-limiting enzyme in GSH synthesis). Further, Sies (1999) has surveyed the role of GSH in multiple cellular functions. However, little emphasis is given in these excellent reviews to the quantitative aspects of GSH synthesis, organ output, uptake and turnover *in vivo*.

Hence, once again, we and others (Jahoor *et al* 1995, 1999; Malmezat *et al*. 2000; Reid *et al*. 2000) have begun to study *in vivo* quantitative aspects of GSH synthesis and status in animals and in human subjects under varying pathophysiological states. Recently, we (Lyons *et al*. 2000) have confirmed in healthy male adults, what has been known in rodents for some time, that the adequacy of the intake of S amino acids (methionine and cystine) determines the rate of whole-blood GSH synthesis (Table 4). Whole-blood concentrations remained unchanged in these studies, suggesting a lower rate of overall GSH turnover and disposal. Also, as summarized here, cysteine was spared under these restrictive conditions as cysteine oxidation was reduced significantly ($P<0{\cdot}001$) with ingestion of the S amino acid-free diet. In contrast, our other recent studies indicate that sepsis in children causes a reduced rate of whole-blood GSH synthesis (Fig. 5) together with a fall in GSH concentration (Lyons *et al*. 2001) suggesting, perhaps not surprisingly, that different physiological mechanisms are responsible for the altered status of GSH homeostasis in response to individual dietary factors *v*. the profoundly different metabolic and cellular characteristics of the reaction to a septic challenge. To date, no comparable pharmacogenomic studies have been able to stratify these kinds of intra-individual variations.

Synthesis of GSH occurs via the γ-glutamyl cycle (Fig. 6) in which an intermediate reaction is catalysed by oxoprolinase that converts L-5-oxoproline (OP) to glutamic acid (Anderson, 1998). This enzymic step can be probed not only with labelled L-5-oxoproline, as we have done recently (Metges *et al*. 1999, 2000), but with L-2-oxothiazolidine-4-carboxylic acid and with the homologous cyclic analogue of homocysteine. The rationale for this investigation brings us back into a focus on the concept of 'metabolic hijacking', which we shall illustrate again by applying our approach of metaprobe biogenomics to homocysteine metabolism.

Current paradigms for assessing homocysteine clearance generally fall into two classes: (a) genotypic assays aimed at predicting the individual's genetic propensity for homocysteinaemia by (1) identifying gene mutations or deletions, such as those governing the folate-dependent thermolabile isoforms of 5,10-methylenetetrahydro-folate reductase, particularly that associated with the common C677→T variant (Weir & Scott, 1998) and (2) assays of plasma homocysteine levels, either by conventional chromatography or by isotope-dilution MS. In this latter case, the result is a static plasma value, which is neither a measure of

Table 4. Glutathione synthesis† and cysteine kinetics in healthy adults (post-absorptive state) given a sulfur amino acid (SAA)-free diet (summarised from Lyons *et al.* 2000)
(Mean values and standard deviations)

	Adequate diet		SAA-free diet	
	Mean	SD	Mean	SD
Glutathione (whole blood)				
FSR (/d)	0·65	0·13	0·49	0·13**
ASR (μmol/l per d)	747	216	579	135**
Concentration (μM)	1142	243	1216	162
Cysteine kinetics (plasma; μmol/kg per h)				
Flux	38·3	6·4	30·9	5·5
Oxidation	5·62	0·6	1·58	0·16

FSR, fractional synthesis rate; ASR, absolute synthesis rate.
† Using L-[1-^{13}C]cysteine incorporation into whole-blood GSH.
Mean values were significantly different from those for adequate diet: **$P<0{\cdot}01$, ***$P<0{\cdot}001$.

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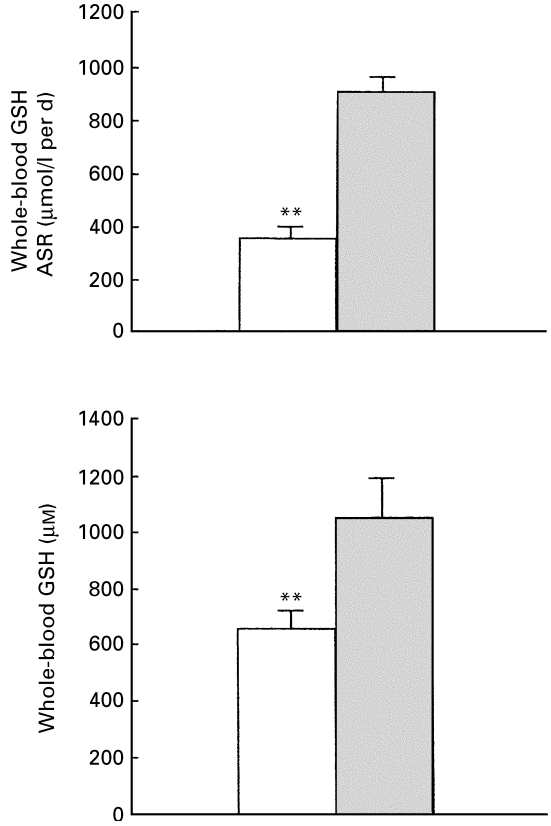

Fig. 5. Whole-blood glutathione (GSH) concentration (μM) and absolute synthesis rate (ASR; mmol/l whole blood per d) in children with sepsis (▭) and their age-matched controls (▩). Values are means with their standard errors represented by vertical bars. Mean values were significantly different from these of controls: $^{**}P < 0{\cdot}01$. (From Lyons *et al.* 2001.)

production or of clearance capacity, and presents with wide variations depending on the dietary history of the host; (b) the more-effective assay procedures are organized around a stimulatory event such as a methionine load, the theory being that a supraphysiological input of methionine given orally (100 mg/kg body weight) will approach or exceed maximal velocities of enzymes in the methionine–homocysteine–serine pathways. Hence, those individuals who cannot effectively clear homocysteine, relative to controls, will present with abnormally-high plasma concentrations (Selhub, 1999). We propose an alternative novel form of *in vivo* enzyme function titration that draws on the metaprobe and 'metabolic hijacking' concepts, giving a direct kinetic assessment of the capacity to clear homocysteine, both before and after a methionine load.

The approach we propose is to quantify the metabolic elimination of a suitable homocysteine metaprobe through the cystathionine β-synthase gateway (Fig. 7) or the trans-sulfuration pathway. This enzyme converts homocysteine and serine into cystathionine, which is subsequently converted, by cystathionase, to cysteine and α-ketobutyrate (or, if reversibly transaminated, as 2-aminobutyric acid which then bears the C skeleton of homocysteine). The K_m values of cystathionase and the enzymes responsible for 2-aminobutyric acid oxidation are two orders of magnitude higher than the values of cystathionine synthase and, therefore, cannot be saturated by products of trans-sulfuration.

The procedural steps are illustrated in Fig. 8, and some potential metaprobes of homocysteine are shown in Fig. 9. Thus, a labelled homocysteine metaprobe is given as a bolus administration (via an intravenous or oral route) and the appearance of the label in blood or expired air measured and the kinetics assessed, using for example the approach described by Fukagawa *et al.* (2000*a*). In their studies the cyclic analogue of cysteine, 2-oxo-4-thazolidinone-4-carboxylic acid was shown to be readily hydrolysed by the ubiquitous enzyme, oxoprolinase, a low-K_m enzymic gateway straddling GSH anabolism and catabolism. A similar metaprobe of homocysteine, i.e. the homologous 2-oxo-tetrahydro-1,3-thiazine-4-carboxylic acid (see Fig. 9), is also an oxoprolinase substrate (Fig. 8). Hence, it can serve as the delivery system for homocysteine that bypasses the complex thiol-mediated chemical reactivity of peripherally-administered homocysteine, or the potential toxicity inherent in the use of homocysteine thiolactone as the pro-drug.

We (Young & Ajami, 1999*a*) reported earlier the application of this metaprobe in a rabbit model of transient hyperhomocysteinaemia induced by methionine load, and have repeated the experiments in a rat model to test the impact of sepsis on the ability to clear homocysteine. The model is based on what is becoming a standard test platform for evaluating therapeutic interventions with breath test probes, including methionine, and as part of pharmaceutical development in Sprague-Dawley rats that are cannulated for intravenous tracer kinetics and intubated for breath collection, as described by Kohno *et al.* (1998) and Ishii *et al.* (1999). This model can also be extended to the study of pathophysiological conditions, either induced by surgery (Ishii *et al.* 1999) or by inoculation with agents such as live *Escherichia coli* (Breullie *et al.* 1993).

We (VR Young and AM Ajami, unpublished results) have initiated our homocysteine metaprobe studies using a labelled L-2-oxo-thiazine-4-carboxylic acid ethyl ester, which was synthesised by phosgene cyclization of L-homocysteine[1–^{13}C]ethyl ester and isolated as a crystalline material. Following this procedure we carried out a two-part experiment. In phase 1 an intravenous bolus of 2 mg probe/kg was administered to male rats according to the methods of Kohno *et al.* (1998) and Ishii *et al.* (1999). Breath was collected at various times and analysed by isotope-ratio MS. The resulting impulse response curve was analysed by non-compartmental modelling (Wagner, 1993; Barrett *et al.* 1998) giving estimates for BMR index, which was the derived fractional catabolic rate in the peripheral (tissue) compartment multiplied by the dose of tracer metaprobe, adjusted for the percentage of isotope release tag recovered. For this latter computation we used the area under the tracer-in-breath exhalation profile extrapolated to a time equal to ten half-lives based on the $^{13}CO_2$ excretion rate constant.

Subsequently, in phase 2 another matched cohort of animals received an infusion of methionine over 1 h, delivering 40 mg/kg, and the homocysteine metaprobe breath test repeated in order to examine the effect of a

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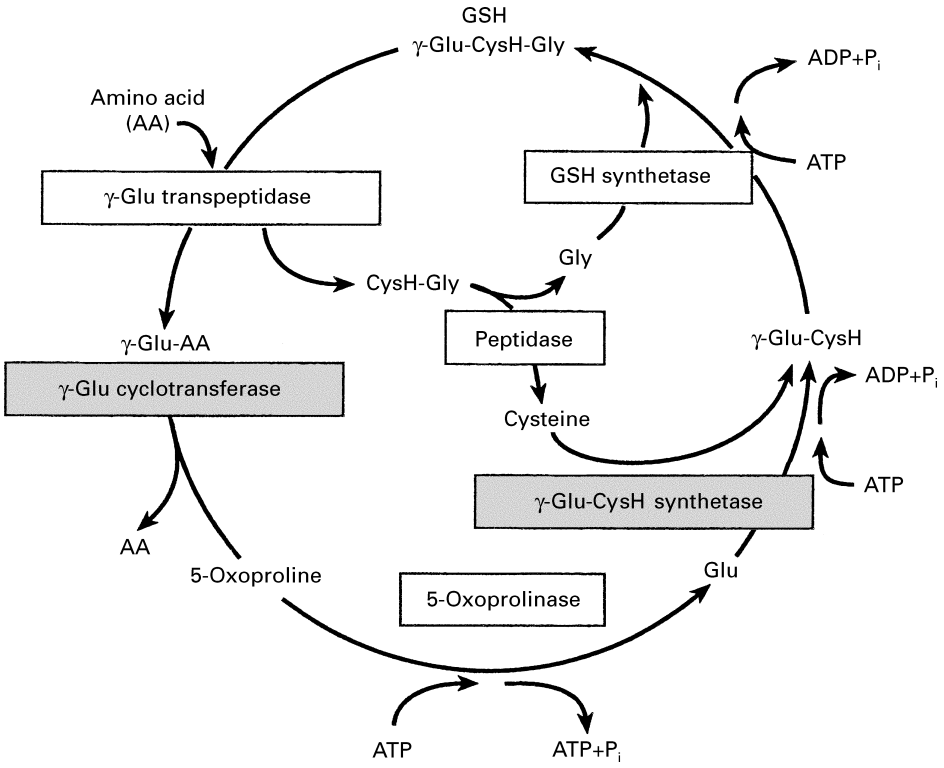

Fig. 6. The synthesis of glutathione (GSH) via the γ-glutamyl (Glu) cycle. CysH, homocysteine; Gly, glycine; P_i, inorganic phosphate. (Modified from Anderson, 1998.)

methionine load on intracellular clearance of derived homocysteine. The results are shown in Fig. 10. Under basal conditions, the apparent metabolic disposal of homocysteine, as determined by oxidative decarboxylation and $^{13}CO_2$ exhalation (adjusted for the normative endogenous CO_2 metabolism determined in sham controls) was 85 (SD 34) μmol/kg per h (n 6). The transient homocysteinaemia induced by the methionine load lowered ($P < 0{\cdot}05$) the index of homocysteine disposal to 28 (SD 13) μmol/kg per h (n 8). The difference between these two metabolic indices (57 (SD 24) μmol/kg per h) can be viewed as the normal adaptive response to a methionine load and represents the range of metabolic capacities that constitute this functional phenotype.

The experiment was repeated 24 h after the induction of sepsis by tail vein injection of a 10^9 live *E. coli* culture. Surviving animals were divided into two cohorts, one serving as the control breath test group and the other cohort receiving the same methionine load described for the basal experimental group. Again, we found that metabolic disposal of homocysteine in animals with sepsis was dramatically ($P < 0{\cdot}01$) impaired, falling to 30 (SD 14) μmol/kg per h (n 8) before the methionine load (Fig. 10) and remaining essentially at the same level after the load, with the difference in the metabolic disposal rates of 2 (SD 8) μmol/kg per h not significant.

These results confirm the generally-accepted literature findings that a methionine load causes a transient hyperhomocysteinaemia, which our findings suggest is caused by a decrease in the metabolic disposal of homocysteine. The biochemistry of sepsis, known to cause numerous dislocations in metabolic disposition and utilization of S amino acids, appears to exacerbate the inability to metabolically process S amino acids provided by replacement therapy, at least when in the form of methionine. While the precise causes or mechanisms for this observed effect remain to be fully characterized (including the impact of uncontrolled variations in the regulation of oxoprolinase and the GSH cycle that are induced by sepsis), our new experimental approach does illustrate how tracer techniques, aided by judicious design of metaprobes, can be used to further characterize functional phenotypes. In the case of studies on the regulation of homocysteine metabolism, the evidence presented here suggests that high metabolizers may be differentiated with relative ease from low metabolizers. Hence, it would be more toward the latter group that therapeutic interventions should be directed and optimized.

In summary, therefore, with reference to S amino acid-replacement therapies for the possible treatment of hyperhomocysteinaemia in sepsis and, by extension, in other disorders of homocysteine clearance, we suggest that the metaprobe approach provides a quantitative metabolic disposal rate for homocysteine and its dependent further adaptive responses. This approach might be particularly useful in testing the hypothesis of Brattström & Wilcken (2000) that the elevation of plasma total homocysteine in patients with atherosclerosis compared with controls is a consequence of impaired renal function and not a cause of atherosclerosis.

'Catalytic promiscuity' and 'moonlighting proteins'

A third example of the application of metaprobes or dynamic biomarkers focuses on what O'Brien & Herschlag

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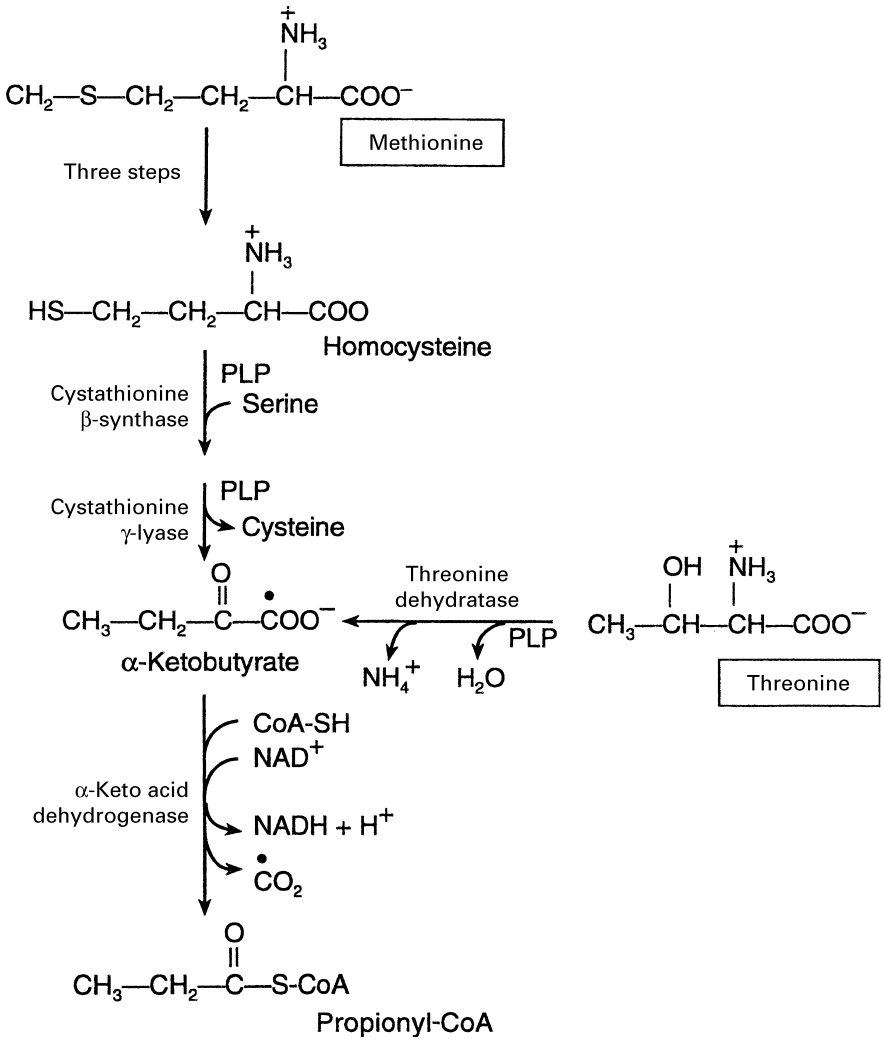

Fig. 7. The pathway of methionine and homocysteine catabolism via the trans-sulfuration pathway. [1-^{13}C]Homocysteine would give rise to [1-^{13}C]α-ketobutyrate which, when decarboxylated, would release the ^{13}C as $^{13}CO_2$. ●, Position of label; PLP, pyridoxal phosphate.

(1999) have referred to as 'catalytic promiscuity' and also on the existence of 'moonlighting proteins' (Jeffery, 1999). The former refers to many enzymes which accept some alternative substrates (usually similar to the normal substrates) and/or catalyse multiple chemical transformations that are normally classified as different types of reactions (e.g. different bonds are broken). Specifically, we are interested here in probing the activity of peptide hydrolases which recognize specific amino acid residues in a peptide chain. These enzymes are involved in the biosynthesis and degradation of peptides, such as hormonal peptides and neurotransmitters, via a highly-ordered series of events. Proline-specific peptidases (Cunningham & O'Connor, 1997), for example, cover practically all situations where a proline residue might occur in a substrate, and the presence of this amino acid, with its unique structural attributes (Kay *et al*. 2000), influences the interaction of the substrate with the enzyme. Parenthetically, proline-rich motifs occur in signalling and cytoskeletal proteins. These motifs serve as a 'molecular Velcro' mediating protein–protein interactions or directing cell compartmentalization. However, in terms of the proline-specific peptides specific metaprobes can, in principle, be designed to probe particular intracellular enzymes, and we (Young & Ajami, 1999*a*) have shown that it is possible, for example, to develop a [^{13}C]leucine-labelled probe for assessment of the lysosomal proline carboxypeptidase.

'Catalytic promiscuity', and by extension 'allosteric promiscuity', permits the existence of what Jeffrey (1999) has termed 'moonlighting proteins'. These proteins have multiple roles, e.g. phosphoglucose isomerase is a key enzyme in glycolysis but when outside of the cell it acts as a nerve growth factor, and thymidine phosphorylase catalyses the dephosphorylation of thymidine, deoxyuridine and their anologues within the cell cytoplasm, but when the protein is in the extracelullar fluid it acts as platelet-derived endothelial cell growth factor, stimulating endothelial growth and chemotaxis. Notably, in the present review, we have described how to probe 5,10-methylenetetrahydrofolate reductase within the compartmentalized pathways of

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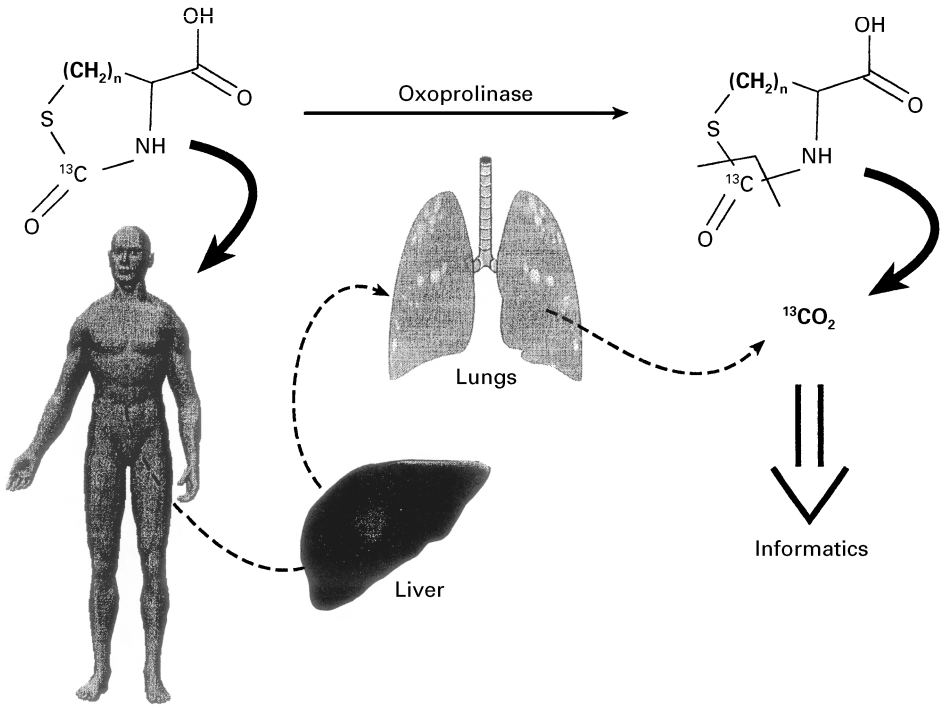

Fig. 8. An outline of the tracer metaprobe approach used for assessing the kinetic status of homocysteine.

homocysteine remethylation. This same enzyme takes on a 'moonlighting' role in biopterin cofactor regeneration, as stated earlier (p. 36), and therefore also becomes an important counter-regulatory effector for NO synthesis. Similarly, the normal role of CD26 is to serve as a T-helper 1 cell activation antigen and signal-binding site, while in its 'moonlighting' capacity as dipeptidyl peptidase IV it hydrolyzes chemokines and regulatory peptides, such as RANTES (regulated on activation, normal T-cell expressed and secreted) and glucagon-like peptide 1 respectively (Young & Ajami, 1999*b*). Function depends on whether the protein (enzyme) appears to act in its normal role or 'moonlighting' role and the 'moonlighting' aspects can be particularly instructive in understanding the natural history of pathophysiologies, which otherwise cannot be predicted by genomic potential alone.

The point worth making here is that this interesting feature of cell biology and metabolism confounds the exercise of ascribing or predicting the functions of encoded proteins from their genomic sequences alone and in reference to the known functions of homologous proteins. It adds considerable complexity to the organization of and therefore understanding of cellular metabolism, and creates greater challenges for post-genomic expectations. However, it seems possible that metaprobes could be designed to assay these multiple activities of specific proteins, especially when they qualify under the definition of 'gateway' enzymes (Young & Ajami, 1999*a*), and that such metaprobes would better help to assess how various proteins come together, and perhaps determine some of the mechanisms underlying disease. In any event, this approach is surely a good example of why the study of metabolism can help us to interpret and integrate genetic–molecular–cellular–proteomic information.

Technology and the interdisciplinary enterprise: a brief comment

We have already mentioned the power and versatility offered to the study of biology by microarray technology, but advances in other areas of technology, along with their increased availability, promise to greatly enhance the study of metabolism, endowing it with an even more worthwhile and exciting purpose. Thus, in *The Structure of Scientific Revolutions* Kuhn (1970) states: 'Because the crafts are one readily accessible source of facts that could not have been casually discovered, technology has often played a vital role in the emergence of new sciences'. Further, Finkelstein (2000) in his historical overview of S amino acid metabolism, with emphasis on homocysteine, states, appropriately; '…the limits on the growth of a research area are likely to result as much from the need for new methodologies as from the need for new concepts'. Indeed, the power achieved by combining new and different advanced technologies is beautifully illustrated, for example, by the studies described by Magistretti *et al.* (1999) on the combined use of positron emission tomography, functional magnetic resonance imaging and NMR. Experimental implementation of *in vivo* spectroscopy has revealed how glucose usage by astrocytes is tightly coupled to glutamate-mediated synaptic transmission. Extrapolating further, this type of approach promises to link a psychological understanding of behaviour and cognition with neurobiology and metabolic events at the synaptic level. This prospect excites us, in view of our long-term interests in amino acid metabolism and the central role of amino acids in neurotransmission. Surely, the expanded and combined application of such molecular imaging (Phelps, 2000) and related major technologies, coupled with gene sequence and

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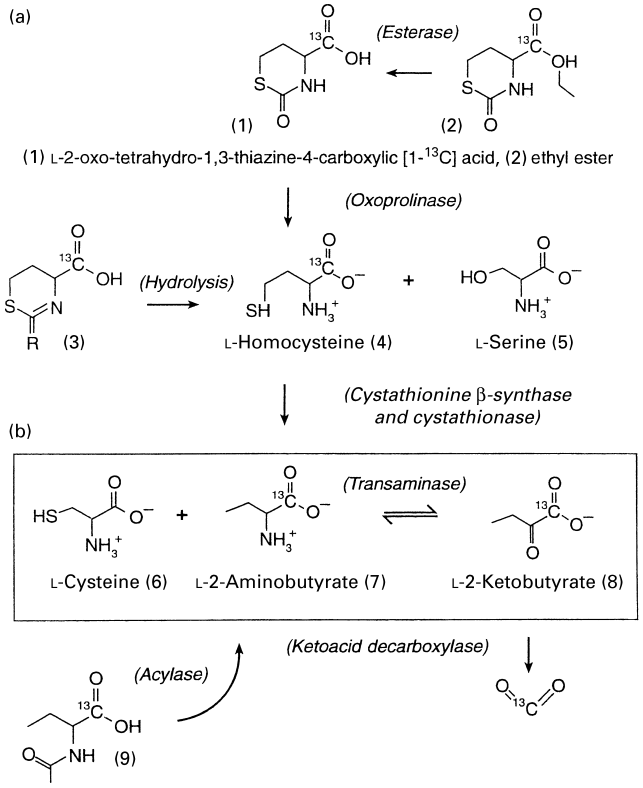

Fig. 9. A schematic presentation of homocysteine metaprobes that encompass two different approaches. (a) A metaprobe configuration involving homocysteine as the core tracer, with the labelled carboxyl group as the release tag, based on either the ester (2) of a tetrahydrothiazine (1) or based on 2-substituted dihydro-1,3-thiazines (3). (b) The indirect metaprobe N-acetyl-amino[1-^{13}C]butyrate (9) is shown, with its relationship to cysteine (6) and 2-ketobutyrate (8). For additional explanation of these metaprobes, see Young & Ajami (1999*a*).

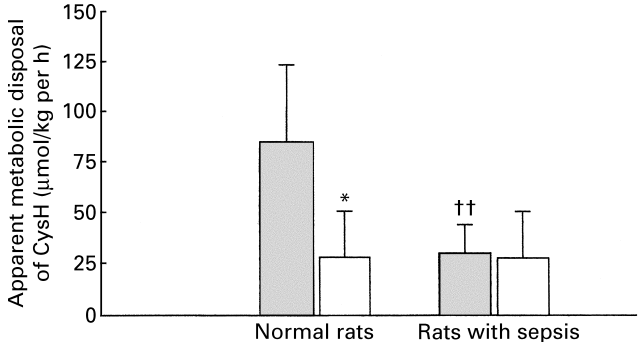

Fig. 10. Apparent metabolic disposal of homocysteine (CysH), using L-2-oxothiazine-4-carboxylic acid (1-^{13}C) ethyl ester as a metaprobe, in normal rats under basal conditions before (▨) and following (▭) a methionine load and in rats with sepsis before and following a methionine load. For details of procedures, see p. 36. Control load effect, δ 57 (SD 24), sepsis load effect, δ 2 (SD 8). Values are means and standard deviations represented by vertical bars. Mean value was significantly different from that before the methionine load: *$P < 0{\cdot}05$. Mean value was significantly different from that for the normal rat: ††$P < 0{\cdot}01$.

expression data, could greatly enhance our understanding of the metabolic, structural, geographical and behavioural corollaries of protein and amino acid metabolism *in vivo*.

With developments, for example, of miniature mass analysers (Badman & Cooks, 2000), 'lab-on-a-chip' devices (Figeys & Pinto, 2000), microfabricated potentiometric electrodes (Linder & Buck, 2000), wearable computers, electronic noses that can sense multiple volatiles in breath (Cyrano™ Sciences, 2000), protein chips (Arenkov *et al.* 2000) and with nanotechnology reducing the size of samples analysed to attolitres (10^{-18}) and detection limits down to zeptomoles (10^{-21}; Guetens *et al.* 2000), to mention just a few, the field of metabolism is now receiving a scientific and technological 'shot-in the arm'. These developments will nourish this aspect of nutritional science, provided the working conditions and academic environment for carrying out, what clearly must be an interdisciplinary research approach, are also present.

Nutrition is a bridging discipline between the molecular and physiological. Its advance requires a varied web of approaches and techniques ranging from genetic, molecular and cellular techniques to those used in integrative and computational biology, on through epidemiological, social behavioural and even economic research. In terms of our interest in these 'new' days of metabolism, as nutritional science continues to advance, the minimal numbers of skills necessary to achieve excellence will expand, putting further pressure on the importance of establishing an interdisciplinary research environment. To paraphrase Wickware (2000): 'Like a circus performer juggling daggers, chairs and flaming clubs, the field of nutritional biochemistry and metabolism stands amid elements ranging from genetics, cellular and molecular biology, organ/interorgan studies to clinical metabolic investigations and on to those in free living populations groups'. We suspect, therefore, that the fullness of potential advances in nutritional metabolism will require a concomitant embrace by academic institutions for true interdisciplinary research. This approach will require, in part as Wickware (2000) and Metzger & Zare (1999) among other researchers have discussed, a reorganization of current research funding approaches and changes in the priorities assigned to research programmes, allocation of space and procedures for professional recognition and advancement. A possibly interesting but relevant point is that there were 196 authors to the paper published in *Science* last March describing the nucleotide sequence of nearly all the 120-megabase euchromatic portion of the genome of *Drosophila melanogaster* (Adams *et al.* 2000) and 117 authors to the recent paper describing the smaller genome sequence of the bacterium *Xylella fastidiosa* (Simpson *et al.* 2000), that causes disease in citrus and other economically-important plants. We can only imagine a much larger number of authors when the metabolic equivalent of the human genome, the human metabolome (i.e. the complete identification and annotation of all the metabolic events and pathways and their interrelationships that serve to promote the functioning of the component organs and tissues of the intact organism in its natural or controlled environment) is published, but who will get the appropriate credit?! These are interesting times for metabolism and nutritional science, and the opportunities are rich with power and promise.

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Coda

The explosion in biological information over the past decade and plethora of new techniques that are now available challenge all of us who are concerned with advancing an understanding of the metabolic basis of mammalian nutrition. We have, by design and also due to our limited intellectual capacity, narrowed our discussion of 'Metabolism 2000' principally to the potential offered by tracers as phenotyping tools. These tools we are comfortable with, and it would be difficult to accurately predict the many other developments that are also relevant to a new fashioning of the metabolic 'Emperor's clothes' in the seasons ahead. As Lucky (2000) points out, no futurist predicted the emergence of the World Wide Web which, in a couple of years, may grow to 100 billion pages (Butler, 2000). Nevertheless, we have tried to make the case for an emphasis on a reconstructive, integrative and technically-advanced metabolic approach as an essential ingredient of contemporary research in nutritional science. We conclude as follows: (1) the metaprobe concept and approach, as outlined here, potentially yields a quantitative physiological (metabolic) phenotype against which to elaborate partial or focused genotypes; (2) physiological (metabolic) phenotypes which have a whole-body or kinetically-discernible inter-organ tissue-directed metabolic signature are an ideal target for this directed tracer-based definition of the corresponding 'functional' genotype; (3) metabolism, probed with tracer tool kits suitable for measuring rates of turnover, change and conversion becomes, in the current sociology of the 'Net', like AOL, Yahoo, Alta Vista, Lycos or Ask Jeeves; in other words, the portal for exploration of the metabolic characteristics of the 'Genomics Internet'.

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