ates of non-specific disciplines, which stood at 52% by the end of the decade,[6] clearly emphasises the need for educators to provide course elements which promote acquisition of transferable personal skills by science students. Biochemistry and biotechnology are rapidly evolving disciplines with considerable potential for future commercial application and we believe that it is equally important to provide a pool of undergraduate scientists who are exposed to the commercial realities of developing a coherent science-based business proposition. It is perhaps significant that a greater proportion (45%) of ABS graduates go directly into employment compared to the 27% (1991 data) of graduates from traditional UK biochemistry degrees.[7]

It is worth highlighting some of the factors which contribute to the successful operation of the Small Business modules in the ABS programme.

(1) The business content is well integrated into the programme. The Small Business Management module in year II serves as a useful preparation for the placement year, while the year of placement provides students with the opportunity to discuss with their work colleagues their ideas for the fourth year Small Business Project. Furthermore there is cooperation between science and business staff at the university: biochemistry staff tend to become involved by suggesting ideas and participating in the assessment.

(2) Careful selection of groups: an intimate knowledge of students is required so that weaker students are put together with more capable and motivated individuals. The optimum size for groups in our experience is three, but the size of the class might necessitate one or two groups of four.

(3) Assessment: It is essential that the assessment 'counts'. The project module contributes 16% to the final honours assessment. In order to prevent fragmentation of groups and to encourage teamwork, assessment is of the group rather than of the individuals within it.

(4) Monitoring: Groups have to present regular reports, written and oral, throughout the duration of the module. The final project report is presented in typed and bound form and presented to a panel consisting of business and science tutors and a small business practitioner.

Acknowledgement

The support of the EHE unit at the University of Ulster is gratefully acknowledged

References

[1] Dickson, M *Financial Times* 24 September 1993, p 7

[2] *Enterprise in Higher Education* Training Agency, Moorfoot, Sheffield, 1989

[3] Green, S (1990) *The Biochemist* **12**, 9–11

[4] Adams, K R (1989) *Biochemical Education* **17**, 26–28

[5] Cannon, T (1991) *Enterprise: Creation, Development and Growth* Butterworth-Heineman, Oxford, p 65

[6] Green, S (1989) *Biologist* **36**, 209–213

[7] *Annual Survey of Graduate Employment 1991* Biochemical Society, London, 1992

The Metabolic Clockwork

GASPAR BANFALVI

Institute of Biochemistry Department I
Semmelweis University Medical School
1444 Budapest PO Box 260, Hungary

Introduction

At least three metabolites are necessary to form a cycle, but in extended cycles the number of components may be more than ten. The combination of cycles in a so-called metabolic clockwork generates new cycles which may not have been described earlier. The central metabolic wheel is the citrate cycle and most of the secondary cycles are coupled to this circle.

Our whole life runs in a cyclic manner. Just think of the cyclic chemical balance between the atmosphere, the continents and the oceans, other processes viewed on a worldwide scale such as the hydrologic cycle, carbon and nitrogen cycles, the change of seasons, hours of the day, life cycles, hormonal cycles, cell cycles, regulatory cycles, metabolic cycles etc. In this paper I deal with metabolic cycles.

A common feature of metabolic cycles is that they contribute to the efficiency of the chemical work which maintains the thermodynamic steady state and help to minimize the increase of entropy (molecular randomness) in living cells. Metabolic cycles are among the most important biochemical pathways which provide a continuous supply of metabolites for anabolic and catabolic processes. Probably the best known among the metabolic cycles are the citrate cycle (also referred to as tricarboxylic acid, TCA, or Krebs cycle) and the urea cycle.[1,2]

Other metabolic cycles may be less familiar, although, from a metabolic point of view they are also important.[3–5] Much less attention has been paid to those metabolites which link different cycles to one another and in this way may form new cycles. Those who are familiar with biochemical pathways may recall the relationship between citrate-urea, citrate-glyoxylate or citrate-purine cycles.[6]

The Metabolic Clockwork

The compilation of the skeletal models of metabolic cycles as commonly described results in a cyclic network which I call the 'metabolic clockwork' (Fig 1). The citrate cycle occupies a central position and role in this metabolic clockwork. The term clockwork sounds rather mechanical and is used mainly to describe the idea of an interwoven network of cyclic metabolic pathways which do not proceed as isolated systems. The citrate cycle itself is believed to have evolved from two major segments involving tricarboxylates and dicarboxylates.[7] Fig 1 shows an 'ideal' clockwork with cycles not all of which may be present in each cell. Obviously some of the cycles are unique to certain organisms, eg the Calvin cycle is found only in photosynthetic cells, the propionate cycle in ruminants and microorganisms, the glyoxylate cycle in certain plant seedlings and microbes. The function of purine nucleotide

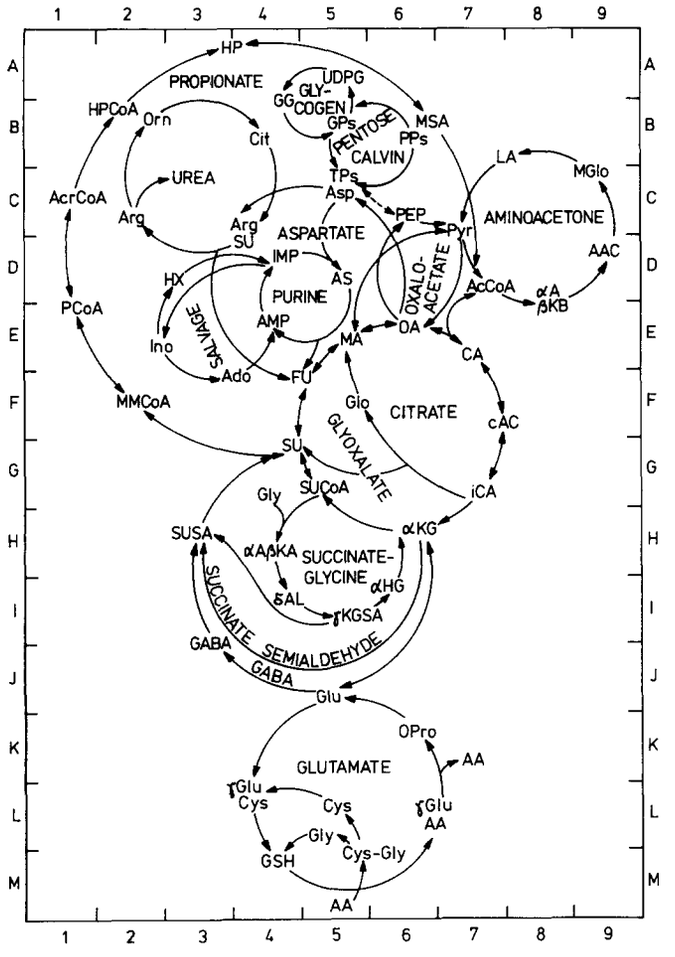

Figure 1 Schematic view of the metabolic clockwork. Metabolic cycles contain the names of their metabolites in abbreviated form. Abbreviations and cycles in alphabetical order are listed below. Position of cycles are indicated in parentheses. Note: not all intermediates in a cycle may be shown. One-headed arrows indicate unidirectional, two-headed arrows bidirectional reactions. Adenylosuccinate cycle: AS, adenylosuccinate; FU, fumarate; MA, malate; OA, oxaloacetate; Asp, aspartate (C–F, 5–6). Amino-acetone cycle: AcCoA, Acetyl Coenzyme A; α-Aβ-KB, α-amino-β-ketobutyrate; AAc, aminoacetone; MGlo, methyl-glyoxale; LA, lactic acid; Pyr, pyruvate (C–D, 7–9). γ-Aminobutyrate cycle: α-KG, α-ketoglutarate; Glu, glutamate; GABA, γ-aminobutyrate; SUSA, succinate semialdehyde; SU, succinate; FU, fumarate; MA, malate; OA, oxaloacetate; CA, citrate; cAC, cis-aconitate; iCA, isocitrate (G–J, 3–6). Aspartate cycle: OA, oxaloacetate; Asp, aspartate; ArgSU, argininosuccinate; FU, fumarate; MA, malate (C–F, 3–6). Aspartate-purine cycle: Asp, aspartate; AS, adenylosuccinate; FU, fumarate; MA, malate; OA, oxaloacetate (C–F, 5–6). Calvin cycle: PPs, pentose phosphates; TPs, triose-phosphates (B–C, 5–6). Citrate cycle: OA, oxaloacetate; CA, citrate; cAC, cis-aconitate; iCA, iso-citrate; α-KG α-ketoglutarate; SUCoA, succinylCoA; SU, succinate; FU, fumarate; MA, malate (E–G, 5–7). Glutamate (Meister) cycle: Glu, glutamate; γ-GluCys, γ-glutamyl cysteine; GSH, glutathione; Cys, cysteine; Gly, glycine; AA, aminoacid; OPro, 5-oxo-proline (K–M, 4–6). Glycogen cycle: GPs, glucose phosphates; UDPG, UDP-glucose; GG, glycogen (A–B5). Glyoxylate cycle: OA, oxaloacetate; CA, citrate; cAC, cis-aconitate; iCA, iso-citrate; Glo, glyoxylate; SU, succinate; FU, fumarate; MA, malate (E–G, 5–7). γ-Ketoglutarate semialdehyde cycle: γ-KGSA, γ-keto-glutarate semialdehyde; SUSA, succinate semialdehyde; SU, succinate; SUCoA, succinylCoA; Gly, glycine; α-AβKA, α-amino β-ketoadipate; δ AL, δ-aminolevulinate (H–I, 3–5). Oxalo-acetate cycle: OA, oxaloacetate; PEP, phosphoenol-pyruvate; Pyr, pyruvate (C–E, 6–7). Pentose cycle: GPs, glucose phosphates; PPs, pentose phosphates; TPs, triose phosphates (B5–6). Propionate cycle: SU, succinate; MMCoA, methylmalonylCoA; PCoA, propionylCoA; AcrCoA, acrylylCoA; HPCoA, hydroxypropionylCoA; HP, hydroxypropionate; MSA, malonate semialdehyde; AcCoA, acetylCoA; CA, citrate; cAC, cis-aconitate; iCA, isocitrate; α-KG, α-ketoglutarate; SUCoA, succinyl CoA (A–G, 1–7). Purine (nucleotide) cycle: IMP, inosine-5'-monophosphate; AS, adenylosuccinate; AMP, adenosine-5'-monophosphate (D–E, 4–5). Pyruvate (pyruvate — malate) cycle: Pyr, pyruvate; OA, oxaloacetate; MA, malate (C-E5-7). Salvage cycle I: AMP, adenosine-5'-monophosphate; IMP, inosine-5'-monophosphate; Ino, inosine; Ado, adenosine (D–E, 3–4). Salvage cycle II: IMP, inosine-5'-monophosphate; Ino, inosine; HX, hypoxanthine (D–E, 3–4). Succinate-glycine cycle: SUCoA, succinylCoA; Gly, glycine; αAβKA, α-amino β-ketoadipate; δ-AL, δ-aminolevulinate; γKGSA, γ-keto-glutarate semialdehyde; αHG, α-hydroxyglutarate; α-KG, α-ketoglutarate (H–I, 5–6). Succinate semialdehyde cycle: αKG, α-ketoglutarate; SUSA, succinate semialdehyde; FU, fumarate; MA, malate, OA, oxaloacetate; CA, citrate; cAC, cis-aconitate; iCA, isocitrate (G–J, 3–6). Urea cycle: Orn, ornithine; Cit, citrulline; ArgSU, argininosuccinate; Arg, arginine (B–C, 3–4).

cycle is to remove ammonia from brain and skeletal muscle in the absence of the urea cycle typical of liver cells. Although, the central and major driving force, ie the citrate cycle is common to most cells, the number of cycles associated with it may vary depending on cell type. Extended cycles typically bypass certain components of the citrate cycle and create new cycles. Among these new cycles are the γ-ketoglutarate semialdehyde, γ-amino-butyrate and the aspartate-purine cycles. Other series of reactions are included here as part of cycles, such as the aspartate, oxaloacetate, pyruvate, glycogen, pentose, and propionate cycles (Fig 1). The combination of cycles such as propionate, aminoacetone, pyruvate, and citrate cycles results in new routes interwoven in a cyclic manner.

It is interesting to note that in the citrate cycle (Fig 1) all of the compounds of the dicarboxylate hemicycle can be bypassed and metabolic demand for them can be satisfied by alternative sources. In contrast the tricarboxylate segment responsible for the conversion of citrate to isocitrate cannot be replaced or bypassed by other cycles.

A logical explanation to the isomerization of citrate to isocitrate is that citrate as a tertiary alcohol is resistant to mild oxidation, while strong oxidation would disrupt the chain, and consequently the cycle also. However, *iso*-citrate is a secondary alcohol which *is* readily oxidized.[8] Extensions, side loops contribute to the supply of basic metabolites and generate new cycles. An intercyclic interaction can occur through one or more intermediates. I propose that one metabolite common to two cycles provides more independence to these cycles than two or more common intermediates if they are to be driven by the clockwork (like teeth of a cogwheel). This intimately interwoven, dynamic network is constantly drained by the utilization of biosynthetic precursors and continuously fed by linear biochemical pathways (eg glycolysis, gluconeogenesis, lipid and amino acid metabolic routes) which are not indicated in the Figure. For the sake of clarity, these pathways are not indicated in Fig 1 and for the same reason the names of metabolites have been abbreviated and some details of the cycles have been omitted. Information on the enzymatic reactions of these cycles can be found in *Enzyme Nomenclature*,[9] detailed description of citric acid cycle in an earlier paper of this Journal,[10] other cycles in biology,[11] molecular biology[12] and biochemistry textbooks[13–16] and in metabolic maps.[17–19]

In this paper only intracellular cycles are dealt with. Intercellular cycles such as carbon dioxide fixation by the C_4-pathway in plants, regulatory cycles, control points involving cycles of phosphorylation and dephosphorylation, unchecked substrate (futile) cycles, the regeneration of coenzymes, enzymes and protein factors are not regarded as parts of the clockwork. None of the inter-organ exchanges of fuel molecules are elements of the clockwork. Examples of such inter-organ exchanges include the glucose-alanine cycle, the glucose-lactate or Cori cycle which occur mostly between skeletal muscle and liver, and the amino acid exchange cycles between organs like liver, muscle, gut, kidney and brain.

Conclusion

This paper describes a metabolic clockwork which: (1) relates a variety of metabolic cycles that occur in cells, (2) contains the citrate cycle as the central element of the clockwork, (3) permits the description of new cycles, (4) considers the tricarboxylate hemicycle to be the most conserved part of the citrate cycle since the conversion of citrate to isocitrate cannot be shunted by alternative loops, and (5) indicates that the number of cycles may vary with cell type.

The presence of this network of cycles provides thermodynamic stability and safety for the cell and metabolic versatility among different cells. Furthermore, the network of cycles contributes to the rapid accommodation of cells to their metabolic needs and to changing nutritional conditions. Many of the cycles and bypasses shown in the Figure have not been reported. Several known series of reactions are discussed here in terms of cycles. These include the aspartate, oxaloacetate, γ-aminobutyrate, glycogen, γ-ketobutyrate semialdehyde, oxaloacetate, propionate, pyruvate-malate, succinate semialdehyde cycles. Based on the idea that cycles generate cycles one can find further extensions by combining cycles such as pyruvate and citrate, propionate and citrate, pyruvate and glyoxylate, propionate and glyoxylate, or the combinations of the succinate-glycine-glyoxylate, GABA-glyoxylate, γ-ketoglutarate semialdehyde-glyoxylate cycles.

Acknowledgement

The critical reading of the manuscript by Professors Henry Paulus and Miklos Toth is gratefully acknowledged

References

[1] Krebs, H A and Johnson, W A (1937) *Enzymologia* **4**, 148–156

[2] Holmes, F L (1980) *Fed Proc* **39**, 216–225

[3] Horecker, B L (1976) in *Unravelling the Pentose Phosphate Pathway* edited by Kornberg, A, Cornudella, L, Horecker, B L and Oro, J, Pergamon Press, pp 65–72

[4] Wood, T (1985) *The Pentose Phosphate Pathway*, Academic Press

[5] Meister, A and Anderson, M E (1983) *Ann Rev Biochem* **52**, 711–760

[6] Baldwin, J E and Krebs, H A (1981) *Nature* **291**, 381–382

[7] Balch, W E, Fox, G E, Magrum, L J, Woose, C R and Wolfe, R S (1979) *Microbiol Rev* **43**, 260–296

[8] Hemming, A and Blotevoge, K H (1985) *Trends Biochem Sci* **10**, 198–200

[9] Webb, E C (1992) *Enzyme Nomenclature, 1992*, Academic Press, San Diego, CA

[10] Banfalvi, G (1991) *Biochem Education* **19**, 24–26

[11] Curtis, H (1986) *Biology* (Fourth edition), Worth, New York, pp 225–227

[12] Alberts, B, Bray, D, Lewis, J, Raff, M, Roberts, K and Watson, J D (1983) *Molecular Biology of the Cell*, Garland, New York, p 73

[13] Watson, J D, Hopkins, N H, Roberts, J W, Argetsinger Steitz, J and Weiner, A M (1983) *Molecular Biology of the Gene* (Fourth edition), Benjamin/Cummings, Menlo Park, pp 38–39

[14] Stryler, L (1988) *Biochemistry*, Freeman, New York

[15] Murray, R K, Granner, D K, Mayes, P A and Rodwell, V W (1988) *Harper's Biochemistry* (21st edition), Prentice-Hall International, Sydney, pp 317, 338, 341, 351

[16] Zubay, G (1988) *Biochemistry* (2nd edition) MacMillan, New York, London, pp 456, 500, 589, 592, 621

[17] Michal, G (1982) *Biochemical Pathways*, Boehringer Mannheim GmbH, Biochimica

[18] Holldorf, A W, Krahl-Mateblowski, V, Mateblowski, M and Wüttrich, S (1989) *Pathways of Metabolism in Liver* (Scheme A1: 8th edition), Ruhr Universität, Bochum

[19] Nicholson, D E (1990) *Metabolic Pathways Map*, 19th edition, Sigma Chemical Co, St Louis, MO