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Branch-point stoichiometry can generate weak links in metabolism: the case of glycine biosynthesis

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Although the metabolic network permits conversion between almost any pair of metabolites, this versatility fails at certain sites because of chemical constraints (kinetic, thermodynamic and stoichiometric) that seriously restrict particular conversions. We call these sites weak links in metabolism, as they can interfere harmfully with management of matter and energy if the network as a whole does not include adequate safeguards. A critical weak link is created in glycine biosynthesis by the stoichiometry of the reaction catalyzed by glycine hydroxymethyltransferase (EC 2.1.2.1), which converts serine into glycine plus one C_1 unit: this produces an absolute dependence of the glycine production flux on the utilization of C_1 units for other metabolic pathways that do not work coordinately with glycine use. It may not be possible, therefore, to ensure that glycine is always synthesized in sufficient quantities to meet optimal metabolic requirements.

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1. Introduction

Despite great progress in understanding nutrition during the 20th century, some important questions remain unanswered (Baker 2005). One of these concerns, for example, how glycine is partitioned between the synthesis of collagen and other metabolic functions when it is deficient in the diet, and, as we shall argue in this paper, this question is just one aspect of a general problem in glycine metabolism. Metabolic networks are highly versatile, and provide many different possibilities for interconverting metabolites. However, although virtually all metabolites are connected by chains of enzyme-catalyzed reactions, there are some restrictions that mean that it is not necessarily possible to convert any metabolite into any other; even conversions that appear to be possible in principle may be prevented by thermodynamic and kinetic constraints (Heinrich *et al* 1997), and others are prevented by pathway stoichiometry (Nuño *et al* 1997). The increasing attention being given to systems biology (Boogerd *et al* 2007) reacts the need to focus attention on entire systems in order to understand them, rather than on small parts.

There are two different types of branch-point in metabolism, as illustrated in figure 1: the more common occurs at a free metabolite, such as acetyl-CoA, pyruvate, etc., that can be freely transferred to the connected pathways in whatever proportions are needed; the fluxes in the different branches can be adapted to metabolic needs, for example by using feedback inhibition to allow the flux of any branch to respond to demand for its end-product (Hofmeyr and Cornish-Bowden 2000), but also by hormonal and genetic regulation. Metabolic pathways with branch-points of this kind have been extensively studied and the major characteristics are well understood (Small and Kacser 1993). A branch-point may also occur at an enzyme-catalyzed reaction that connects several different products and substrates, and here the situation is more complex. The products *B* and *C* do not then come from different enzymes but from the same one, an

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enzyme that catalyzes a break-down reaction. The reaction stoichiometry then requires the substrates and products to react in definite proportions to permit mass conservation. This may create a conflict of interest between the different pathways that converge on or diverge from the reaction in question, if the necessary fluxes of each branch for using or supplying the substrates or the products do not correspond to the stoichiometric constraints.

Branch-points at enzymes have been much less studied than branch-points at metabolites, and we shall show that they can generate strong metabolic limitations, with potentially severe repercussions for the health of the organism if the rest of the network does not provide mechanisms to avoid them. There is a constraint of this kind in the synthesis of glycine, which is catalyzed by the enzyme glycine hydroxymethyltransferase (GHMT) (EC 2.1.2.1) with tetrahydrofolate (THF) as coenzyme in the following reversible reaction:

$$\text{L-serine} + \text{THF} \rightleftarrows \text{5,10-methylene-THF} + \text{glycine} + \text{H}_2\text{O}$$

As will be shown, the stoichiometry of this reaction severely limits the capacity of glycine synthesis to respond to metabolic demand, so that it may be less than what a human or a large animal requires. This may explain why although glycine is usually classed as a non-essential amino acid, because it can be synthesized by human metabolism, there is evidence that human metabolism cannot supply it in sufficient quantities (Jackson 1991). Furthermore, certain health problems are related to glycine deficiency, and clinical and nutritional studies have also shown that it should be considered a conditionally essential amino acid (Jackson 1991; Jackson *et al* 1996; de Koning *et al* 1998; Matilla *et al* 2002; de Koning *et al* 2003; Lewis *et al* 2005).

2. Metabolic implications of different designs of branch-points: possibility of weak links

Figure 1 shows all possible ways in which a substrate *A* can be converted into two products *B* and *C,* and figure 2 illustrates how the different types of branch-point may be embedded in a metabolic pathway. The independent-flux system (figure 1a) is a typical bifurcation point at which three fluxes v_1, v_3 and v_4 are connected by the common metabolite *A.* This is a typical metabolic crossroads where each emerging pathway can be regulated independently according to the demand for its end-product. By contrast, in the other three designs

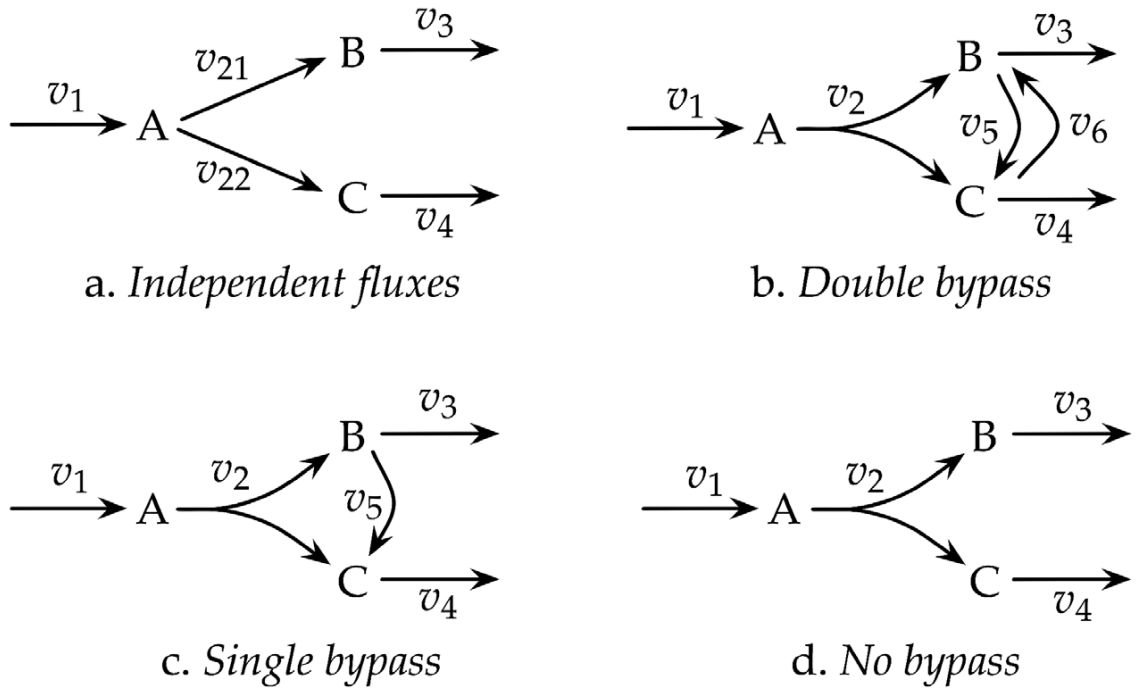

Figure 1. Types of branch-point in metabolism. (**a**) Branch-point at a metabolite *A,* with two reactions to *B* and *C* that can be regulated independently; (b, c, d) branch-point at an enzyme-catalyzed reaction that requires *B* and *C* to be produced in equimolar quantities via v_2, with (**b**) a two-way bypass reaction that allows interconversion of *B* and *C* in either direction; (**c**) a one-way bypass reaction that allows *B* to be converted into *C,* but not vice versa; or (**d**) no bypass, with no possibility of interconversion of the products.

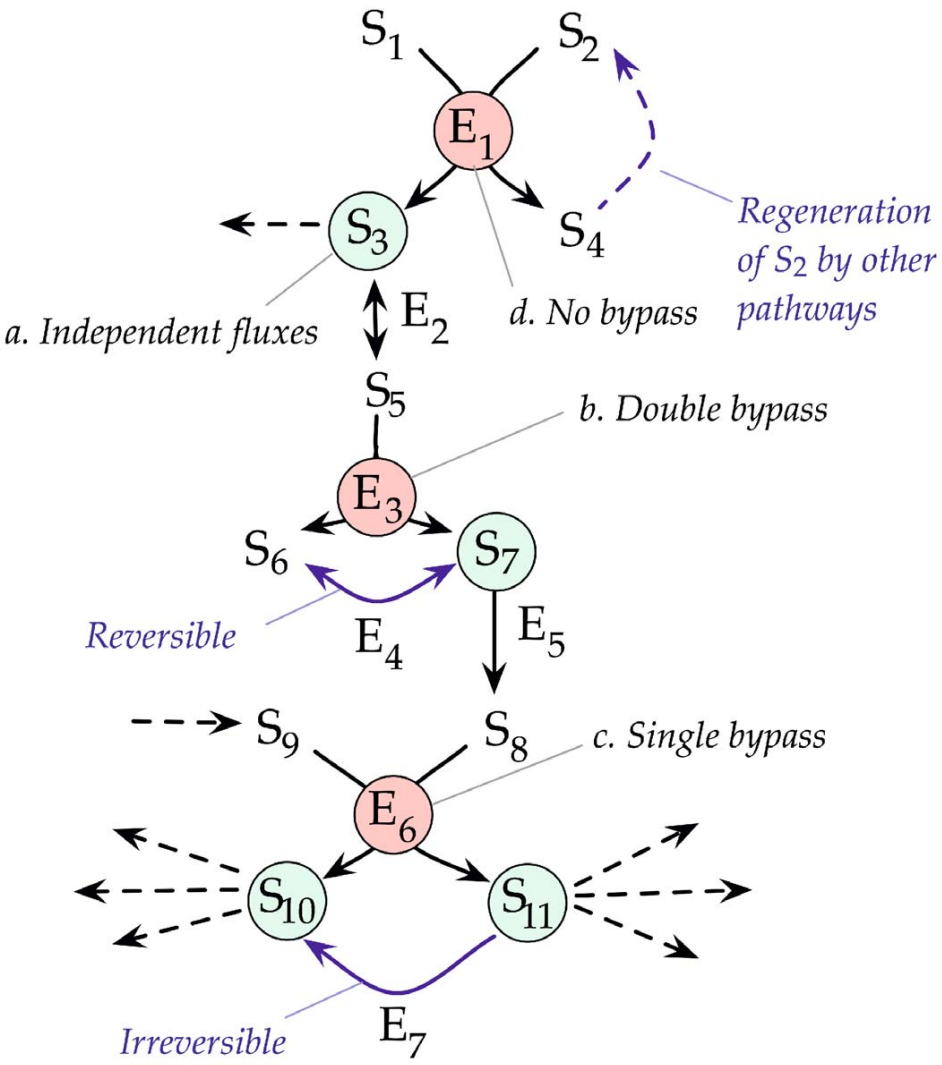

Figure 2. Illustration of the different kinds of branch-point in a metabolic pathway. Nodes shown in blue represent branch-points at metabolites (case a of figure 1). Nodes shown in pink represent branch-points at enzyme-catalyzed reactions, and there are three kinds of these, labelled as in figure 1: (b) The branch-point at E_3 produces two metabolites S_6 and S_7 that are freely interconvertible. (c) The branch-point at E_6 produces two metabolites S_{10} and S_{11} in equimolar quantities, but the bypass allows S_{11} to be converted into S_{10}, but not vice versa. This case corresponds to synthesis of glycine (corresponding to S_{11}) and tetrahydrofolate-C_1 units (S_{10}). (d) The branch-point at E_1 produces two metabolites S_3 and S_4 that are not interconvertible, but regeneration of the substrate S_2 by pathways that consume S_4 allows production of S_3 to continue.

the three fluxes v_1, v_3 and v_4 are connected by the enzyme catalyzing v_2, and in each of them the flux from *A* to yield *B* + *C* is the same, so there is no freedom to yield independent amounts of *B* and *C*. There are, however, some important differences between these last three cases. In the double-bypass design (figure 1b) the fluxes v_5 and v_6 (which may be a single reversible reaction) allow free conversion between *B* and *C*: an example in glycolysis is the interconversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, the products of the aldolase-catalyzed breakdown of fructose 1,6-bisphosphate. In the single-bypass design (figure 1c) conversion of *B* to *C* is possible, at a rate v_5, but there is no provision for the reverse conversion of *C* to *B*. This is exemplified by glycine biosynthesis, in which the products of the reaction catalyzed by glycine hydroxymethyltransferase can be repartitioned by the glycine cleavage system, as analyzed below. Finally, the no-bypass design (figure 1d) is at the opposite extreme from the independent-flux design, with no possibility of interconversion between *B* and *C* in either direction.

We now analyze the stoichiometric constraints imposed by each of the different designs of branch-point. The fluxes involved in all of these conversions, expressed as changes in metabolite concentrations, can be written as differential equations, which can be converted into algebraic equations by introducing the steady-state condition.

2.1 *Independent fluxes*

In the case of figure 1a the set of differential equations can be converted into algebraic equations by setting each expression to zero for the steady state:

$$\frac{dA}{dt} = v_1 - v_{21} - v_{22} = 0$$

$$\frac{dB}{dt} = v_{21} - v_3 = 0$$

$$\frac{dC}{dt} = v_{22} - v_4 = 0$$

At steady state $dA/dt = dB/dt = dC/dt = 0$, therefore,

$$v_1 = v_{21} + v_{22};\ v_{21} = v_3;\ v_{22} = v_4$$

As there is no restriction on the values of v_3 and v_4 (or on those of v_{21} and v_{22} other than the stoichiometric relationship $v_1 = v_{21} + v_{22}$), any of them can take any value up to v_1.

2.2 *Single bypass*

We shall consider the case of a single bypass (figure 1c) in detail as it is the one that applies to glycine biosynthesis, and will simply summarize the corresponding analysis for the branch-points with a double bypass or with no bypass. If there is a bypass reaction from *B* to *C* but not from *C* to *B* the set of equations at steady state is as follows:

$$\frac{dA}{dt} = v_1 - v_2 = 0;\ v_1 = v_2 \quad (1)$$

$$\frac{dB}{dt} = v_2 - v_3 - v_5 = 0;\ v_3 = v_2 - v_5 \quad (2)$$

$$\frac{dC}{dt} = v_2 + v_5 - v_4 = 0;\ v_4 = v_2 + v_5. \quad (3)$$

At steady state ($dA/dt = dB/dt = dC/dt = 0$) the lack of a bypass in one direction imposes some constraints, as can be seen by solving these equations. Elimination of v_2 between Eqns. (2) and (3) gives:

$$v_3 = v_4 - 2 \cdot v_5 \quad (4)$$

or, equivalently,

$$v_4 = v_3 + 2 \cdot v_5 \quad (5)$$

As all fluxes must be positive (or zero), Eqn. (4) implies that v_4 cannot be less than $2 \cdot v_5$ and v_3 cannot be greater than v_4, as the upper limit of v_3 [and also of v_2, from Eqn. (3)] occurs when $v_5 = 0$, whereas Eqn. (5) allows v_4 to be greater than v_3. Thus $v_3 \leq v_1$, whereas $2 \cdot v_1 \geq v_4 \geq v_1$. So v_3 can never exceed v_4, and very importantly, v_2 (glycine biosynthesis) cannot be greater than v_4 (consumption of C_1 units). This restriction may have a very important metabolic consequence, because it means that the rate of biosynthesis of the intermediate *B* (v_2), cannot exceed the flux for consumption of *C* (v_4); if the demand of *B* is much greater than that for *C,* there can be a shortage of *B* and a failure in the normal regulation of metabolism, provoking a lack of precursors or a perturbation in the dynamics of the network. This type of design can thus lead to a *weak link in metabolism*, and we shall argue that it does produce such a weak link in the case of glycine biosynthesis.

2.3 *Double bypass*

When bypasses between the products exist in both directions (figure 1b) the analysis is similar, except that the expressions 'for and now' depend also on v_6. As there is no restriction on the relative values of v_5 and v_6 other than those imposed by thermodynamics, any of them can take any value less than or equal to v_2, and the constraint that applies to a single bypass is weakened. There is full freedom of conversion of *A* into *B* or *C,* as with independent fluxes. The fluxes are stoichiometrically related by $v_3 + v_4 = 2 \cdot v_1$; one of the exit fluxes can be zero, which would give $v_3 = 2 \cdot v_1$ or $v_4 = 2 \cdot v_1$. The combination of aldolase and triose-phosphate isomerase in glycolysis is an example of this design.

2.4 *No bypass*

When there is no bypass reaction in either direction (figure 1d) the set of equations follows from those for a single bypass by omitting v_5 throughout, and the conclusion is that there is absolutely no freedom of interconversion between the two products, the 1:1 ratio of these fluxes being imposed by the stoichiometry of the reaction v_2. There are many cases like this in metabolism, including most decarboxylating enzymes, kinases, etc. Specific examples are the arginase reaction that converts arginine into ornithine and urea, and, in the reverse direction, the condensation of acetyl-CoA with oxaloacetate to give citryl-CoA. However, although this design is abundant in metabolism, it does not usually create conflicts in pathway fluxes, as at least one of the products resulting from the branch (e.g. ATP or acetyl-CoA) can normally be produced or consumed by many other reactions. Examples of metabolic weak links produced by lack of bypasses may exist, of course, but further investigation will be needed to identify them; in any case the need to consider the network as a whole remains fundamental.

3. Single-bypass design and the glycine biosynthesis constraint

Glycine biosynthesis exemplifies the single-bypass design and illustrates the metabolic constraints that it imposes, as a weak link in metabolism. Glycine is synthesized by the enzyme glycine hydroxymethyltransferase (GHMT), with the elimination of a C_1 unit from C-3 of serine (*see* section 1). This is a rather complex reaction, described in detail elsewhere (Schirch and Szebenyi 2005). In mammalian cells it is found both in the cytoplasm and in the mitochondria. This compartmentation does not affect the glycine constraint, as the stoichiometric constraint continues to apply. If anything, compartmentation will intensify the constraint. Several lines of investigation have shown that the inability of mitochondria to produce glycine results in glycine-auxotrophic cells, despite the presence of GHMT in the cytoplasm. The reaction appears as reaction 3 in figure 3, which illustrates the pathways involved in the biosynthesis of glycine, together with those involved in its use for different metabolic functions. The central part of the metabolic scheme is shown in more detail in figure 4, with the fluxes labelled v_1 to v_5 as in section 2.2 and figure 1c. In addition, Figure 4 makes explicit a point that is only implicit in figure 3, namely

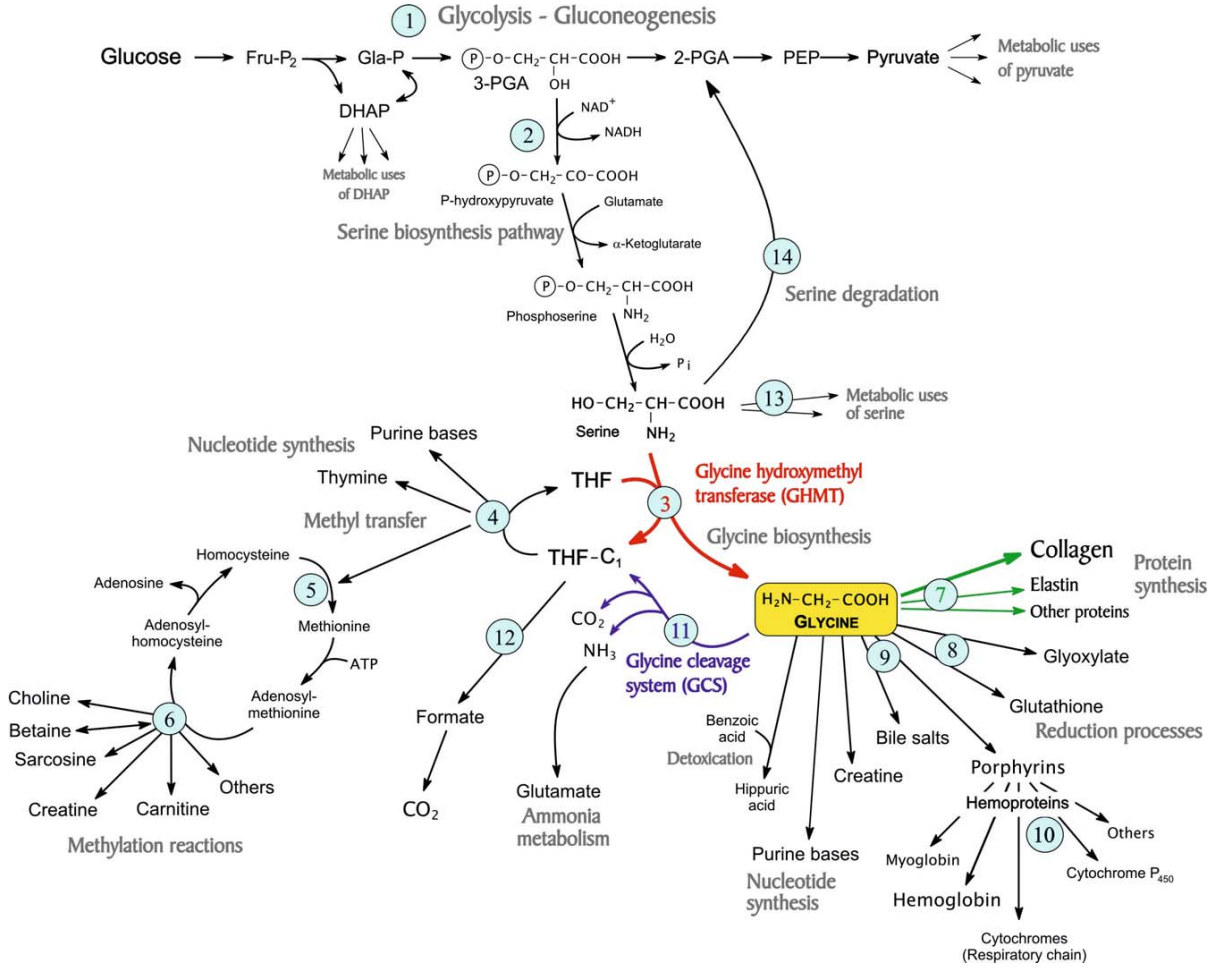

Figure 3. Metabolic pathways involved in the biosynthesis of glycine from serine, and its use for different metabolic functions. Pathways: 1, glycolysis-gluconeogenesis system; 2, branch to synthesis of serine and glycine; 3, reaction of glycine hydroxymethyltransferase (GHMT) (EC 2.1.2.1) (shown in red), which converts serine into glycine with the transfer of a C_1 unit to tetrahydrofolate (THF); 4-6, set of reactions of C_1 transfer, necessary for glycine synthesis as they unload the C_1 unit from THF-C1, regenerating free THF so that it can participate again in reaction 3; 7-10, processes of biosynthesis involving glycine, especially the synthesis of collagen and other proteins (reactions 7, shown in green); 11, irreversible reaction of the glycine cleavage system, (EC 1.4.4.2) (shown in blue), which converts glycine into a C_1 unit; 12, oxidation of the C_1 unit to formate and CO_2, which works as a sink of C_1 units, allowing the capability for glycine synthesis to increase; 13, set of reactions that use serine for other processes, e.g. for choline synthesis; 14, pathways for serine degradation. Symbols: DHAP, dihydroxyacetone-phosphate; Fru-P_2, fructose 1,6-bisphosphate; Gla-P, glyceraldehyde 3-phosphate; 3-PGA, 3-phosphoglycerate; 2-PGA, 2-phosphoglycerate; PEP, phosphoenolpyruvate; THF-C_1, 5, 10-methylene-THF. *See* also figure 4.

that the glycine cleavage system consumes tetrahydrofolate as co-substrate, but as the glycine molecule is wholly derived from serine this does not affect the relationships derived in section 2.2. So the constraint in glycine biosynthesis is accurately represented by the scheme in figure 1c.

In glycine metabolism a bypass reaction does exist, provided by the glycine cleavage system, a multienzyme complex consisting of glycine dehydrogenase (decarboxylating) (EC 1.4.4.2), aminomethyltransferase (EC 2.1.2.10) and dihydrolipoyl dehydrogenase (EC 1.8.1.4). This catalyzes the following overall reaction, shown as Reaction 11 in figure 3:

$$\text{glycine} + \text{THF} + \text{NAD}^+ \rightarrow \text{5,10-methylene-THF} + \text{CO}_2 + \text{NH}_3 + \text{NADH}$$

This does not work as a bypass in both directions, because the release of CO_2 and NH_3 makes it thermodynamically irreversible *in vivo* (Xue *et al* 1999). It cannot account for glycine synthesis, therefore, but only for the production of a C_1 unit by glycine degradation (Xue *et al* 1999; Lamers *et al* 2007). This metabolic design leads to an important stoichiometric problem, because the C_1 unit must be released from THF-C_1 before THF can be used as a carrier in the reaction again. The unloading of C_1 can satisfy several purposes, such as biosynthesis of purines, thymine, or the transfer of the methyl group to adenosyl-methionine, which is, in turn, a coenzyme for most methyl transfer reactions (Christensen and MacKenzie 2006).

As we have shown, this design cannot yield glycine or THF-C_1 independently, but is forced to supply the two of them stoichiometrically, so there is no possibility of varying the fluxes in the two branches independently according to the demand for their products (Hofmeyr and Cornish-Bowden 2000). Reaction 3 is not a bifurcation point, therefore, but a breakdown with a fixed stoichiometry. The C_1-transfer flux must therefore work in coordination with glycine usage if a collapse in glycine metabolism is to be avoided.

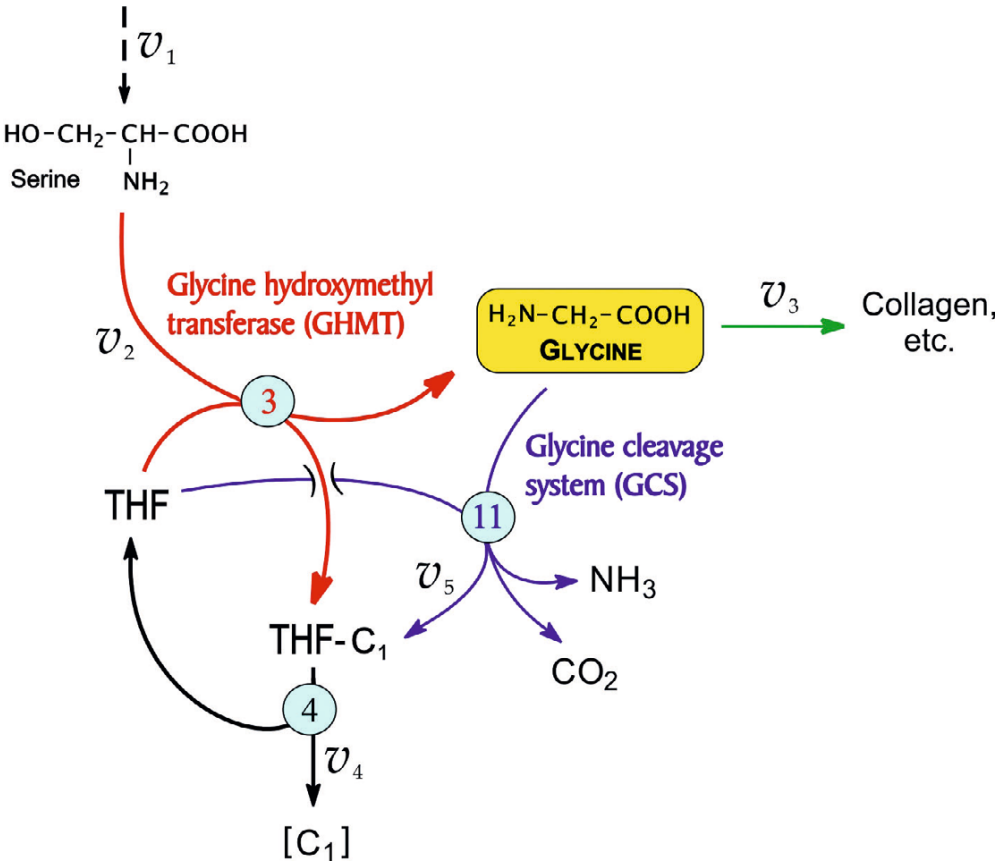

Figure 4. Metabolic production of glycine. This shows the central part of figure 3 with fluxes labelled with the symbols used in figure 1c, and also shows explicitly that tetrahydrofolate is a co-substrate with glycine in reaction 11, the glycine cleavage system. However, as the glycine molecule is entirely derived from serine, this addition does not affect the relationships between the fluxes discussed in section 2.2. In particular, it remains true that v_3 cannot exceed v_4, and that v_2 (glycine biosynthesis) cannot be greater than v_4 (consumption of C_1 units).

There are other possibilities for glycine biosynthesis, for example from choline (via sarcosine), threonine degradation, carnitine synthesis, and the transamination of glyoxylate, which can be produced from several sources. However, biosynthesis of glycine via glycine hydroxymethyltransferase is by far the most important process, so treating this as the only important source of glycine will not lead to large errors. We can then express the stoichiometric constraint in the following words, which we shall call the *glycine biosynthesis constraint:*

If the unique significant metabolic pathway for glycine synthesis is the GHMT reaction, the steady-state metabolic flux for net glycine production cannot in any conditions exceed the flux for consumption of C_1 units transferred via THF.

The bypass provided by the glycine cleavage system emphasizes the constraint rather than providing a way to circumvent it, as demonstrated for the case of the single bypass analyzed above.

As the stoichiometry requires glycine to be produced from serine with equimolar production of tetrahydrofolate-C_1, this ratio cannot be modified under any kinetic conditions. Therefore, although the behaviour is easiest to analyse in the steady state, the glycine biosynthesis constraint also applies when the metabolism is not in steady state.

4. Discussion

It follows from the analysis that glycine biosynthesis catalyzed by glycine hydroxymethyltransferase depends stoichiometrically on the total set of reactions dependent on THF-C_1 (reactions 4–6 and 12 in figure 3). However, the physiological need for C_1 units is independent of the need for glycine (with the exception of purine bases and creatine synthesis), and so there is no obvious reason for the consumption of glycine and C_1 units to be closely coupled. A high consumption of glycine for biosynthesis of other products (reactions 7–10 in figure 3), especially collagen, elastin and other proteins (reactions 7), would therefore have to force an equal or higher production of C_1 units carried by THF. But this is not possible, as production of the THF-C_1 intermediate will not depend on the activity of other unrelated pathways, but must be made according to the specific need for this metabolite. As every one of these pathways has its specific regulation mechanisms, glycine biosynthesis is not possible when there is no expenditure of C_1 units, because tetrahydrofolate will not be regenerated.

The physiological reality is more complicated than we have implied, however, because not all tissues are identical, and because folates do not move between cellular compartments at rates commensurate with metabolism, and the folate pools are thus to a considerable extent separate. In mitochondria in adult cells, for example, the restrictions implied by our analysis can become very important, because accumulation of THF must prevent further production of glycine. In many cells there is no glycine cleavage system and the properties will be those of no-bypass (section 2.4). In most of these cases the complications do not alleviate the problem of insufficient glycine production but make it worse. On the other hand, as synthesis of nucleic acids requires a high expenditure of THF-C_1 to produce purines and thymine, these processes act as net producers of glycine. Thus the stoichiometric constraint are net producers of glycine, and the stoichiometric constraint may be attenuated in rapidly growing cells such as embryos and cancer cells.

These complications undoubtedly add to the difficulties of making a detailed analysis of all the fluxes, but the problems created by the stoichiometric constraint in the GHMT reaction remain even if there is extensive partitioning of the different processes of glycine production and utilization. This is because the constraint affects every molecule of glycine produced by this reaction, regardless of any channelling, compartmentation etc. What is ultimately important is to compare the total amount of glycine available in the whole organism with the total amount needed for the metabolic functions of the whole organism: if the former is less than the latter there must be a problem. Even if channelling or compartmentation may alleviate it locally, this can only be at the expense of aggravating it elsewhere. Thus, although these complications must ultimately be taken into account in a full analysis of glycine fluxes *in vivo,* they do not affect the major point of this paper.

Glycine is particularly important for synthesis of collagen and elastin, as it accounts for one third of the residues of both proteins. In addition, it is used extensively in metabolism as a chemical reagent for the synthesis of many compounds, such as glutathione, which plays a major role in combating redox stress. Glycine also participates in the detoxification of benzoic acid (Jackson 1991), and acts as a neurotransmitter. It is important to make a detailed assessment of all the routes to glycine (not shown in figure 3), apart from synthesis from serine, and to determine whether they are sufficient for the metabolic requirements of the organism. Calculation is made difficult by complexities in the operation of the procollagen cycle, and by the re-use of free amino acids produced by protein turnover, but our calculations (not shown) suggest a glycine (Bienkowski *et al* 1978), and by the re-use of deficit as high as 10 g/day or even higher. The detailed calculation falls outside the scope of the present paper and will be the subject of a future publication.

This existence of a glycine deficit is supported by clinical evidence, as in some conditions glycine synthesis is insufficient for metabolic requirements (Jackson 1991), and a daily glycine deficiency impairs glutathione synthesis and causes increased excretion of 5-oxoproline in the urine (Gibson *et al* 2002; Persaud *et al* 1996). Progressive increases in 5-oxoproline excretion by pregnant women suggest that

the availability of glycine becomes insufficient as pregnancy advances (Jackson *et al* 1996). These observations support the idea that glycine is a semi-essential (or conditionally essential) amino acid in some conditions and suggest that problems such as osteoarthritis that are related to connective tissues, where collagen and elastin are major components, may be due in part to glycine deficiency.

If glycine biosynthesis is a weak link in metabolism, then diseases such as osteoarthritis should be found in the wild, because the glycine biosynthesis constraint applies to any animal, and these diseases should increase in severity with the size of the animal in accordance with allometric considerations. Osteoarthritis is, in fact, one of the rare diseases that are common in the wild: it has been detected in the skeletons of large dinosaurs from the Jurassic Age, 100-150 million years ago (Rothschild 1987, 1993); it occurs in present-day large mammals, such as elephants (Weissengruber *et al* 2006) and rhinoceroses (Bonard 1987; Wallach 1967). In general, osteoarthritis has been found in a broad variety of present-day mammals, both in the wild and in captivity (Greer *et al* 1977), and specifically in great apes, such as chimpanzees, gorillas and bonobos (Jurmain 2000). It has also been found in fossil Hominidae, including Neanderthals (Straus and Cave 1957), in Upper Paleolithic and Neolithic human fossils (Ackernecht 1953), and in human fossils from the Middle Pleistocene in Atapuerca (Spain) (Pérez and Martínez 1989).

The stoichiometric constraint of the GHMT reaction is not unique in metabolism, and there are many other reactions that produce two metabolites in equimolar amounts without bypasses in the two directions (in many cases there is no bypass in either direction) to allow production of the two to be regulated independently. Why then, should glycine biosynthesis constitute a potentially serious problem whereas these other apparently similar examples do not? This cannot be deduced by confining attention to one reaction and its immediate neighborhood, but requires examination of the whole metabolic network, in accordance with the increasingly recognized principle that understanding the properties of a complex system requires study of the complete system (Kacser 1987; Fell 1997; Cornish-Bowden 2006; Daran-Lapujade *et al* 2007). The glycine problem arises not just from the glycine hydroxymethyltransferase reaction and the glycine cleavage system, but from the lack of any downstream connections between the pathways that consume glycine and C_1 units and the lack of any mechanism to transfer information between the two (Cornish-Bowden and Cárdenas 2001). Most other reactions that generate two products in equimolar amounts are protected by mechanisms that ensure that no problem arises: bypasses may exist, as provided by the triose-phosphate isomerase reaction, for example, or one of the two products is continuously converted back to the corresponding substrate, as the ADP released by a kinase is converted back to ATP by other pathways in the network, or one of them is excreted, as for example the CO_2 released by decarboxylases. It follows that the special problem in glycine synthesis is not simply a consequence of the glycine hydroxymethyltransferase reaction, but of the absence of adequate compensating processes in the entire metabolic system. Although a sink for surplus C_1 units does exist, in the form of the production of CO_2 from formate shown as Reaction 12 in Figure 3, the activity of this process (Case and Benevenga 1977) appears to be insufficient to overcome the problem. In addition, in principle, formate itself could be excreted. A small deficit in glycine production might well be overcome in this way, but a daily deficit of several grams would require a correspondingly high level of formate excretion.

If the glycine weak link is as old as we suggest, arriving with the need for collagen synthesis by the first Metazoa, another question that arises is why natural selection has not found a solution. However, although evolution involves some optimization (Meléndez- Hevia 1993; Cornish-Bowden 2004), it is mainly a combination of natural selection and opportunism, taking materials and processes that originally served other purposes and adapting them to new ones (Jacob 1977; Meléndez-Hevia *et al* 1996). The inability of natural selection to foresee future needs is also seen in the problem of ribulose-bisphosphate carboxylase in the face of the increases in the atmospheric O_2 concentration over the course of evolution (Edwards *et al* 2001). The selective pressure to eliminate the glycine constraint is probably low, because it does not affect survival or reproduction of most individuals. Nonetheless, the existence of the constraint predicts a reduction in the quality of life, especially in old age.

The question of whether glycine is an essential amino acid will eventually be decided only by facts, but facts can only tell us about particular cases, whereas here we have developed a theoretical argument that can be applied in general.

A major aim in Systems Biology is to emphasize the necessity of studying biological systems as a whole. A reason behind that purpose is that complex systems are also strongly constrained systems; therefore it is inappropriate to look for adaptations at the level of single reactions or even pathways. A global perspective is necessary, and that takes us far from a reductionistic vision of the organic word (*see* the essay by Michel Morange in this issue; Morange 2008). The case of glycine metabolism presented here is a good example to illustrate this concept: it shows that pleiotropy must be expected in mutational investigations of metabolic networks wherever there are ‘single bypass’ reactions.

As to the specific case of glycine metabolism, the stoichiometric constraint discussed here seems to provide

clear grounds to make it 'conditionally essential', a fact that several authors had noticed on the basis of empirical studies (see, e.g. Jackson 1991). The stoichiometric constraint in glycine synthesis shown in this work implies that although human metabolism can synthesize glycine, its capability has a fixed limit. This agrees with Christensen's (1982) remark that "the fact that a given amino acid can be synthesized in metabolism does not mean it is enough to sustain all metabolic needs". Glycine deficiency could be a major cause of a number of health problems caused by an insufficient supply of glycine, such as arthrosis, osteoporosis and others related to insufficient synthesis and renovation of collagen.

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References

Ackernecht E H 1953 Paleopathology; in *Anthropology today: an Encyclopedic inventory* (ed.) A L Kroeber (Chicago: University of Chicago Press)

Baker D H 2005 Comparative nutrition and metabolism: explication of open questions with emphasis on protein and amino acids; *Proc. Natl. Acad. Sci. USA* **102** 17897–17902

Bienkowski R S, Baum B J and Crystal R G 1978 Fibroblasts degrade newly synthesized collagen within the cell before secretion; *Nature (London)* **276** 413–416

Bonard E C 1987 The rhinoceros horn syndrome (an unusual form of neuropathy and arthrosis of the feet); *Rev. Med. Suisse Romande* **107** 855–856

Boogerd F, Bruggeman F J, Hofmeyr J H S and Westerhoff H V 2007 *Systems biology: philosophical foundations* (New York: Wiley)

Case, G L and Benevenga, N J 1977 Significance of formate as an intermediate in the oxidation of the methionine, S-methyl-L-cysteine and sarcosine methyl carbons to CO_2 in the rat; *J. Nutr.* **107** 1665–1676

Christensen H N 1982 Interorgan amino acid nutrition; *Physiol. Rev.* **62** 1193–1233

Christensen K E and MacKenzie R E 2006 Mitochondrial one-carbon metabolism is adapted to the specific needs of yeast, plants and mammals; *Bioessays* **28** 595–605

Cornish-Bowden A 2004 *The pursuit of perfection* (Oxford: Oxford University Press)

Cornish-Bowden A 2006 Putting the systems back into systems biology; *Persp. Biol. Med.* **49** 475–489

Cornish-Bowden A and Cárdenas M L 2001 Information transfer in metabolic pathways: effects of irreversible steps in computer models; *Eur. J. Biochem*. **268** 6616–6624

Daran-Lapujade P, Rossell S, van Gulik W M, Luttik M A H, de Groot M J L, Slijper M, Heck A J R, Daran J M, de Winde J H, Westerhoff H V, Pronk J T and Bakker B M 2007 The fluxes through glycolytic enzymesin Saccharomyces cerevisiae are predominantly regulated at posttranscriptional levels; *Proc. Natl. Acad. Sci. USA* **104** 15753–15758

de Koning T J, Duran M, Dorland L, Gooskens R, Van Schaftingen E, Jaeken J, Blau N, Berger R and Poll-The B T 1998 Beneficial effects of L-serine and glycine in the management of seizures in 3-phosphoglycerate dehydrogenase deficiency; *Ann. Neurol*. **44** 261–265

de Koning T J, Snell K, Duran M, Berger R, Poll-The B T and Surtees R 2003 L-serine in disease and development; *Biochem. J*. **371** 653–661

Edwards G E, Furbank R T, Hatch M D and Osmond C B 2001 What does it take to be C_4? Lessons from the evolution of C_4 photosynthesis; *Plant Physiol*. **125** 4646

Fell D 1997 *Understanding the control of metabolism* (London: Portland Press)

Gibson N R, Jahoor F, Ware L and Jackson A A 2002 Endo-genous glycine and tyrosine production is maintained in adults consuming a marginal-protein diet; *Am. J. Clin. Nutr*. **75** 511–518

Greer M, Greer J K and Gillingham G 1977 Osteoarthritis in selected wild animals; *Proc. Okla. Acad. Sci*. **57** 39–44

Heinrich R, Montero F, Klipp E, Waddell T G and Meléndez-Hevia E 1997 Theoretical approaches to the evolutionary optimization of glycolysis: thermodynamic and kinetic constraints; *Eur. J. Biochem*. **243** 191–201

Hofmeyr J H S and Cornish-Bowden A 2000 Regulating the cellular economy of supply and demand; *FEBS Lett*. **476** 47–51

Jacob F 1977 Evolution and tinkering; *Science* **196** 1161–1166

Jackson A A 1991 The glycine story; *Eur. J. Clin. Nutr*. **45** 59–65

Jackson A A, Persaud C, Meakins T S and Bundy R 1996 Urinary excretion of 5-L-oxoproline (pyroglutamic acid) is increased in normal adults consuming vegetarian or low protein diets; *J. Nutr.* **126** 2813–2822

Jurmain R 2000 Degenerative joint disease in African great apes: an evolutionary perspective; *J. Hum. Evol*. **39** 185–203

Kacser H 1987 On parts and wholes in metabolism; in *The organization of cell metabolism* (ed.) G R Welch and J S Clegg (New York: Plenum Press) pp 327–337

Lamers Y, Williamson J, Gilbert L R, Stacpoole P W and Gregory J F 3rd 2007 Glycine turnover and decarboxylation rate quanti_ed in healthy men and women using primed, constant infusions of [1,2-$^{13}C_2$]glycine and [$^2H_3$]leucine; *J. Nutr*. **137** 2647–2652

Lewis R M, Godfrey K M, Jackson A A and Cameron I T, Hanson, M A 2005 Low serine hydroxymethyltransferase activity in the human placenta has important implicat-ions for fetal glycine supply; *J. Clin. Endocrinol. Metab*. **90** 1594–1598

Matilla B, Mauriz J L, Culebras J M, Gonzalez-Gallego J and Gonzalez P 2002 Glycine: a cell-protecting anti-oxidant nutrient; *Nutr. Hosp*. **17** 2–9

Meléndez-Hevia E 1993 *La Evolución del Metabolismo: hacia la Simplicidad* (Madrid: Eudema)

Meléndez-Hevia E, Waddell T G and Cascante M 1996 The puzzle of the Krebs citric acid cycle: assembling the pieces of chemically feasible reactions, and opportunism in the design of metabolic pathways during evolution; *J. Mol. Evol*. **43** 293–303

Morange M 2008 What history tells us XV. Cyril Norman Hinshalwood (1897–1967) – A chemical dynamic vision of the organic world; *J. Biosci.* **34** 669–672

Nuño J C, Sánchez-Valdenebro I, Pérez-Iratxeta C, Meléndez-Hevia E and Montero F 1997 Network organization of cell metabolism: monosaccharide interconversion; *Biochem. J.* **324** 103–111

Pérez P J and Martínez I 1989 Evidence of temporomandibular arthrosis in the Middle Pleistocene human fossils from Atapuerca/Ibeas (Spain); *J. Paleopathol*. **3** 15–18

Persaud C, Forrester T and Jackson A A 1996 Urinary excretion of 5-L-oxoproline (pyroglutamic acid) is increased during recovery from severe childhood malnutrition and responds to supplemental glycine; *J. Nutr*. **126** 2823–2830

Rothschild B M 1987 Diffuse idiopathic skeletal hyperostosis as reected in the paleontologic record: dinosaurs and early mammals; *Semin. Arthritis Rheum*. **17** 119–125

Rothschild B M and Martin L 1993 *Paleopathology: Disease in the fossil record* (New York: CRC Press)

Schirch V and Szebenyi D M 2005 Serine hydroxymethyltransferase revisited; *Curr. Opin. Chem. Biol.* **9** 482–487

Small J R and Kacser H 1993 Responses of metabolic system to large changes in enzyme activities and effectors. 2. The linear treatment of branched pathways and metabolite concentrations: assessment of the general non-linear case; *Eur. J. Biochem*. **213** 625–640

Straus W and Cave A J E 1957 Pathology and the posture of Neanderthal man; *Q. Rev. Biol*. **32** 348–363

Wallach J D 1967 Degenerative arthritis in a black rhinoceros; *J. Am. Vet. Med. Assoc*. **151** 887–889

Weissengruber G E, Fuss F K, Egger G, Stanek G and Hittmair K M, Forstenpointner, G 2006 The elephant knee joint: morphological and biomechanical considerations; *J. Anat*. **208** 59–72

Xue H H, Sakaguchi T, Fujie M, Ogawa H and Ichiyama A 1999 Flux of the L-serine metabolism in rabbit, human, and dog livers Substantial contributions of both mitochondrial and peroxisomal serine:pyruvate/alanine:glyoxylate aminotransferase; *J. Biol. Chem*. **274** 16028–16033

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