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# A weak link in metabolism: the metabolic capacity for glycine biosynthesis does not satisfy the need for collagen synthesis

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In a previous paper, we pointed out that the capability to synthesize glycine from serine is constrained by the stoichiometry of the glycine hydroxymethyltransferase reaction, which limits the amount of glycine produced to be no more than equimolar with the amount of $C_1$ units produced. This constraint predicts a shortage of available glycine if there are no adequate compensating processes. Here, we test this prediction by comparing all reported fluxes for the production and consumption of glycine in a human adult. Detailed assessment of all possible sources of glycine shows that synthesis from serine accounts for more than 85% of the total, and that the amount of glycine available from synthesis, about 3 g/day, together with that available from the diet, in the range 1.5–3.0 g/day, may fall significantly short of the amount needed for all metabolic uses, including collagen synthesis by about 10 g per day for a 70 kg human. This result supports earlier suggestions in the literature that glycine is a semi-essential amino acid and that it should be taken as a nutritional supplement to guarantee a healthy metabolism.

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## 1. Introduction

Collagen is the protein that characterizes the animal world, as it is the material that allows multicellular flexibility. It appeared with the origin of animal life in the Precambrian explosion (Aouacheria *et al.* 2006) and its history thus spans the same time period as that of animals, around 580 million years (Li *et al.* 1998). It is the most abundant protein in the human body and constitutes more than a quarter of the total protein (Waterlow 2006). The high content of glycine in collagen (one-third of the amino acid residues) implies an important requirement for the availability of this amino acid to support a healthy turnover of collagen, high enough to avoid problems of accumulation of undesirable chemical modifications (glycation and others), as a protein-deficient diet induces decreased turnover of protein, especially collagen (Gibson *et al.* 2002).

Renewal of collagen is not usually regarded as a problem, not only because collagen turnover was long believed to be very slow, but also because glycine is conventionally classified as a non-essential amino acid, as it can be synthesized from serine in the reaction catalysed by glycine hydroxymethyltransferase (EC 2.1.2.1). However, clinical and nutritional studies during the past twenty years indicate that the amount of glycine available in humans is not enough to meet metabolic needs and that a dietary supplement is appropriate (Jackson *et al.* 1987; Jackson 1991; Persaud *et al.* 1996; Li *et al.* 2001; Brawley *et al.* 2003; Waterlow 2006). According to these authors, therefore, glycine should be considered a conditionally essential amino acid. These observations are puzzling, however, because the existence of a pathway to synthesize glycine conflicts with the idea that there could be a glycine deficiency sufficiently large to need a specific dietary supplement, and because it is thought

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that in general pathways can satisfy metabolic demands (Fell 1997).

It was widely believed in the past that collagen turnover is very slow, with a life-span of several years for the molecule, but today it is recognized that it is a very significant proportion of the whole daily protein turnover (Waterlow 2006). Collagen synthesis is a very complex process, rather different from that of most other proteins. The regular ribosomal protein synthesis makes a precursor, the procollagen peptide, and during this process a number of residues of proline and lysine are hydroxylated by procollagen–proline dioxygenase (EC 1.14.11.2), and procollagen–lysine 5-dioxygenase (EC 1.14.11.4). Three propeptide molecules (identical or non-identical, according to the tissue and the collagen type) are then assembled to give the triple helix of procollagen, a soluble molecule that is secreted outside the cell; this is converted into collagen by removal of the *N*- and *C*-propeptides, which are not integrated into the helix structure, by procollagen *N*-endopeptidase (EC 3.4.24.14) and procollagen *C*-endopeptidase (EC 3.4.24.19). Finally, the resulting collagen fibrils are assembled to yield the collagen fibre, the fibrils in the final structure being stabilized by covalent cross-linking (Kadler *et al.* 1996).

Hydroxylation of proline is strictly necessary for the three propeptides of procollagen to be assembled correctly in the triple helix (Perret *et al.* 2001; Mizuno *et al.* 2003). However, this mechanism sometimes fails and a process of fine quality control that we shall call the *procollagen cycle* occurs inside the cell to check the structure. It involves degrading all newly synthesized molecules that are not correctly folded in the triple helix, resynthesizing them, checking their correct triple helix structure, and repeating the degradation–synthesis cycle until the correct structure is achieved, and so a high fraction (30–90%, depending on the tissues and the age of individuals) of the newly synthesized collagen is degraded in this cycle within minutes of its synthesis (Woessner and Brewer 1963; Van Venrooij *et al.* 1974; Bienkowski *et al.* 1978a, b; Bienkowski 1983; Schneir *et al.* 1984; Mays *et al.* 1991; Woessner 1991; Krane *et al.* 1996; Lucattelli *et al.* 2003; Inoue *et al.* 2006). Although there is substantial reutilization of glycine in the procollagen cycle, this still adds to the amount of glycine required.

In a previous paper (Meléndez-Hevia and de Paz-Lugo 2008), we explained the theoretical basis of a constraint that prevents the synthetic pathway from responding to the demand for glycine, and could thus explain the observations of glycine deficiency mentioned above. The stoichiometry of the reaction catalysed by glycine hydroxymethyltransferase (figure 1) requires glycine and tetrahydrofolate-$C_1$ (THF-$C_1$) to be produced in equimolar amounts, regardless of any differences in the metabolic demand of the two products, and regardless of compartmentation or differences in enzyme activity between tissues. As far as THF-$C_1$ is concerned, the potential problem is avoided by the glycine cleavage system, which allows glycine to be diverted to the production of THF-$C_1$. However, the reaction is thermodynamically irreversible, and no known corresponding process allows glycine to be recovered from unwanted THF-$C_1$. As the $C_1$ unit must be released from THF-$C_1$ before THF can be used again for the synthesis of glycine (Krebs *et al.* 1976; N'Diaye *et al.* 1980), the $C_1$ transfer flux must work in coordination with glycine usage if a collapse in glycine synthesis is to be avoided. However, there appear to be no adequate compensating processes in the metabolic network to allow this. As the glycine hydroxymethyltransferase reaction is the principal source of glycine, the maximal capacity for glycine production at steady state cannot significantly exceed the net production of $C_1$ units minus double the flux through the glycine cleavage system (Meléndez-Hevia and de Paz-Lugo 2008). The question that now needs to be answered is whether this quantity is enough to satisfy all the metabolic demand for glycine because, if it is not sufficient, this could resolve the paradox whereby nutritional studies suggest a deficit in a supposedly non-essential amino acid.

It may in fact be impossible for an organism to obtain as much glycine as it needs for the synthesis of porphyrins, purine bases, glutathione and bile salts, detoxification of benzoic acid and, especially, synthesis of collagen and other proteins. This limitation could of course be purely theoretical, as there could be a compensatory mechanism in the metabolic network to counteract the limitation. To assess

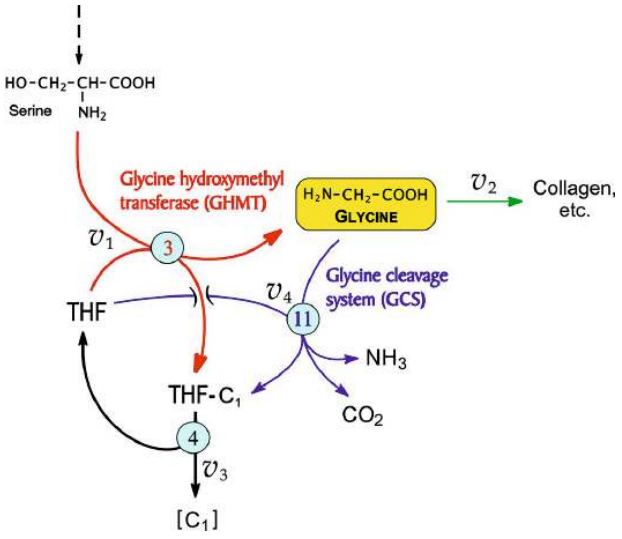



**Figure 1.** Reactions involved in glycine metabolism. THF, tetrahydrofolate; $v_1$, reaction catalysed by glycine hydroxymethyltransferase (EC 2.1.2.1), marked in red; $v_2$, net glycine production; $v_3$, net production of $C_1$ units transferred by tetrahydrofolate; $v_4$, reaction catalysed by the glycine cleavage system (EC 1.4.4.2), marked in blue. In terms of these symbols the glycine constraint requires that $v_2 = v_3 - 2v_4$.

whether it has practical consequences, therefore, we made a thorough assessment of the amount of glycine available from all sources (synthesis as well as diet), and compared it with the amount required for metabolism. Our calculations indicate that the synthesis of glycine is indeed insufficient, in agreement with the nutritional evidence from the past 20 years that it is a conditionally essential amino acid in humans. Insufficiency of glycine is not life-threatening, even in the worst of cases, but a chronic shortage may result in a glycine deficiency syndrome, with detrimental effects on the quality of life.

## 2. Methodological approach

It might appear that experimental studies of collagen turnover and glycine production in rats or other small animals would definitively resolve the question. Unfortunately, however, the problem is greatly complicated by allometric scaling. A 70 kg human has 350 times the mass of a 200 g rat, but neither skeletal mass nor metabolic capacity increase in proportion to body mass. Skeletal mass is proportional to the 1.1 power of body mass (Reynolds and Karlotski 1977), so a human has about $350^{1.1}$, i.e. about 630, times the skeletal mass of a rat, implying about 630 times as much collagen. On the other hand, metabolic capacity increases as the 0.75 power of body mass (Kleiber 1947) and so a human has only $350^{0.75}$, or 81, times the metabolic capacity of a rat. As a result, collagen-related problems are typically found in large animals, not in small ones. These problems accumulate with age, and are especially acute in large animals that live for many years. All of this means that very little information about glycine requirements can be obtained by studying small laboratory animals with short lifetimes.

What is needed, therefore, is a detailed study of the available data on the sources, both dietary and metabolic, and requirements of glycine in the human, and we have accordingly analysed the fluxes of all pathways related to glycine metabolism.

### 2.1 *Glycine synthesis*

Glycine can be synthesized in two ways: (i) the main pathway is synthesis from serine by means of the glycine hydroxymethyltransferase reaction (figure 1); but (ii) some glycine also comes from other sources, sarcosine, glyoxylate, threonine and trimethyllysine (carnitine synthesis), most of them coming directly from dietary compounds. In the hydroxymethyltransferase route, the $C_1$-unit is produced as methylene-THF and, as the amount of glycine produced is directly related to the consumption of $C_1$ units, we need to make a detailed assessment of this consumption in the synthesis of many compounds, including lecithins, creatine and purine bases. Where the data indicate a range, we have taken the value that indicates the maximal glycine production. In addition to the synthesis of glycine, hydrolysis of dietary protein provides between 1.5 and 3 g per day, according to the quality of the diet (Gibson *et al.* 2002).

### 2.2 *Metabolic consumption of glycine*

Glycine is used in metabolism in two ways: (i) as material to build porphyrins, purine bases, creatine, glutathione, bile salts and hippuric acid (as a pathway for detoxification of benzoic acid); and (ii) as amino acid for the synthesis of proteins, especially collagen.

We thoroughly analysed the fluxes of these pathways with data from the literature to obtain a broad view of their metabolic activity in humans *in vivo*. Many of the data have been described by different groups of researchers, not always using the same methodology, but with reasonable agreement. We calculated the daily amount of synthesis or degradation of each product.

As far as possible, the data refer to humans *in vivo*, with measurement, for example, of the kinetics of $^{13}C$-labelled precursors. Where data were available only for other animals (rat, pig or rabbit), we extrapolated the values to the human according to body size, and the results presented here correspond to a hypothetical 70 kg human adult in the age range of 30–50 years. Age and sex differences are discussed where they are significant.

## 3. Results for glycine synthesis

### 3.1 *Synthesis via glycine hydroxymethyltransferase: pathways that consume THF-$C_1$*

The glycine hydroxymethyltransferase reaction yields the $C_1$ unit as methylene-THF, which is the origin of four kinds of reactions:

(i) Reduction by nicotinamide adenine dinucleotide phosphate (NAD[P]H) to methyl-THF, the methyl group being then transferred to adenosyl-homocysteine to give adenosylmethionine, the source of all methylation reactions apart from those in the synthesis of thymine (pathway B in figure 2);

(ii) Transfer of the $C_1$-unit as formyl-THF in two reactions of the purine ring synthesis (pathway shown in red in figure 4);

(iii) Transfer of the $C_1$ unit directly from methylene-THF to deoxyuridylate dUMP by thymidylate synthase (EC 2.1.1.45) to give deoxythymydilate (dTMP); in this reaction the methylene group of

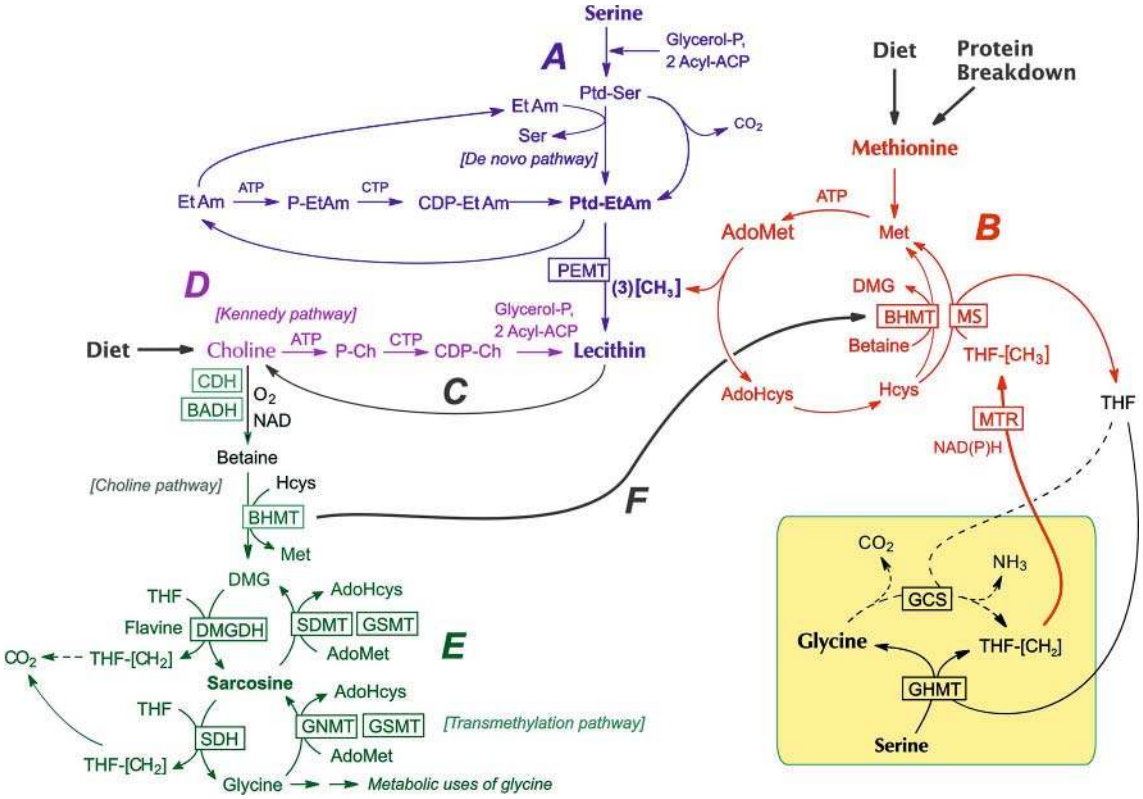
THF-$C_1$ is reduced to methyl by oxidation of THF to dihydrofolate, which is then reduced to THF by NADPH by dihydrofolate reductase (EC 1.5.1.3); *see* figure 4.

(iv) Oxidation of the formyl group to $CO_2$ (pathway shown in red in figure 4).

As all these reactions involve unloading $C_1$ units from THF-$C_1$, they regenerate THF, allowing the production of glycine during human metabolism. Their fluxes need to be analysed, therefore, to know the maximum amount of glycine that can be produced from serine.

The production of glycine and THF-$C_1$ traffic in humans is summarized in table 1, and in the remainder of this section we shall explain the basis for the values given there.

3.1.1 *Traffic of methyl groups by adenosylmethionine:* Lecithin synthesis is a major consumer of adenosylmethionine and so has a major effect on glycine synthesis. It involves the rather complex network shown as pathways A and D in figure 2. There are two pathways, of which the first, *de novo* choline synthesis, starts from serine (pathway A), and this synthesis is achieved by three methyl transfers to phosphatidylethanolamine from adenosylmethionine. The methyl groups of adenosylmethionine can come from THF-$C_1$, which comes in turn from glycine hydroxymethyltransferase, or from betaine. When they come from THF-$C_1$,




**Figures 2.** Metabolic pathways involved in methyl transfers (Cook and Wagner 1984; Mudd and Poole 1975; Mudd *et al.* 1980, 2001). **Pathways: A** (blue), pathway that involves *de novo* synthesis of choline; **B** (red), origin of $C_1$ units, which involves net glycine synthesis by glycine hydroxymethyltransferase, and the transfer of $C_1$ units to homocysteine in the adenosylmethionine cycle; **C**, degradation of lecithins as their regular turnover yields free choline; **D** (purple), synthesis of lecithin from free choline by the Kennedy pathway; **E** (green), degradation of choline that yields glycine directly from choline skeleton carbon, and pathway of sarcosine synthesis; **F**, remethylation of homocysteine by betaine. **Intermediates:** AdoHcys, adenosylhomocysteine; AdoMet, adenosylmethionine; DMG, dimethylglycine; EtAm, ethanolamine; Fal, formaldehyde; Hcys, homocysteine; Met, methionine; Ptd-EtAm, phosphatidylethanolamine; Ptd-Ser, phosphatidylserine; THF, tetrahydrofolate; THF-[$CH_2$], methylene-THF; THF-[$CH_3$], methyl-THF. **Enzymes**: BADH; betaine-aldehyde dehydrogenase (EC 1.2.1.8); BHMT, betaine-homocysteine methyl transferase (EC 2.1.1.5); CDH, choline dehydrogenase (EC 1.1.99.1) coupled with betaine-aldehyde dehydrogenase (EC 1.2.1.8); DMGDH, dimethylglycine dehydrogenase (EC 1.5.99.2); GCS, glycine cleavage system (EC 1.4.4.2); GNMT, glycine *N*-methyltransferase (EC 2.1.1.20); GHMT, glycine hydroxymethyltransferase (EC 2.1.2.1); GSMT, glycine/sarcosine *N*-methyltransferase (EC 2.1.1.156); pathway of sarcosine synthesis by transmethylation. MS, methionine synthase (EC 2.1.1.13); MTR, methylene-THF reductase (NAD(P)H) (EC 1.5.1.20); PEMT, phosphatidylethanolamine *N*-methyltransferase (EC 2.1.1.17); SDH, sarcosine dehydrogenase (EC 1.5.99.1); SDMT, sarcosine-dimethylglycine *N*-methyltransferase (EC 2.1.1.157).

**Table 1.** Daily glycine production and tetrahydrofolate (THF)-$C_1$ traffic in humans[a]

| Source | THF-$C_1$ flux | Glycine production | |
|---|---|---|---|
| | mmol/day | mmol/day | mg/day |
| Methyl groups (AdoMet)[b] | 14.55 | 14.55 | 1092 |
| Purine bases[c] | 5.50 | 5.50 | 413 |
| Thymine | 6.22 | 6.22 | 467 |
| $C_1$ units excreted as $CO_2$[d] | 10.73 | 10.73 | 805 |
| Histidine degradation | –3.21 | –3.21 | –240 |
| *Subtotal from serine via GHMT* | | 33.69 | 2537 |
| Endogenous sarcosine | | 1.89 | 142 |
| Glyoxylate | | 1.17 | 88 |
| Carnitine | | 0.08 | 6 |
| *Subtotal from endogenous sources independent of GHMT* | | 3.14 | 236 |
| Sarcosine from dietary choline | | 1.43 | 107 |
| Dietary threonine | | 0.29 | 22 |
| *Subtotal from dietary sources* | | 1.72 | 129 |
| *Total glycine production from all sources* | | 38.55 | 2902 |

[a]Sources of the data and calculations are explained in the text.
[b]Glycine synthesis by glycine hydroxymethyltransferase (GHMT). Expenditure of $C_1$ equivalents signifies an equimolar glycine production as maximum (*see* figure 1).
[c]Purine base formation consumes one glycine equivalent and releases two glycine equivalents (section 3.1.2). To be as explicit as possible, we show 5.50 mmol/day produced in this table, but later (table 2) we shall subtract the 2.75 mmol/day consumed.
[d]All $C_1$ units excreted as $CO_2$ must be balanced by production of an equivalent amount of THF-$C_1$, and hence of glycine.

this synthesis means a net production of three glycine molecules per choline residue. The second route is the Kennedy pathway (pathway D), which facilitates recycling of the choline released from lecithin turnover (pathway C) without consuming methyl groups, and thus does not affect glycine synthesis.

The magnitudes of most of these fluxes and the kinetics of the pathways have been extensively studied *in vivo* in humans, rats, pigs and rabbits. Net choline synthesis in rat has been reported to be 70 $\mu$mol/day per 100 g of rat (Wise and Elwyn 1965), which corresponds to 49 mmol/day for a 70 kg human adult. Total methyl group traffic has been reviewed recently by Stead *et al.* (2006) and Mudd *et al.* (2007). Based on studies of infusion of stable isotopes, these last authors concluded that the transmethyl rate in a young person is 17–23 mmol/day. However, the net production of methyl groups is less than the total flux, as a significant fraction is recycled through the betaine pathway (pathway E, which can include dietary choline as donor), without involving new donations from THF-$C_1$.

The key to evaluating the net expenditure of THF-$C_1$ is the flux of homocysteine remethylation by methionine synthase (EC 2.1.1.13) or by homocysteine methyltransferase (EC 2.1.1.14). However, since this $C_1$ can also be supplied by histidine catabolism (*see* section 3.1.5), we have to take into account only the $C_1$ flux by THF from serine. This has been determined in humans by Davis *et al.* (2004) by infusing [3-$^{13}$C]serine and assaying its incorporation in the methyl group of methionine. They measured the rate of folate-dependent homocysteine remethylation from serine directly, and so distinguished it from other THF-$C_1$ sources, as well as from homocysteine remethylation from betaine. They reported the flux for this process to be about 15 mmol/day (about 30% of the total methyl-methionine flux), which means a glycine synthesis of about 1.1 g/day.

This flux is the net origin of newly synthesized methyl groups transferred by adenosylmethionine, and includes all methyl transfers that involve glycine synthesis apart from that of thymine, which is supplied directly by THF-$C_1$, namely, for the synthesis of lecithins (including those excreted through biliary secretion), creatine, trimethyllysine, carnitine and others, as well as any methylated compounds (such as trimethylamine and dimethylamine) that are excreted in the urine, and also oxidation of synthesized methyl groups to $CO_2$ by the sarcosine system. We consider all these fluxes below.

3.1.2 *Biosynthesis of purine bases:* Purine synthesis is also a net source of glycine, despite the fact that it consumes glycine. Synthesis of each purine molecule consumes one glycine plus two $C_1$ units supplied from THF-$C_1$ (figure 3).

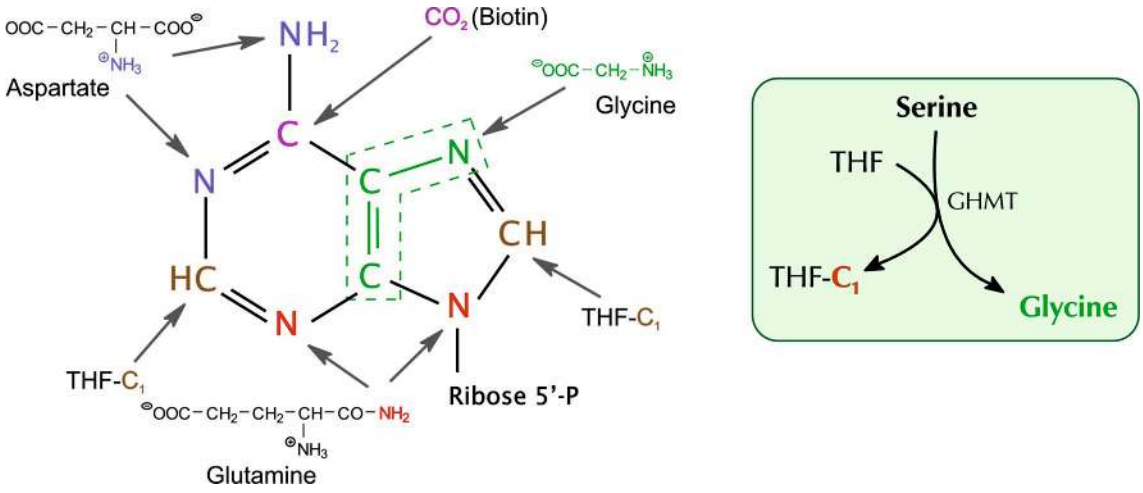



**Figure 3.** Metabolic origin of the atoms in the purine ring. Glycine is incorporated in the ring synthesis by phosphoribosylamine–glycine ligase (EC 6.3.4.13). $C_1$ units are transferred from formyl-tetrahydrofolate (THF) by phosphoribosylglycinamide formyltransferase (EC 2.1.2.2) and 5-amino-4-imidazole carboxamimide ribotide (AICAR) formyltransferase (EC 2.1.2.3). As each molecule of serine yields one glycine plus one $C_1$ unit stoichiometrically (green box), the expenditure of two $C_1$ units for the synthesis of purine means that two molecules of serine are consumed with the net production of one glycine molecule.

Thus, two molecules of serine, yielding two $C_1$ units plus two glycine molecules, are used in the synthesis of each purine molecule, leaving one molecule of glycine available for other purposes.

The amount of glycine produced daily through this process may be calculated as follows. Nucleotide synthesis occurs continuously in metabolism, with a net synthesis at all times, even though an important amount of nucleotides derived from RNA and DNA degradation is recycled. As humans cannot degrade purines beyond uric acid, measurement of the excretion of uric acid allows the purine synthesis flux to be calculated. Bowering *et al.* (1970) reported a daily uric acid excretion of about 2.75 mmol in individuals with a low purine diet. This means that purine synthesis also amounts to 2.75 mmol/day, and therefore the amount of $C_1$ transferred is double that (5.5 mmol/day), corresponding to a net daily synthesis of glycine of 2.75 mmol.

3.1.3 *Biosynthesis of thymine*: Thymine synthesis consumes $C_1$ units for its methyl group, which is provided directly from methyl-THF by thymidylate synthase (EC 2.1.1.45), or by thymidylate synthase (FAD) (EC 2.1.1.148). As the expenditure of each methyl group implies net production of one glycine molecule, the synthesis of thymine implies equimolar glycine production.

Thymine is used only for DNA synthesis. From data on the lifetimes of different tissues, the total daily cell division can be estimated at around $1.3 \times 10^{12}$ cells/day, about 2.6% of all the cells in the human body (around $5 \times 10^{13}$), as given, for example, by Baserga (1976). Assuming that every cell has the same genome with $6.4 \times 10^9$ base pairs (diploid), and that A–T pairs make up 60% of these, each cell contains $3.8 \times 10^9$ thymine residues in its genome. The total daily consumption of thymine is thus $3.8 \times 10^9 \times 1.3 \times 10^{12} = 5 \times 10^{21}$ molecules = 8.3 mmol. However, this does not mean a net synthesis of thymine as such, because some is recycled through the salvage pathway, estimated to represent 25–60% (Taheri *et al.* 1981). Let us take the minimal value (25%) of recycling (as the most favourable conditions for glycine synthesis), and so 75% of synthesis is *de novo*. The daily synthesis of thymine is therefore 6.2 mmol, which produces an equimolar amount of glycine.

3.1.4 *Oxidation of formyl and formate to $CO_2$: a sink of $C_1$ units*: Figure 4 shows the pathways involved in formyl-THF and formate metabolism, as well as some related pathways involving methionine metabolism and traffic of methyl groups. Methyl groups are formed by the reduction of $N^5$,$N^{10}$-methylene-THF by 5,10-methylene-THF reductase, and are transferred to homocysteine for all methylation reactions by adenosylmethionine.

A way to escape from the glycine hydroxymethyltransferase constraint, which makes glycine synthesis strongly dependent on the transfer of $C_1$ units from THF-$C_1$ to the different acceptors, is to oxidize the formyl groups of THF-$C_1$ to $CO_2$, which is a real sink of $C_1$ units. This can be made from $N^{10}$-formyl-THF by formyl-THF dehydrogenase, or from free formate by formate dehydrogenase or by the peroxidase–catalase system (*see* figure 4). In principle, free formate could also be released from formyl-THF by formyl-THF synthetase, but this is unlikely because it is thermodynamically unfavoured, and this reaction mainly works as a way to metabolize free formate coming from diet precursors. However, as we analyse below, the effect of this

sink is modest (a maximum of 30%) and is insignificant in relation to the total demand for glycine.

The complexity of this metabolic network makes a full evaluation of the sink *in vivo* very difficult, for three reasons: (i) assay of labelled carbon in $CO_2$ coming from a source of $C_1$ units such as serine or glycine does not give us the sink rate, because that $CO_2$ can be produced by serine or glycine catabolism via the Krebs cycle through the serine oxidation pathways, which is obviously not a sink of THF-$C_1$ units; (ii) formate and its precursors (methanol and formaldehyde) can also come from exogenous sources, as they are present in certain components of the diet; (iii) free formate is also

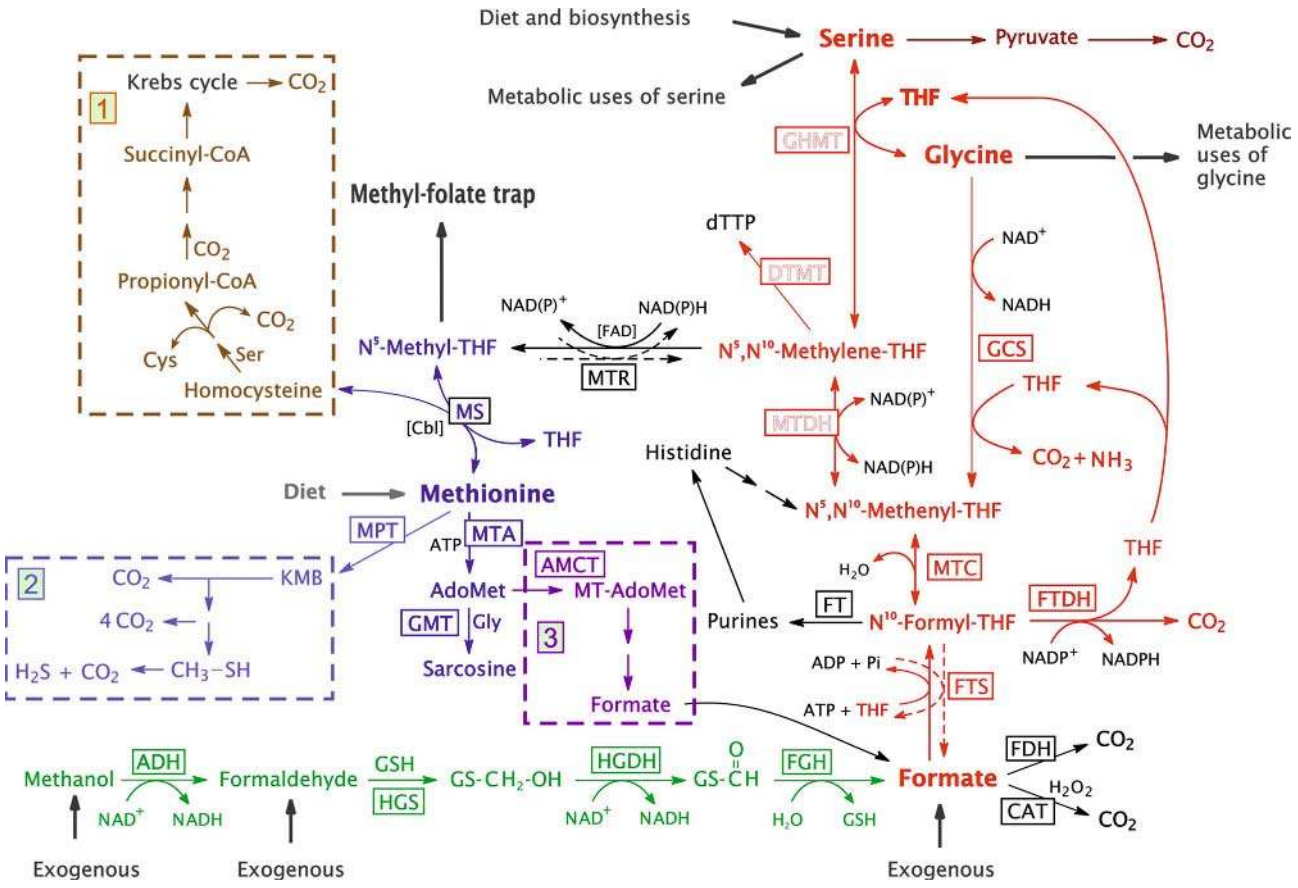



**Figure 4.** Pathways involved in the metabolism of formate, formyl-tetrahydrofolate (THF) and methionine oxidation. This network is also a means for methanol and formaldehyde (or even formate) detoxification by incorporating them into the THF pathway, or by direct oxidation of the free formate to $CO_2$ (pathway marked in green). Oxidation of endogenous formate or formyl group to $CO_2$ works as a sink of $C_1$ units, allowing some increase in the capability for glycine synthesis (pathway marked in green). **Intermediates**: AdoMet, adenosylmethionine; GS-, glutathione derivative; GSH, glutathione; KMB, *α*-keto-*γ*-methylthiobutyrate; THF, tetrahydrofolate. **Enzymes**: ADH, alcohol dehydrogenase (EC 1.1.1.1); AMCT, adenosylmethionine cyclotransferase (EC 2.5.1.4); CAT, peroxidase-catalase system, which has virtually no activity in humans and primates (McMartin *et al.* 1977); DTMT, deoxycytidylate *C*-methyltransferase (EC 2.1.1.54); FDH, formate dehydrogenase (EC 1.2.1.2); FGH, *S*-formylglutathione hydrolase (EC 3.1.2.12); FT, formyl transferases involved in the purine synthesis pathway: phosphoribosylglycinamide formyltransferase (EC 2.1.2.2) and AICAR formyltransferase (EC 2.1.2.3); FTDH, formyl-THF dehydrogenase (EC 1.5.1.6); FTS, formyl-THF synthetase (EC 6.3.4.3); GCS, glycine cleavage system (EC 1.4.4.2); GHMT, glycine hydroxymethyltransferase (EC 2.1.2.1); GMT, glycine *N*-methyltransferase (EC 2.1.1.20); HGDH, *S*-hydroxy-methylglutathione dehydrogenase (EC 1.1.1.284); HGS, *S*-hydroxy-methylglutathione synthase (EC 4.4.1.22); MPT, methionine pyruvate transaminase (EC 2.6.1.41), a conversion that can also be made by methionine glyoxylate transaminase (EC 2.6.1.73); MTA, methionine adenosyl-transferase (EC 2.5.1.6); MTC, methenyl-THF cyclohydrolase (EC 3.5.4.9); MTDH, methylene-THF dehydrogenase ($NADP^+$) (EC 1.5.1.5), or ($NAD^+$) (EC 1.5.1.15); MTR, 5,10-methylene-THF reductase (EC 1.5.1.20), a reaction that has classically been considered irreversible (Lumb *et al.* 1988, 1989a, b), although there is evidence that it can work in the opposite direction (Case and Benevenga 1977); MS, methionine synthase (EC 2.1.1.13). The dashed boxes indicate additional routes that complicate the calculation of oxidation of $C_1$ units (*see* text): Pathways of methionine degradation: 1 (brown border), classical trans-sulphuration pathway; 2 (light blue border), transamination/trans-sulphuration pathway (most of the reactions of this route are still not well characterized); 3 (purple border), adenosylmethionine–formate pathway.

produced during metabolism (mainly from the methyl group of methionine) and it can be oxidized to $CO_2$ independently of THF metabolism.

Case and Benevenga (1977) have assayed the fluxes of oxidation of several carbon precursors to $CO_2$ in rats by including different $^{14}C$-substrates in the diet. As the C-2 of the imidazole ring of histidine (which yields the $C_1$ unit: *see* figure 4) is only catabolized to THF-$C_1$, as a formimino residue, the design of the experiments using histidine labelled in this atom allowed the total oxidation of the $C_1$ units to $CO_2$ via formyl-THF to be calculated. In contrast, other amino acids, such as methionine and serine, are not useful for this estimation because the carbon can follow other routes that do not involve THF-$C_1$ traffic (*see* boxes 1 and 2 in figure 4). Their results gave an oxidation rate of this carbon to $CO_2$ of about 30% of the total histidine catabolism flux which, as they state, represents the maximum percentage release of formate from carbon oxidized solely by way of THF.

3.1.5 *Histidine catabolism*: Histidine catabolism releases one $C_1$ unit as a formimino residue, which is directly incorporated in the THF-$C_1$ pool. Thus, dietary histidine causes a net reduction in the capacity for synthesizing glycine. The recommended dietary intake of histidine is 700 mg (4.5 mmol) per day (FAO/WHO/UNU 1985), which agrees with its regular intake in adult humans. This implies that the same amount of histidine is degraded, yielding an equimolar amount of $C_1$ units, and reducing the glycine synthesis capacity by the same extent. However, as in the THF-$C_1$ flux, 30% of these $C_1$ units are oxidized to $CO_2$, and so the actual reduction of glycine synthesis is 70% of this flux (3.2 mmol, or 240 mg).

### 3.2 *Synthesis of glycine independent of glycine hydroxymethyltransferase*

3.2.1 *Sarcosine metabolism*: Sarcosine (methylglycine) is a source of glycine independent of glycine hydroxymethyltransferase, by oxidative transfer of the methyl group to THF (pathway E in figure 2). On the other hand, sarcosine synthesis goes through two pathways: (a) from glycine by transfer of a methyl group from adenosylmethionine, and (b) from choline, by oxidation of its hydroxymethyl group to carboxylate giving betaine, and then elimination of two methyl groups by adenosylhomocysteine and THF. Coupling of the first sarcosine synthesis pathway with its degradation makes the glycine–sarcosine cycle which, obviously, does not imply net glycine synthesis. Thus, the choline pathway is the only one in this scheme capable of producing net glycine synthesis. This choline can, in turn, come from endogenous synthesis (from serine), or from the diet.

For the purpose of our calculations, we need to know the flux from choline to sarcosine, as it means a net glycine synthesis flux independent of glycine hydroxymethyltransferase. The methyl group fluxes involved in these conversions must not be taken into account a second time for our calculations, as they have already been considered above. Our calculations on these pathways must, therefore, be limited to the direct production of glycine from choline (endogenous or exogenous) via sarcosine.

The fluxes involved in sarcosine metabolism have been studied in patients who excrete large amounts of sarcosine in their urine on account of specific genetic defects in their sarcosine-oxidizing systems (Mudd *et al.* 1980, 2007; Mudd and Poole 1975). As sarcosine is not excreted to any significant extent in the urine of normal subjects (about 0.01–0.02 mmol/day in normal urine), the sarcosine excreted by these patients provided a measure of sarcosine endogenously formed and subsequently metabolized by sarcosine dehydrogenase in the normal human. Maintaining these patients on constant diets with differing amounts of methionine, choline (or choline derivatives) and glycine, Mudd *et al.* (2007) concluded that normal daily sarcosine synthesis (normalized for a 70 kg person) is about 6.2 mmol. On the other hand, using kinetic data and metabolite concentrations, and knowing that most of the total flux of these pathways occurs in the liver, they estimated a possible distribution of the two contributions to the total flux of sarcosine synthesis as 53.5% from choline and 46.5% from glycine, on a normal equilibrated diet containing about 3.6 mmol of choline daily (Mudd *et al.* 1980). Thus, according to their conclusions, the fluxes of sarcosine, normalized for a 70 kg person, would be a total synthesis of 6.2 mmol/day, of which 3.3 mmol/day comes from choline, and 2.9 mmol/day from glycine. As all sarcosine is converted ultimately into glycine, this last value gives us the flux of the glycine–sarcosine cycle, and the previous one (3.3 mmol/day) the net conversion of choline (dietary and endogenous) to glycine. This value varies according to the amount of choline in the diet, and may decrease to 1.9 mmol/day with a minimal choline diet (Mudd *et al.* 1980), representing endogenous sarcosine synthesis, the difference of 1.4 mmol/day (3.3–1.9) corresponding to sarcosine derived from dietary choline.

3.2.2 *Glyoxylate*: The main pathways of glyoxylate metabolism in humans are shown in figure 5 (Baker *et al.* 2004; Behnam *et al.* 2006; Salido *et al.* 2006). Alanine-glyoxylate transaminase (reaction 5 in figure 5) catalyses the main reaction of that route, working in the direction of glycine production, with glycolate being the main source of glyoxylate. Although certain pathways of glycolate production have not been fully explored at present, despite their obvious importance, the general glycolate elimination pathway is its conversion into glyoxylate and its elimination through conversion into glycine. Glyoxylate is also a product of hydroxyproline degradation, so it can be a source of glycine, as Ruiz-Torres and Kürten (1976) pointed out. Some glyoxylate is in any case oxidized to oxalate

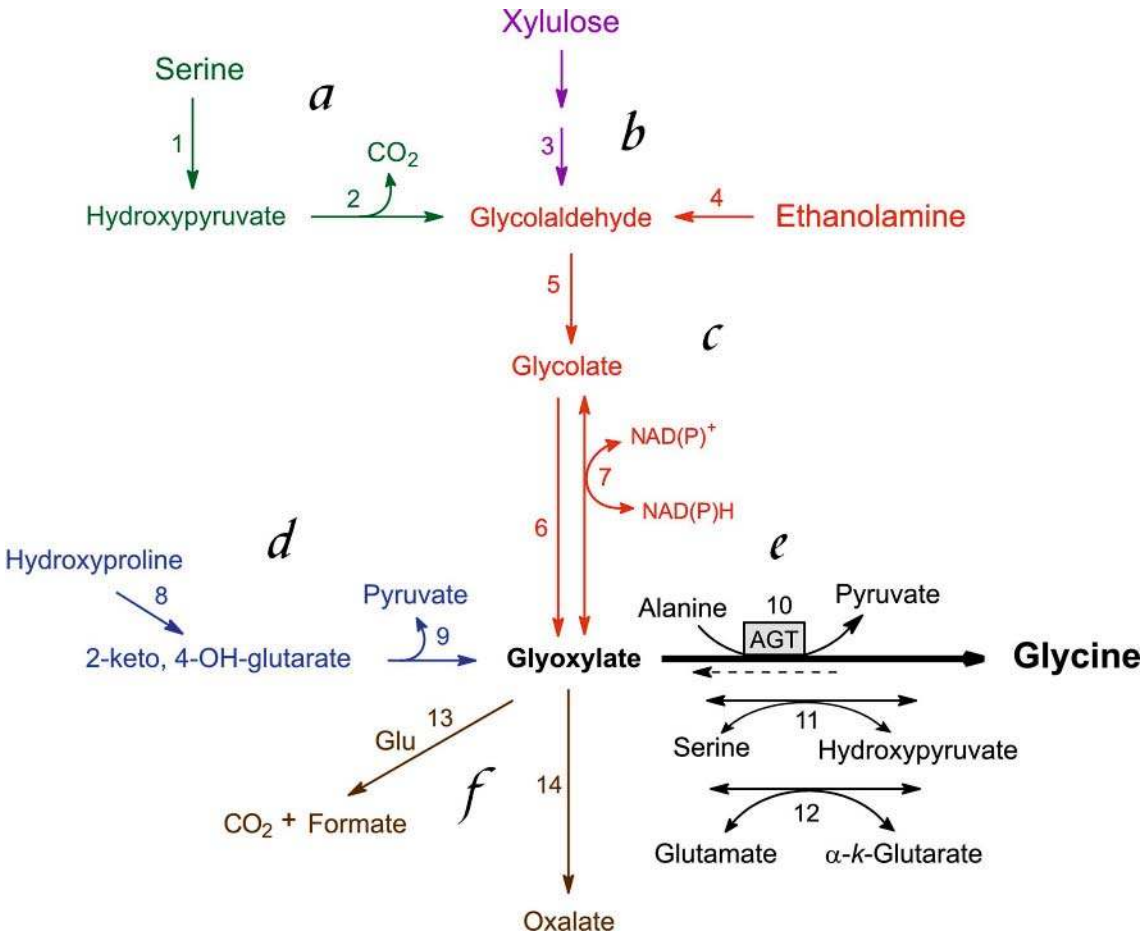



**Figure 5.** Pathways of glyoxylate metabolism. Routes that yield glycine through glyoxylate from several sources, independently of glycine hydroxymethyltransferase activity. ***(a)*** *Production of glycolaldehyde from serine* (marked in green): 1, serine 2-dehydrogenase (EC 1.4.1.7); 2, hydroxypyruvate carboxylase (EC 4.1.1.40); ***(b)*** *Production of glycolaldehyde from xylulose derivatives* (marked in purple): 3, reaction catalysed by fructose bis-phosphate aldolase (EC 4.1.2.13); ***(c)*** *Glycolate pathway* (marked in red): 4, ethanolamine oxidase (EC 1.4.3.8); 5, aldehyde dehydrogenase (EC 1.2.1.3); 6, glycolate oxidase (EC 1.1.3.15); 7, glyoxylate reductase ($NAD^+$) (EC 1.1.1.26) or glyoxylate reductase ($NADP^+$) (EC 1.1.1.79); ***(d)*** *Hydroxyproline pathway* (marked in blue): 8, hydroxyproline degradation from collagen breakdown; 9, 4-hydroxy-2-oxoglutarate aldolase (EC 4.1.3.16). ***(e)*** *Transamination reactions that convert glyoxylate into glycine:* 10, alanine-glyoxylate transaminase (AGT) (EC 2.6.1.44), the main reaction for this step; 11, serine glyoxylate transaminase (EC 2.6.1.45); also catalysed by aromatic amino acid-glyoxylate transaminase (EC 2.6.1.60); 12, glyoxylate-glutamate transminase (EC 2.6.1.4); ***(f)*** *Glyoxylate degradation* (marked in brown): 13, decarboxylation of glyoxylate (Crawhall and Watts 1962); 14, oxidation of glyoxylate to oxaloacetate by glyoxylate oxidase (EC 1.2.3.5). Notice that as hydroxypyruvate is produced in reaction 11 it can in principle be recycled in the production of glycolaldehyde (reaction 2), implying an additional source of glycine.

or decarboxylated to formate (without yielding glycine). Glyoxylate production is, therefore, an additional source of glycine, as alanine glyoxylate transaminase operates *in vivo* from glyoxylate to glycine.

The flux of the transaminase reaction that converts glyoxylate into glycine can be estimated from experimental data. The oxalate concentration in human urine is usually low, but is increased in a series of diseases known as hyperoxalurias. Primary hyperoxaluria type I is a rare autosomal recessive disease caused by mutations in the transaminase gene, provoking a lack or diminished activity of the enzyme, which leads to an increase in the conversion of glyoxylate into oxalate, this being excreted in urine. Salido *et al.* (2006) have presented an interesting experimental model of this disease by cloning mice without expression of the transaminase gene. These mice showed severe hyperoxaluria, with calcium oxalate calculi in the urinary tract. Thus, the concentration of oxalate in the urine of mutant mice compared with that in normal ones provides a base for estimating the normal rate of flux through the transaminase. The oxalate concentration in the urine of normal mice was 0.31–0.34 mM, in agreement with the

human reference data (0.1–0.5 mmol/day), whereas mutant mice yielded 1.0–1.2 mM. The difference of 0.8 mM is, therefore, the total amount of glyoxylate converted into glycine in normal subjects. Considering a human urine secretion of 1.5 l/day, the value calculated gives 1.2 mmol/day. This means that an equimolar amount of glycine is synthesized through this reaction in normal subjects.

3.2.3 *Degradation of threonine:* As threonine is a totally indispensable amino acid in the diet for human metabolism, its metabolic flux in an adult human is just its degradation flux, which equals its intake. The three possible pathways for threonine degradation are shown in figure 6. Only one of the three threonine degradation pathways produces glycine (threonine dehydrogenase and glycine C-acetyl transferase [TDH-GAT]) as threonine aldolase (TAL) is inactive in mammals. These fluxes have been assayed in human metabolism *in vivo* by several authors, and there is a general agreement that threonine is a poor source of glycine (Yeung 1986; Parimi *et al.* 2005), and no more than 7% of the threonine catabolized is converted to glycine (House *et al.* 2001). The recommended regular intake of threonine (FAO/WHO/UNU 1985) for a 70 kg human adult is 490 mg (4.1 mmol) per day, and 7% of this (0.3 mmol) is the daily production of glycine in this pathway.

3.2.4 *Biosynthesis of carnitine*: The pathway for synthesis of L-carnitine is shown in figure 7. It is synthesized from trimethyllysine, which comes from protein breakdown, yielding a glycine molecule as a byproduct. Carnitine is excreted in the urine, as no pathway for its degradation exists in humans (Vaz and Wanders 2002; Rebouche and Seim 1998). An unusual feature of this pathway is that its precursor (trimethyllysine) is only supplied from protein breakdown, as *N*-methylation of lysine residues occurs at a post-translational stage. Although the net carnitine synthesis implies the incorporation of three methyl groups from adenosylmethionine to lysine, we do not have to consider these transfers now for our calculations, as they have been taken into account already in the total adenosylmethionine flux.

Carnitine synthesis and excretion vary with the amount in the diet. It is only present in foods of animal origin, and its synthesis in strict vegetarian humans (Rebouche and Engel 1984; Rebouche *et al.* 1993) is about 0.084 mmol/day, which is insignificant for glycine production.

### 3.3 *Summary of the capability for glycine synthesis*

The results of the calculations explained above on the capability for glycine synthesis are listed in table 1, considering separately those that depend on glycine hydroxymethyltransferase, those from other metabolic sources, and those that depend on dietary components. It may be seen that the total glycine production amounts to about 3 g/day. Values are shown with the precision reported by the original authors, though in most cases there must be more uncertainty

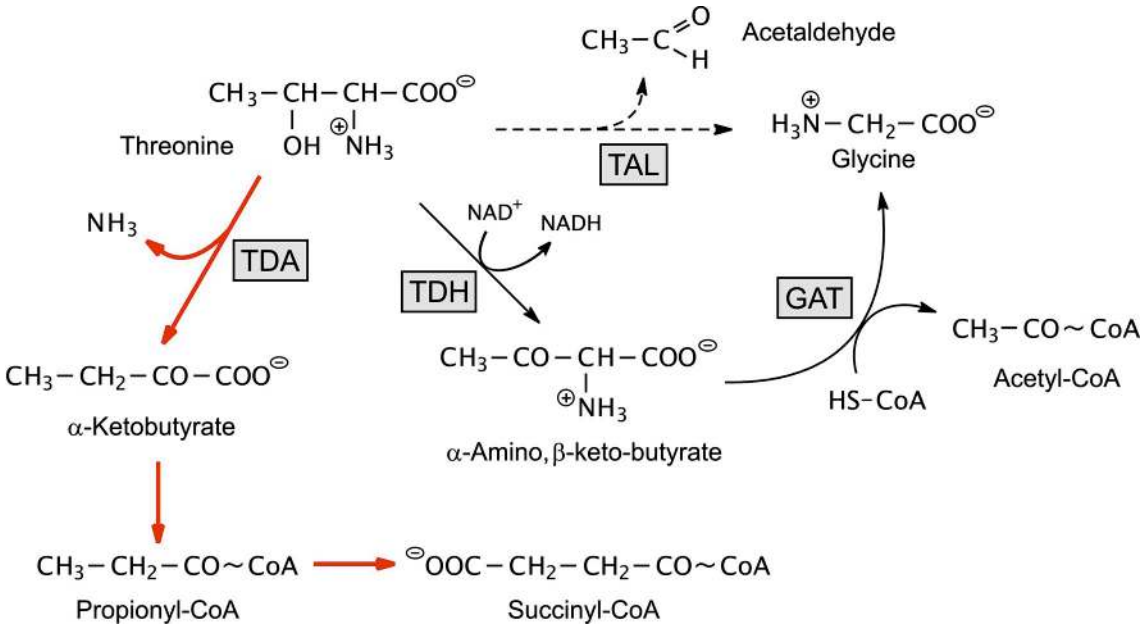



**Figure 6.** Pathways for threonine degradation. TAL, threonine aldolase (EC 4.1.2.42), virtually inactive in mammals (Yeung 1986); TDA, threonine deaminase (or threonine dehydratase) (EC 4.3.1.19), the main pathway in human metabolism, accounting for 93% of the total flux (shown in red); TDH, threonine dehydrogenase (EC 1.1.1.103); GAT, glycine C-acetyl transferase (EC 2.3.1.29), the only pathway that yields glycine, but its activity is very low, accounting for only 7% of the threonine degradation flux (House *et al.* 2001).

than the numbers suggest. To the 3 g/day available from metabolism may be added about 2.5 g/day from hydrolysis of dietary protein, the exact amount varying between about 1.5 g/day for a low-protein diet to about 3 g/day for a high-protein diet (Gibson *et al.* 2002).

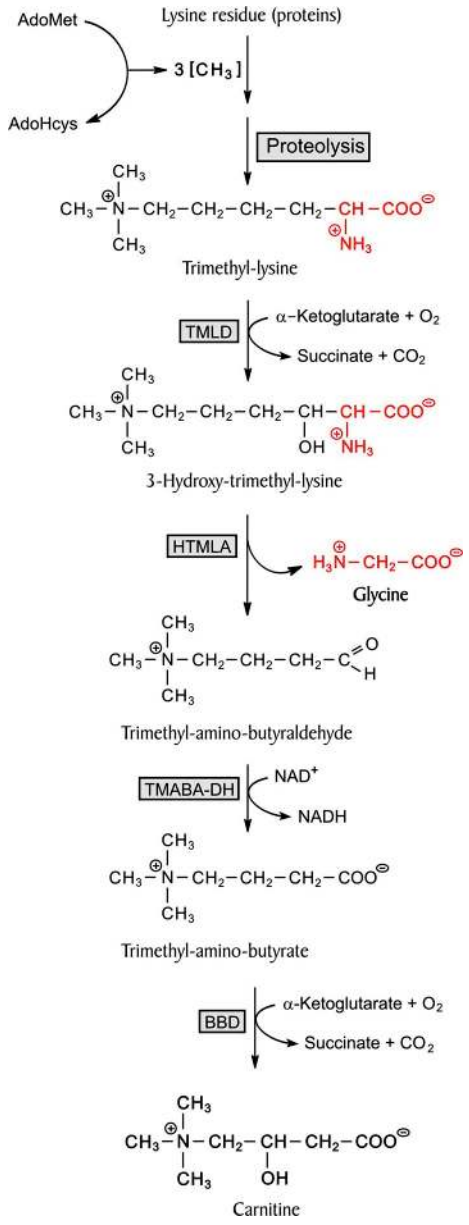



**Figure 7.** Pathway of carnitine biosynthesis (Vaz and Wanders 2002). Lysine is methylated as a protein residue, so trimethyllysine originates from proteolysis. Taking into account that the production of glycine by the production of the methyl groups (which come from tetrahydrofolate [THF]-$C_1$ via adenosylmethionine) have already been considered inside the methyl traffic in our calculations (*see* Section 3.1), this pathway has a net yield of one glycine molecule. TMLD, trimethyllysine dioxygenase (EC 1.14.11.8); HTMLA, hydroxytrimethyllysine aldolase (no EC number assigned); TMABA-DH, trimethylaminobutyraldehyde dehydrogenase (EC 1.2.1.47); BBD, γ-butyrobetaine dioxygenase (EC 1.14.11.1).

This completes the first part of the investigation, and we now study the magnitude of daily consumption, to determine if 5.5 g/day is sufficient for human needs. In fact, if glycine is truly a non-essential amino acid, the glycine from dietary protein hydrolysis is not needed, and 3 g alone per day should be sufficient.

## 4. Results for the metabolic consumption of glycine

Glycine has several metabolic uses, on the one hand as a precursor of other metabolites, such as porphyrins, purines, creatine, glutathione and bile salts and, on the other hand, for synthesis of proteins, especially collagen and elastin, of which it constitutes about a third of all the residues.

### 4.1 *Glycine as a precursor of other metabolites*

Table 2 summarizes the consumption of glycine in the production of other metabolites, not including synthesis of collagen and other proteins. The values shown in the table are explained in the remainder of this section.

4.1.1 *Biosynthesis of porphyrins*: Synthesis of porphyrins constitutes one of the most important uses of glycine, as eight molecules of glycine are needed for the synthesis of each haem group (ferroporphyrin) (figure 8). Most of the porphyrins synthesized daily are used to make the haem group of haemoglobin, but they are also used for the haem group of myoglobin, as well as for cytochromes and other haem proteins, such as catalase and peroxidase. The total porphyrin turnover can be estimated, because porphyrins are degraded only to bile pigments (biliverdin, bilirubin, stercobilin, etc.) which are excreted in the bile secreted. From data on these excretion products, iron turnover, and the life span of red blood cells, London *et al.* (1950) estimated the daily turnover of total haem groups to be around 0.4 mmol

**Table 2.** Daily glycine expenditure for metabolite synthesis in humans[a]

| Metabolite synthesized | Glycine consumption | |
|---|---|---|
| | mmol/day | mg/day |
| Porphyrins | 3.20 | 240 |
| Purines[b] | 2.75 | 206 |
| Creatine | 5.60 | 420 |
| Glutathione | 7.56 | 567 |
| Bile salts | 0.80 | 60 |
| *Total metabolic uses* | 19.91 | 1493 |

[a]Sources of the data and calculations are explained in the text.

[b]The consumption of glycine for purine production cancels half of the amount produced (table 1).

(about 90% of them coming from haemoglobin, and 10% from other compounds). This means a daily expenditure of glycine of 0.4 × 8 = 3.2 mmol.

4.1.2 *Biosynthesis of purine bases*: Purine synthesis consumes equimolar glycine, as mentioned above (*see* figure 3). Thus, the value of this flux (2.75 mmol/day) implies equimolar glycine expenditure in this pathway (206 mg/day).

4.1.3 *Biosynthesis of creatine:* Creatine synthesis consumes glycine and $C_1$ units in equimolar amounts (figure 9). It is neutral with respect to glycine, therefore, with no net expenditure or production. However, we have to take

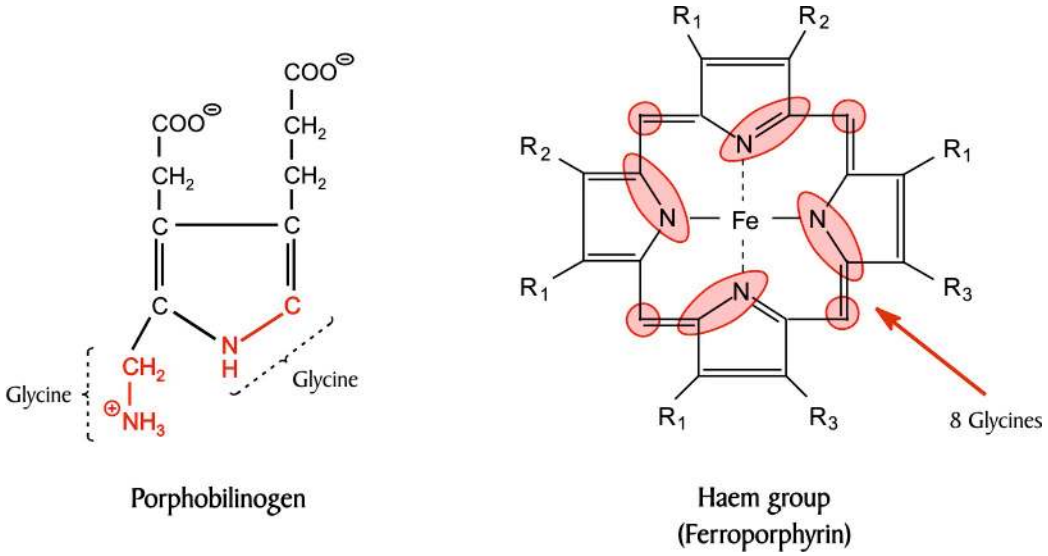



**Figure 8.** Participation of glycine in porphyrin biosynthesis. Eight glycine molecules (shown in red) are consumed in the biosynthesis of each haem group, or any other porphyrin.

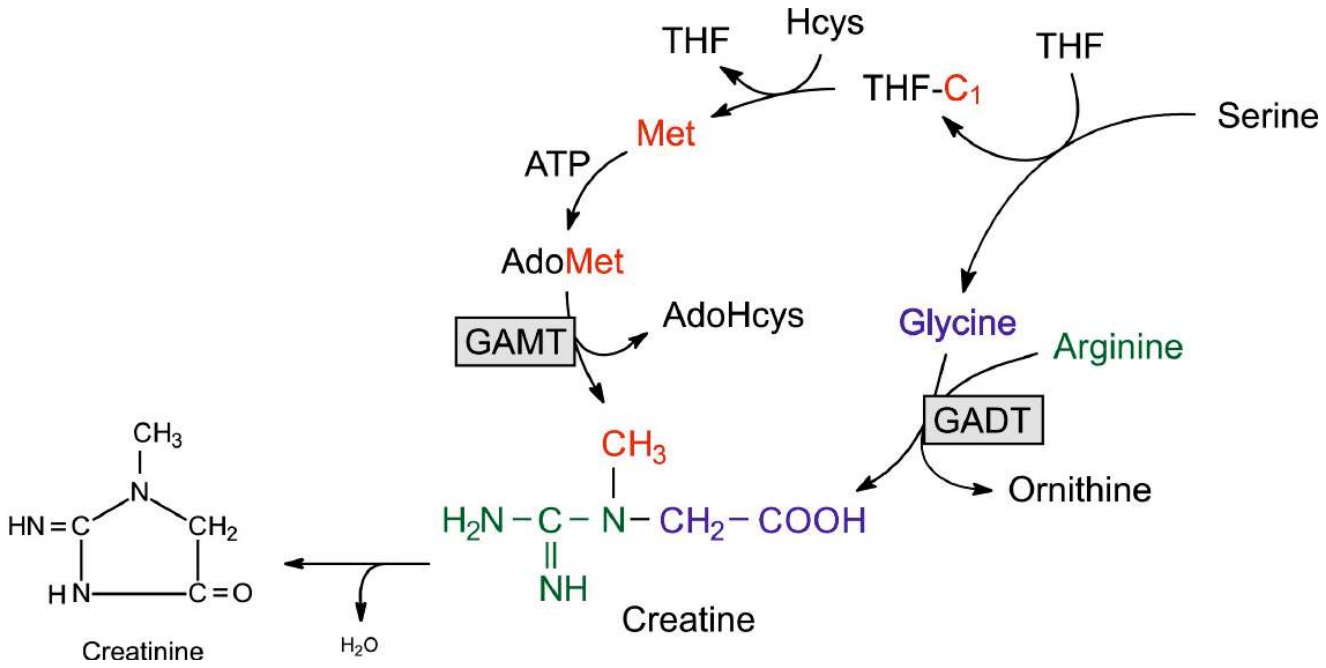



**Figure 9.** Scheme of the pathway for creatine synthesis, and its conversion to creatinine. Creatine synthesis results from the sequential activity of two enzymes: GADT, glycine amidinotransferase (EC 2.1.4.1) and GAMT, guanidinoacetate N-methyltransferase (EC 2.1.1.2). As one glycine molecule and one methyl group (both being derived in equimolar proportions from serine) are consumed, creatine synthesis does not involve net consumption or production of glycine. Nevertheless, this traffic must be taken into account, as it has repercussions for the glycine systemic flux. Creatine is spontaneously converted into creatinine, which is excreted in the urine.

the glycine expenditure into account here, as the whole adenosylmethionine methyl traffic was considered above. The daily synthesis of creatine can be calculated from the data for creatine and creatinine excretion, as creatine is not degraded but spontaneously converted into creatinine, which is excreted in the urine, or excreted directly as creatine. The amount of creatinine plus creatine excreted depends on the muscle mass, age (the younger the more), sex (more in males) and on the creatine intake. Taking these variables into consideration, and subtracting the creatine from the diet, Stead *et al.* (2006) estimated a daily creatine synthesis in men of 7.7 mmol at 20–39 years, 5.6 mmol at 40–59 years, and 3.7 mmol at 60–80 years. We shall take 5.6 mmol as the average amount of daily glycine consumption.

4.1.4 *Glutathione synthesis:* Glutathione, γ-Glu-Cys-Gly (GSH), is an important reducing reagent in metabolism, whose oxidation involves the formation of a thiol–disulphide bond, as GS–SG. Glutathione plays an important role as an antioxidant in many metabolic processes, especially in the red blood cells, red muscle and heart for the detoxification of free radicals and toxic oxygen radicals. It is present at a high concentration (up to 10 mM) in many cells, accounting for more than 90% of the total non-protein sulphur (Meister 1988). The pathway of glutathione synthesis, shown in figure 10, is a route of two steps catalysed by glutamate-cysteine ligase that condenses glutamic acid with cysteine to yield γ-glutamylcysteine, consuming ATP, followed by glutathione synthase, which adds glycine to this dipeptide. Glutamylcysteine is cleaved by γ-glutamylcyclotransferase, yielding cysteine and 5-oxoproline (pyroglutamate). The latter is converted back to glutamate by 5-oxoprolinase or excreted in the urine (Jackson *et al.* 1987, 1996).

The glutathione turnover rate (oxidation–reduction cycle) has been determined in humans *in vivo* as 30 micromol/(kg·hr) (Fukagawa *et al.* 1996), i.e. 50 mmol/day for a 70 kg human; and *de novo* glutathione synthesis, assayed by the conversion of methionine to cysteine in the route, was estimated to be about 15% of this flux, 85% of the cysteine being, therefore, recycled. Assuming the same recycling rate for glycine gives a net daily glycine expenditure of 7.5 mmol.

4.1.5 *Synthesis of bile salts:* The synthesis of bile salts uses glycine to make glycocholate and other glycine derivatives (figure 11). In total, about 20 g/day of bile salts are produced, containing around 75% of glycine conjugates, although most of them (19.5 g) are reabsorbed (*see*, for example, Meisenberg and Simmons 1998). Of the resulting net excretion of 0.5 g/day, 25% consists of taurine derivatives, which do not imply any glycine excretion, and 75%, or 375 mg/day, is sodium glycocholate, equivalent to about 0.8 mmol/day of glycine.

### 4.2 *Summary of glycine expenditure for metabolite synthesis*

The summary of these results in table 2 shows that the expenditure of glycine for metabolite synthesis, excluding collagen and protein synthesis, is about half the production (table 1).

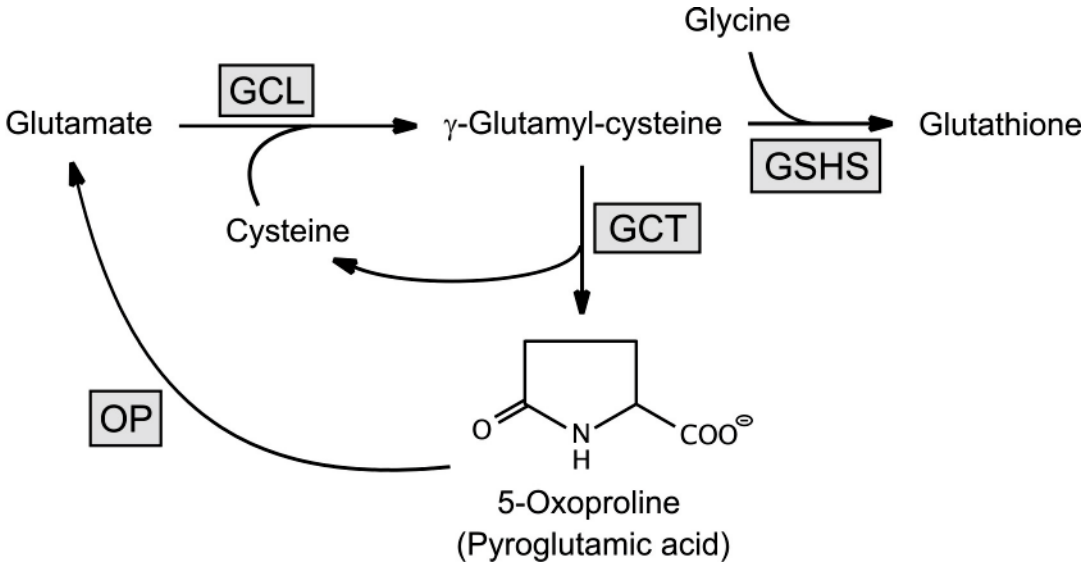



**Figure 10.** Pathway of glutathione metabolism. Synthesis of glutathione (GSH) and production of 5-oxoproline. GCL, glutamate–cysteine ligase (EC 6.3.2.2); GSHS, glutathione synthase (EC 6.3.2.3); GCT, γ-glutamyl cyclotransferase (EC 2.3.2.4); OP, 5-oxoprolinase (EC 3.5.2.9). When glycine is scarce, the concentration of 5-oxoproline is increased, and a greater amount is excreted in urine.

### 4.3 S*ynthesis of proteins*

4.3.1 *Collagen synthesis*: Data for the daily production of collagen are shown in table 3. The data for some of the tissues relate to measurements on small animals, such as rats and rabbits, and underestimate the needs of a 70 kg human, because of allomeric scaling. As first noted by Galileo in 1638, the skeletal mass of a terrestrial animal does not increase in proportion to the body mass, but approximately in proportion to the body mass raised to the power 1.1; for example, it is about 4% of the body mass of a squirrel, but about 20% of that of an elephant (Reynolds and Karlotski 1977). Fortunately, however, the tissues that account for the large bulk of collagen production – skeleton, skin and muscle – were studied, at least in part, with human subjects. On the other hand, the data for replacement of bone collagen (Babraj *et al.* 2005) refer to 'healthy young men' – by implication, subjects with a high-quality diet – and may be unrepresentative of collagen turnover in older and less healthy people.

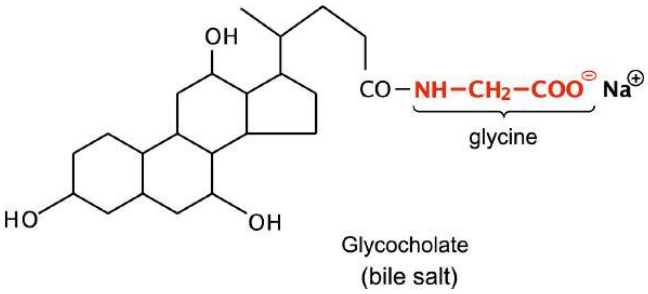



**Figure 11.** Structure of glycocholate, one of the principal bile salts. Its synthesis from cholesterol consumes one molecule of glycine (shown in red).

The procollagen cycle uses amino acids from the general pool (exogenous and metabolically synthesized) together with those coming from collagen degradation, and increases the demand for glycine, so this cycle makes the calculations of the net synthesis of collagen difficult, and its complexity is probably one reason for the disparity of data on collagen turnover reported over time. Laurent (1982) has remarked on this fact, concluding that the value obtained will underestimate the true rate of collagen synthesis by the proportion of newly synthesized collagen that is degraded within the period of measurement. It was widely supposed some years ago that collagen turnover was a slow process, with a lifespan of several years for the molecule. Today it is recognized that this is a very significant proportion of the whole daily protein turnover (Waterlow 2006).

Taking account of the varying production of collagen in different tissues, the data in table 3 allow an estimate

**Table 3.** Collagen content, maximal daily turnover and maximal procollagen cycle rates in the principal tissues[a]

| Organ | Collagen content[b] g [ref] | Fractional synthesis[c] %/day [ref] | Net collagen synthesis g/day | Average lifetime[d] days | Procollagen cycle[e] % [ref] | Gross procollagen synthesis[f] g/day | Glycine equivalent[g] g/day |
|---|---|---|---|---|---|---|---|
| Skeleton | 1640 [1] | 2.64 [9] | 43.3 | 38 | 90 [19] | 433 | 5.65 |
| Skin | 1390 [2,3] | 2 [10–13] | 27.8 | 50 | 56.7 [10] | 64.2 | 0.85 |
| Muscle | 523 [2] | 3.52 [11,14,15] | 18.4 | 28 | 95.3 [10] | 391.5 | 5.10 |
| Cartilage | 109 [4,5] | 4.16 [16] | 4.54 | 24 | 75 [20,21] | 18.2 | 0.23 |
| Heart | 3.36 [2] | 9.8 [10,11] | 0.33 | 10 | 96.2 [10] | 8.7 | 0.11 |
| Lung | 18.4 [2,6,7,8] | 7.1 [6,10,17] | 1.3 | 14 | 82.3 [10] | 7.3 | 0.09 |
| Ligaments | 45 [2] | 1.85 [18] | 0.83 | 54 | 20 [22,23] | 1.0 | 0.01 |
| *Total* | 3720 | | 96.5 | | | 924 | 12.04 |

[a] Sources of the data are as follows: 1. Rogers *et al.* (1952); 2. Neuman and Logan (1950); 3. Tobin (2006); 4. Atencia *et al.* (1989); 5. Eyre *et al.* (2006); 6. Hance *et al.* (1976); 7. Huang (1977); 8. Pierce and Hocott (1960); 9. Babraj *et al.* (2005); 10. Mays *et al.* (1991); 11. Laurent (1982); 12. el-Harake *et al.* (1998); 13. Waterlow (1984); 14. Waterlow (2006); 15. Palmer *et al.* (1980); 16. Repo and Mitchell (1971); 17. Bradley *et al.* (1975); 18. Miller *et al.* (2005); 19. Bienkowski *et al.* (1978a); 20. Flanagan and Nichols (1969); 21. Daughaday and Mariz (1962); 22. Berg (1980); 23. Bienkowski and Engels (1981).

[b] Total collagen content of the tissues.

[c] Collagen secreted to the extracellular matrix.

[d] As the data are presented in various ways in the original sources, calculation of the values shown may be illustrated for the case of bone. Babraj *et al.* (2005) give a value of 0.11%/h for the fractional synthesis rate in bone in the absorptive state, or 2.64%/day, with a daily synthesis of 2.64 × 1640/100 = 43.3 g, and an average life of 100/2.64 = 38 days.

[e] Collagen resynthesized in the procollagen cycle. Subtraction of the values given from 100% gives the percentages secreted to the matrix.

[f] Procollagen synthesis, of which a large proportion (as given in the preceding column) is degraded.

[g] The amount of glycine needed to account for the collagen synthesized, assuming 95% recycling of glycine. The total amount needed is highly dependent on this assumption, as discussed in the text.

that the maximal activity of the procollagen cycle is about 90% of the total procollagen synthesized. This activity varies substantially according to age (Mays *et al.* 1991) and, in particular, to diet, with smaller effects due to other conditions; more generally, therefore, it varies between 30% and 90%. This obviously produces a broad variation of glycine flux involved in the whole of protein breakdown, which can clearly account for the range of total systemic glycine flux. The gross total of 924 g collagen corresponds to a consumption of 240 g glycine (if there is no recycling of glycine), a huge amount, far in excess of the approximately 4 g available from metabolism and the diet after taking into account the 1.5 g needed for metabolic functions other than synthesis of collagen and other proteins. Clearly, therefore, much of the glycine produced from collagen breakdown and from the procollagen cycle must be recycled, more than 98%, if there is to be no deficit at all. It is very unlikely that the proportion can be as high as this, however, especially as the large increases in collagen production that follow administration of extra amino acids (Babraj *et al*. 2005) imply that collagen production is affected by the diet. Moreover, the measured value for cysteine reutilization is only 85% (section 4.1.4), and although the rate for glycine may be higher, there is no reason to suppose it is so much higher. The right-hand column of table 3 gives the amount of glycine that is needed if 95% of the glycine released in the procollagen cycle is recycled, and even a reutilization rate as high as this implies a substantial glycine deficit. It is clear that even though the true need cannot be estimated with any precision, it must amount to several grams and there are no grounds for believing that the supply of glycine is adequate to meet the demand for collagen. Thus, the response of the organism to the deficit will involve a decrease in protein turnover.

4.3.2 *Elastin synthesis:* Elastin is another protein of the connective tissue; it is different from collagen, but its content of glycine is also about 33% (Boudier *et al.* 1981). From data on elastin content in different tissues (Lowry *et al.* 1941; Neuman and Logan 1950; Rosenbloom *et al.* 1993), the total elastin in the human body can be estimated as about 75 g. It is usually considered to turn over very slowly, however, about 1–2% per year in the total human body (Rucker and Dubick 1984; Shapiro *et al.* 1991), though there have been reports of turnover as high as 455 mg/day in aortic tissue, which would mean an average lifetime of about 11 days (Keeley and Johnson 1983). Clearly, further work is needed to resolve the major discrepancy in estimates, and to establish the true rate of turnover. On the other hand, it has been reported that elastin has a high synthesis rate only in embryonic development and in fast-growing tissues such as the uterus (Rosenbloom *et al.* 1993). In fact, in human uterus development, the content of both collagen and elastin increases substantially (Woessner and Brewer 1963). However, as elastin turnover and synthesis are very low in adult humans in most circumstances, we shall not take into account the glycine expenditure related to it. Note, however, that it increases substantially in pregnancy, and should then probably be considered.

4.3.3 *Synthesis of other proteins:* The glycine content of non-collagen proteins is around 5% in mass (Gibson *et al.* 2002; Block and Weiss 1956), and so their turnover also influences glycine traffic. The net protein synthesis rate (the same for protein breakdown at steady state in adults), has been estimated to be in the range of 200–300 g/day for a 70 kg adult human (Norton *et al.* 1981; Nissim *et al.* 1983; Waterlow 1984; Young and Pellett 1987; Duggleby and Waterlow 2005). There is no general consensus on this matter, probably because the different methods used lead to rather different results in similar subjects, as discussed by Nissim and Lapidot (1979). We shall assume for our calculations a basic rate of 300 g/day for total protein turnover. From this quantity, a rate of 97 g/day corresponds to collagen turnover, according to the experimental data shown in table 3, and the rest (203 g/day) to non-collagen proteins. It follows that around 1 g/day of glycine for synthesis of other proteins must be added to the amount given in table 3.

## 5. Discussion

This first detailed calculation of the supply and consumption of glycine, summarized in table 4, indicates that the capacity of human metabolism to supply glycine is insufficient to account for the glycine needed for metabolism as a precursor of other metabolites and, in particular, for sustaining an adequate synthesis of collagen. This result agrees with the suggestion from numerous nutritional studies over the past 20 years (Persaud *et al.* 1996; de Koning *et al.* 1998; Jackson 1991; Jackson *et al.* 1996, 2002; Lewis *et al.* 2005) that glycine is a semi-essential amino acid in human

**Table 4.** Glycine balance sheet

| Process | Glycine flux | Source of data |
|---|---|---|
| Synthesis in metabolism | 3 g/day | Table 1 |
| Hydrolysis of dietary proteins | 1.5 to 3 g/day | Section 2.1 |
| Synthesis of metabolites | –1.5 g/day | Table 2 |
| Synthesis of collagen | –12 g/day* | Table 3, section 4.3.1 |
| Synthesis of other proteins | –1 g/day | Section 4.3.3 |
| Balance | –8.5 to –10 g/day | |

*The value of –12 g/day is derived from table 3, i.e. it assumes that 95% of the glycine released in the procollagen cycle is recycled. If the real recycling rate is lower than 95% the glycine flux needs to be increased accordingly.

nutrition, and that the stoichiometric problem discussed previously (Meléndez-Hevia and de Paz-Lugo 2008) is not purely theoretical but has practical consequences for human nutrition, because glycine production cannot be regulated according to demand in the same way as most other biosynthetic routes (*see* Cornish-Bowden *et al.* 1995; Hofmeyr and Cornish-Bowden 2000). The observation of Babraj *et al.* (2005) that intravenous administration of extra amino acids results in a large and rapid increase in collagen synthesis strongly supports the conclusion of table 4, because if glycine was truly a non-essential amino acid, the amount available from the diet would be irrelevant.

The disagreement between supply and demand of glycine can explain the otherwise puzzling results of nutritional studies, for example, that the human foetus may suffer from an insufficiency of glycine (Lewis *et al.* 2005). As Christensen (1982) remarked, the fact that a given amino acid can be synthesized during metabolism does not mean that there is enough to sustain all metabolic needs. In fact, in normal adults on either a low-protein diet or a diet limited in the intake of non-essential nitrogen, *de novo* synthesis of glycine does not satisfy metabolic demand (Jackson 1991). These and other results point in the same direction as our calculations, and a thorough survey of the literature has not revealed any contradictory results.

Two clear signs of glycine deficiency come from studies of the metabolism of glutathione and benzoic acid. Under normal conditions, only a small fraction of 5-oxoproline, a product of glutathione metabolism (figure 10), is excreted, but, in the case of a congenital absence of glutathione synthase, it is excreted in the urine in large amounts. It is also excreted in the urine in large quantities in conditions of glycine deficiency (Persaud *et al.* 1996), and this symptom can be corrected by supplementing the diet with glycine; the authors proposed that assay of 5-oxoproline in urine supplies a way of measuring glycine deficiency. High amounts of this metabolite have been found in the urine of vegetarians (186% of normal values for males, and 136% for females), similar to the amounts in subjects with a low-protein diet (25 g/day), who had 141% of the 5-oxoproline found in subjects with a high-protein diet (70 g/day) (Jackson *et al.* 1996). Note that a glycine deficit must affect all uses of glycine, not just collagen production, and an adverse effect on glutathione synthesis, for example, will have deleterious effects on the response to oxidative stress.

As mentioned in section 2.2, glycine is used in the benzoic acid elimination pathway, which converts it into hippurate. Benzoic acid is a regular component of fruits such as prunes and cranberries, and is used regularly in the food industry as a preservative. Administration of sodium benzoate to adult humans at an oral dose of 4–10 g caused depletion of the glycine metabolic pool, as assayed by 5-oxoproline excretion. The subjects excreted hippurate in amounts that were increased 1.5–5-fold after ingestion of equimolar glycine, and on the basis of these results, Jackson *et al.* (1996) proposed that a major cause of benzoic acid toxicity was the lack of enough glycine to permit it to be eliminated, and so the toxicity of benzoic acid also suggests that current glycine synthesis is minimal for metabolic requirements. Despite this toxicity, benzoic acid has been used to treat inborn errors of urea synthesis, because the excretion of waste nitrogen can then be achieved by synthesis and excretion of hippurate after sodium benzoate administration. Children with inborn errors of urea synthesis exhibit high mortality, and although elimination of glycine creates its own problems, these are less serious than death.

The reutilization of amino acids in protein turnover has been extensively studied (Fern and Garlick 1973; Fern *et al.* 1985; Mays *et al.* 1991; Johnson *et al.* 1999; Martini 2004; Miller *et al.* 2005), but there is still no clear consensus on the extent of reutilization of amino acids released from protein breakdown, and the maximum value of 90% assumed in our calculations may overestimate the true value. Additional complications come from the fact that although collagen is an extracellular protein it can be degraded inside or outside the cells, depending on the tissue and the cells involved in its degradation (Woessner 1991; Krane *et al.* 1996; Blair *et al.* 2002; Lucattelli *et al.* 2003; Bhide *et al.* 2005; Inoue *et al.* 2006). Several different mechanisms are involved, so there are likely to be differences in reutilization of systemic glycine compared with intracellular glycine.

The shortage of glycine may become serious in conditions such as pregnancy and old age, especially if accompanied by malnutrition. So, even though glycine cannot be regarded as indispensable for survival, because failure to maintain collagen in a healthy state is not lethal, it is required for adequate synthesis of collagen and for a healthy level of protein turnover. In fact, people with a protein-deficient diet adapt by decreasing protein turnover (Gibson *et al.* 2002). However, although this adaptation allows survival it has secondary effects, because the increased lifetimes of proteins increase the probability of their undergoing undesirable chemical modifications, such as oxidation, glycation and cross-linking, which can alter their activities. Even though the turnover of collagen may be slow, it is increased in elderly people (Mays *et al.* 1991; Lohmander *et al.* 1996, 2003; Passeri *et al.* 2003), which may be explained by the increase in modified collagen which is more susceptible to collagenases. Thus, even though survival is not threatened by a shortage of glycine, the quality of life certainly is.

Conditions such as a protein-deficient diet that decrease protein turnover, and consequently increase the life-span of collagen (Gibson *et al.* 2002), also decrease the need for glycine, of course; in extreme conditions the glycine shortage may even disappear, as collagen turnover decreases enormously. However, this glycine dispensability is more

apparent than real because with time the glycation of collagen promotes extra covalent cross-links between chains, reducing plasticity (Finkelstein 2004). A healthy physiology of the organism therefore requires collagen to be renewed. So a decrease in collagen turnover, although it is a mechanism to allow survival of the organism, has important secondary effects, including ageing. On the other hand, the shortage of glycine may increase substantially during pregnancy, as collagen and elastin synthesis increases with development of the uterus.

The natural conclusion from our analysis is that the quality of life can be improved by taking glycine as a nutritional supplement according to the calculated deficiency (about 10 g daily), to guarantee adequate synthesis and renovation of collagen. Before reaching this conclusion, we need to ask whether an excess of glycine in the diet may have secondary effects, especially as it is a neurotransmitter. However, non-essential amino acids such as glycine and aspartic acid do not significantly penetrate the blood–brain barrier (Baños *et al.* 1975; Davson 1976). Furthermore, Directive 67/548/EEC of the European Union describes glycine as 'not hazardous', as it does not become toxic in rats when taken orally until a gigantic dose of 8 g/kg is reached, corresponding to around 600 g in a human.

There is also an evolutionary puzzle: if glycine deficiency is a serious problem in all large animals, as these studies suggest, why has it not been overcome by natural selection? Despite the importance of this question, we have not discussed it here, in part because we have done so in a previous paper (Meléndez-Hevia and de Paz-Lugo 2008), and in part because it requires a more profound discussion than is appropriate in a paper that is mainly concerned with nutritional aspects. In addition, animals in the wild have substantially more exercise than is common for many modern humans, and it will therefore be desirable in the future to study and analyse the effects of exercise.

It is surprising that despite the reports from different nutritional studies of shortages of glycine, the implication that it is an essential amino acid has not been readily accepted, though the list of essential amino acids has been revised in other respects within the past 25 years. For example, the question of whether histidine is an essential amino acid was controversial for many years, until 1985, when FAO/WHO/UNU (1985) stated in a Technical Report on the basis of the clinical nutritional studies of Kopple and Swendseid (1975) and Stifel and Herman (1972) that it is essential for humans. In the case of glycine, the resistance is probably due to the fact that glycine deficiency is difficult or impossible to detect in the short term, especially in a small animal such as a rat. The effects are in the long term, and shortage will contribute to the development of osteoarthritis and osteoporosis, typical degenerative diseases of old age, and among the few health problems found in large wild animals – in general, animals over 40 kg in adult weight – including elephants (Weissengruber *et al.* 2006), dogs (Mahan 1978), rhinoceros, giraffes, etc. Collagen-related problems are well documented in nature, and are much more ancient than human culture (Ackernecht 1953). They cannot therefore be attributed to unhealthy modern dietary habits, or even to the modification in human diet brought about by the spread of agriculture in the past 5000 years. However, a task for the future will be to estimate the amount of glycine that can be supplied by microorganisms present in the digestive tract, which we have been unable to consider in the present study due to the absence of suitable data, but which may well affect the varying frequency of collagen-related diseases in animals with different systems of digestion.

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