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The Synthesis of Glycine and Serine by the Rat

By H. R. V. Arnstein and A. Neuberger
National Institute for Medical Research, Mill Hill, London, N.W. 7

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It has been known for almost 50 years that glycine can be synthesized by mammals. Thus Wiechowski (1906) and Magnus-Levy (1907) showed that animals fed benzoate over long periods excreted more hippuric acid than could be accounted for either by the glycine present in their body proteins or that ingested. Later, McCoy & Rose (1937) and Rose, Burr & Sallach (1952) using diets containing pure amino acids demonstrated clearly that neither glycine nor serine has to be supplied to the young rat in order to obtain a good rate of growth. It has also been shown that other mammalian species, including man, can synthesize amounts of glycine sufficient to maintain body weight and nitrogen equilibrium and to support a normal growth rate. The hen also can synthesize glycine, but not at a rate permitting optimum growth, as shown by the fact that growth can be greatly improved by addition of glycine to a diet deficient in this amino acid (Almquist, Stokstad, Mecchi & Manning, 1940; Almquist & Grau, 1944). The turkey, too, requires glycine for optimum growth (Jukes, Stokstad & Belt, 1947), although the amounts needed appear to be lower than in the hen (Kratzer & Williams, 1948). It thus appears likely that glycine is a 'semi-essential' amino acid for all birds.

Even in mammals, there is good evidence that the rate of synthesis of glycine cannot greatly surpass that required by the demands of growth. Thus it is uncertain whether the growth inhibition and general toxicity of benzoate are entirely due to the removal from the body of glycine in the form of hippurate; but the addition of glycine to the diet abolishes the toxic effects of benzoate and restores the growth rate to a normal value (Griffith, 1929; White, 1941), indicating clearly that the rate of synthesis of this amino acid in the rat is not large enough to supply sufficient glycine both for growth and for conjugation of quantities of benzoate of 1–2 m-moles per day. Similar conclusions can be drawn from the earlier observations of Griffith & Lewis (1923) and Csonka (1924) on the effect of the glycine content of the diet on the extent of hippuric acid excretion after a given dose of benzoate. Experiments of this type do not, however, permit any quantitative conclusions concerning the rate of synthesis of glycine under normal conditions, and it was expected that the use of isotope methods would afford a more satisfactory approach to this problem.

In experiments primarily designed for another purpose (Arnstein & Neuberger, 1953) known amounts of glycine and serine were fed to young rats over relatively long periods. It was thought that a comparison of the radioactivity of the fed amino acid with that of the amino acid isolated from the tissues of the animal at the end of the experiment should yield information of the amounts of these two amino acids synthesized by the rat. The effect of the amount of glycine in the diet on the rate of synthesis was also examined and this also afforded an opportunity to study some quantitative aspects of the conversion of glycine to serine, particularly that of the α-carbon of the former to the β-carbon of the latter.

EXPERIMENTAL

Animals and diet. Albino rats of Institute strain were placed on the experimental diets soon after weaning, with the exception of Expt. 2, in which adult animals were used.

The amino-acid diets AAD 1 and AAD 2 have been described previously (Arnstein & Neuberger, 1953). The casein diet used in Expts. 1–3 had the following composition (g./kg. diet): [α-^{14}C]glycine, 20; casein (light, white, unextracted), 125; salt mixture (Glaxo Laboratories, no. DL 6), 40; cod-liver oil, 16; arachis oil, 64; cane sugar, 340; maize starch, 395. Where indicated, cobalamin (2·5 μg./rat/day) was given orally. In Expts. 6 and 7 choline chloride (5 mg./rat/day) was given orally, but in later experiments (nos. 9, 10 and 12–15) it was fed with the diet. DL-Methionine (25 mg./rat/day) was also fed with the diet (Expts. 3 and 4).

Food consumption and body weight. Except where otherwise indicated, the food consumption of each animal was measured daily and the body weight on alternate days.

Isolation of serine and glycine

A. *By chromatography of the benzamido acids.* The animals were killed under anaesthesia and the minced viscera (liver, kidney, spleen, reproductive organs, washed gastro-intestinal tract, heart and lungs) of each group which had received the same diet were extracted with ethanol. After two to three extractions with trichloroacetic acid (TCA; 6%, w/v) the tissues (usually 2–4 g.) were again extracted with boiling ethanol and ether and dried at 80°. The protein-rich residue was hydrolysed with 6 N-HCl (10 ml./g. tissue) at 100° for 20 hr. Excess HCl was removed by evaporating to dryness *in vacuo* and the amino acids were precipitated with mercuric acetate (Campbell & Work, 1952*a*; cf. Neuberg & Kerb, 1912). The free amino acids were regenerated with H_2S and chromatographed on a Zeo-Karb 225 (Permutit Co. Ltd., London) column (55 × 4 cm. diam., approx. 900 g. wet resin), essentially as described for Dowex 50 resin (Stein & Moore, 1949; Ehrensvärd, Reio, Saluste & Stjernholm, 1951). Fractions containing aspartic and glutamic acids, serine and threonine were combined and the acidic amino acids were removed by adsorption on Amberlite IR-4B resin (Resinous Products Corp., Philadelphia, Pa.). The remaining serine and threonine were converted to the *N*-benzoyl derivatives (Steiger, 1944). The mixture was separated on a column of Hyflo Super-cel (75 g.; Johns Manville Co., London) containing 0·5 N-H_2SO_4 (37·5 ml.). The effluent was collected in fractions (30 ml.) which were titrated with 0·02 N-KOH in methanol, using cresol red as indicator. With 2% (v/v) *n*-butanol in chloroform there were successively eluted benzoic acid present in the crude mixture of benzamido acids (peak I; fractions 3–6) and *N*-benzoylthreonine (peak II; fractions 9–14); after a further 120 ml. this was followed by 10% (v/v) *n*-butanol in chloroform which eluted *N*-benzoylserine (peak III; fractions 23–27). The fractions containing the potassium salt of *N*-benzoylserine were combined and evaporated to dryness *in vacuo*. The salt was dissolved in water (2–5 ml.), the solution was filtered and acidified with a slight excess of N-HCl. *N*-Benzoylserine crystallized after a few days at 0°. (Yield: approx. 20 mg./g. tissue + equal amount in mother liquors.) Recrystallization from ethyl acetate afforded pure *N*-benzoyl-L-serine, m.p. 148–149° (microblock, uncorrected), of unchanged radioactivity on subsequent recrystallization. *N*-Benzoyl-L-threonine isolated from the appropriate fraction by a similar procedure had m.p. 191°; it was non-radioactive.

The fractions from the Zeo-Karb column containing glycine and alanine were similarly combined and benzoylated. Benzoylalanine and hippuric acid were separated on a Hyflo Super-cel column (75 g.) containing 0·5 N-H_2SO_4 (37·5 ml.). Chloroform was used to elute benzoic acid (peak I; fractions 2–3), benzoylalanine (peak II; fractions 4–6) and hippuric acid (peak III; fractions 8–15). Hippuric acid was, however, eluted more conveniently by changing to 10% (v/v) *n*-butanol in chloroform as soon as the benzoylalanine had been washed off the column. All fractions (30 ml.) were titrated with 0·02 N-KOH in methanol, appropriate fractions were combined and the hippuric acid was isolated by acidifying the aqueous solution of the potassium salt, as described above; after recrystallization from ethyl acetate:chloroform it had m.p. 190–191° and its radioactivity was unchanged on subsequent recrystallization.

The purity of the benzamido acids was further checked by paper chromatography of the amino acids obtained by hydrolysis with 2 N-HCl.

B. *As the dinitrophenyl (DNP) derivative.* In most experiments, glycine was isolated directly from the protein

hydrolysate by treating the mixture with 1-fluoro-2:4-dinitrobenzene and isolating the dinitrophenyl derivative, m.p. 203–205°, after chromatography on Hyflo Super-cel (Perrone, 1951; Campbell & Work, 1952*b*). The radioactivity of this derivative agreed well with that of the hippuric acid isolated by method A.

Degradation of serine

A. *From chromatographically isolated* N-*benzoylserine.* The following modification of the procedure described by Sakami (1950) was used. *N*-Benzoylserine (0·35 m-mole), isolated as described above, was refluxed with 4 N-HCl (approx. 50 ml.) for 8 hr. The solution was evaporated to dryness and benzoic acid was extracted with light petroleum (b.p. 60–80°). The residue was dissolved in N-NaOH (0·3 ml.) and phosphate buffer (4 ml.; prepared by adding conc. H_3PO_4 to 0·5 M-Na_2HPO_4 to pH = 5·8). The pH was readjusted, if necessary, to 5·8 with 2 N-NaOH, CO_2 was removed by passing CO_2-free N_2 through the solution which was boiled under reflux. To the cooled solution 0·5 M-$NaIO_4$ (2·8 ml.) was added and CO_2-free N_2 was passed through the solution for 2 hr., CO_2 arising from the carboxyl carbon atom of serine being collected as $BaCO_3$. M-$SrCl_2$ (6 ml.) was added and the pH was adjusted to 7 with 2 N-NaOH in order to remove iodate and excess periodate. Formate, derived from the α-carbon atom of serine, was oxidized with $HgCl_2$ to CO_2 and converted into $BaCO_3$ as described by Sakami (1950).

H_2S was passed through the solution, HgS was removed by filtration and the filtrate was concentrated *in vacuo* to approx. 50 ml. Formaldehyde, derived from the β-carbon atom of serine, was isolated as the dimedon derivative by adding dimedon (120 mg.) to the above solution, adjusting the pH to 5 with 2 N-NaOH and warming the mixture to 100° for 15 min. The solution was cooled and the dimedon derivative was filtered off. After recrystallization (aq. ethanol or ethanol) it had m.p. 191°.

B. *Isolation of the β-carbon atom as formaldehyde dimedon from protein hydrolysate.* The mixed viscera were minced and extracted with ethanol and TCA as described above. The dried tissue (1 g.) was hydrolysed with 6 N-HCl for 20 hr. at 100° and the amino acids were purified by precipitation as the Hg complexes, as before (p. 272). After regeneration with H_2S, the amino-acid mixture was oxidized with periodate and the formaldehyde arising from the β-carbon atom of serine was isolated as the dimedon derivative (Rees, 1946).

In one experiment (no. 10, Table 3) the radioactivity of the β-carbon atom of serine obtained by this method was compared with that using method A. The good agreement of the two values confirms the validity of the direct periodate oxidation of the amino-acid mixture for estimating the radioactivity in the β-carbon atom of serine.

RESULTS

Changes of body weight and variations of food consumption with the different diets

Some of the experiments (nos. 6–14, Table 1) had been designed primarily for a study of the biosynthesis of labile methyl groups. For this purpose the cobalamin and choline contents of the diet were varied; the effects of these supplements on growth and food consumption have been discussed in the

Table 1. *Changes of body weight and average food consumption of rats fed various diets containing* [α-^{14}C]*glycine or* L-[β-^{14}C]*serine*

(AAD 1 and AAD 2 are synthetic amino-acid diets (Arnstein & Neuberger, 1953). Two rats were used in each experiment; in Expt. 11 one rat (no. 11*a*) grew exceptionally well, probably owing to intestinal synthesis of cobalamin, whilst the other (no. 11*b*) showed the expected, poor, growth rate for this diet. In this experiment only, the two rats were worked up separately. The diets were supplemented, as indicated, by cobalamin (2·5 μg./rat/day), DL-methionine (25 mg./rat/day) or choline chloride (5 mg./rat/day) designated by the abbreviations B_{12}, Met and Ch respectively.)

Expt. no.	Labelled component fed	Basal diet	Supplement	Duration of experiment (days)	Aggregate body wt. (g.) Initial	Aggregate body wt. (g.) Final	Average food consumption (g./day/rat)
1		Casein	B_{12}	39	107	266	8·9
2			B_{12}	35	686	716	16·6*
3			None	35	93	189	6·6
4†	2% [α-^{14}C]Glycine (57 μc/mole)	AAD 1	Met + B_{12}	21	94	131	4·5
5			Met	23	96	139	4·7
6			B_{12}	23	109	139	4·5
7			Ch + B_{12}	39	108	181	5·7
8			Ch	39	105	90	3·5
9	0·5% [α-^{14}C]Glycine (740 μc/mole)	AAD 2	B_{12}	39	125	171	6·7
10			Ch + B_{12}	39	158	278	9·4
11*a*			Ch	39	79	136	9·9
11*b*			Ch	20	59	68	5·0
12	0·7% L-[β-^{14}C]Serine (220 μc/mole)		B_{12}	20	101	145	5·9
13			Ch + B_{12}	20	134	251	9·1
14			Ch	20	121	143	5·2
15	0·1% [α-^{14}C]Glycine (160 μc/mole)		Ch + B_{12}	21	128	197	8·0
16	0·01% [α-^{14}C]Glycine (160 μc/mole)		Ch + B_{12}	21	128	198	7·9

* Adult rats; daily food consumption was measured during 28 days only.
† The animals in this experiment suffered from a respiratory infection from which one died.

preceding paper (Arnstein & Neuberger, 1953). The growth and food consumption data of the remaining animals (Expts. nos. 1–3, 15–16; Table 1) were of the expected order.

Radioactivities of the glycine and serine isolated from viscera and carcass

In Table 2 the specific radioactivities of the glycine and of the β-carbon atom of serine of the proteins of the viscera and of the carcass respectively are compared with the radioactivities of the corresponding amino acids supplied in the diet. The basal amino-acid diets AAD 1 and AAD 2 contained no glycine or serine, and the labelled amino acids were added to the diets at the concentrations indicated. Casein of cow's milk is reported to have a glycine content of 2 % (Gordon, Semmett & Bender, 1950) and a serine content of 5·9 % (Rees, 1946). In order to allow for the dilution of the labelled glycine by the unlabelled serine and glycine residues in casein, it is assumed that the serine is, on a molar basis, metabolically equivalent to glycine (see Shemin, 1946). The crude casein contained 8 % of ash and water and the corrected casein content of the diet was therefore 11·5 %. The corrected glycine content of the diets used in Expts. 1–3 was calculated to be 2·7 %.

The radioactivity of the glycine isolated from the visceral proteins was remarkably constant in Expts. 4–8 in which diets containing 2 % labelled glycine were used; the values varied between 37 and 44 % of that of the dietary amino acid. There was no apparent effect of cobalamin or choline, or significant variation with the rate of growth. As would be expected, the relative activity of the visceral glycine to that of the fed glycine decreased, as the content of the labelled amino acid in the diet was reduced, being approx. 17 % at the 0·5 % level, 6 % at the 0·1 % level and 0·6 % at the level of 0·01 %. The corrected values with the casein diets (Expts. 1–3; bracketed) were of the order expected for a diet containing about 2·7 % labelled glycine. With 0·7 % [β-^{14}C]serine, the dilution was similar to that found with 0·5 % [α-^{14}C]glycine.

The glycine obtained from the mixed carcass proteins always had a lower specific radioactivity than the glycine isolated from the hydrolysates of the proteins of the viscera. The ratio of the two activities was high and approached unity in experiments in which growth was very good, especially in the two casein experiments in which young rats were used, but in Expt. 8, in which the young rats used lost weight during the experiment, the radioactivity of the carcass glycine was 62 % of that of the glycine of the viscera. By contrast, in the adult rats (Expt. 2) the radioactivity of the carcass glycine remained low, both absolutely and relatively to the visceral glycine, although the

Table 2. *Specific radioactivity of glycine and of the β-carbon atom of serine of the hydrolysed proteins of rats fed* [α-^{14}C]*glycine at various dietary levels or* L-[β-^{14}C]*serine*

(A dietary level of 0·7 % of serine is equivalent on a molar basis to 0·5 % of glycine. Details of the diets, isolation procedures and radioactivity assay are given in the Experimental section; data on growth and food consumption are given in Table 1. The specific radioactivity of the isolated compounds or carbon atoms is expressed as μc/mole or μc/g. atom and has been calculated for a uniform radioactivity, 1 mc/mole, of the dietary precursor. The values in parentheses in columns 5 and 6 are corrected also for the glycine and serine present in the casein fed; for details of the correction see text.)

Expt. no.	Basal diet	Dietary level of labelled compound fed (%)	Supplement*	Specific radioactivity of isolated compounds (μc/mole) or carbon atom (μc/g. atom) Viscera (*a*)	Carcass (*b*)	Ratio 100*b*/*a*
			Glycine experiments			
1	Casein	2	B_{12}	406 (544)	374 (500)	92
2	Casein	2	B_{12}	375 (501)	168 (225)	45
3	Casein	2	None	426 (570)	372 (497)	87
4	AAD 1	2	Met + B_{12}	433	279	64
5	AAD 1	2	Met	433	277	64
6	AAD 1	2	B_{12}	374	258	69
7	AAD 1	2	Ch + B_{12}	440	328	75
8	AAD 1	2	Ch	406	251	62
10	AAD 2	0·5	Ch + B_{12}	157	—	—
11*b*	AAD 2	0·5	Ch	179	—	—
15	AAD 2	0·1	Ch + B_{12}	59·5	34·5	58
16	AAD 2	0·01	Ch + B_{12}	6·5	2·9	45
			Serine experiments			
12	AAD 2	0·7	B_{12}	144	—	—
13	AAD 2	0·7	Ch + B_{12}	153	—	—
14	AAD 2	0·7	Ch	129	—	—

— Signifies value not determined. * For abbreviations used see Table 1.

experiment was extended for 35 days. The low ratio in Expt. 16 is possibly due to errors involved in counting the carcass DNP-glycine sample, which had a very low activity.

Comparing experiments on young rats given similar diets, it is worthy of note that extension of the period of feeding of the labelled compound from 21 or 23 to 39 days does not significantly increase the specific radioactivity of either the visceral or the carcass glycine. The radioactivity found in these experiments for the glycine of the viscera appears to depend entirely on the level of the labelled glycine in the diet and its radioactivity, in contrast to the radioactivity of the glycine of the carcass which depends also on the rate of growth.

The conversion of the α-carbon atom of glycine into the β-carbon atom of serine

Table 3 shows that the extent of this conversion is a function of the glycine content of the diet. The radioactivities of the two carbon atoms of glycine have not been separately determined in this work, but the available evidence, including the distribution of radioactivity in the three carbon atoms of serine shown in Table 3, indicates clearly that feeding α-labelled glycine gives rise only to insignificant radioactivity in the carboxyl group of glycine. It was therefore assumed that the whole of the radioactivity of the glycine was located in the α-carbon atom.

With a glycine content of the diet of 2%, the radioactivity of the β-carbon atom of serine was between 50 and 60% of that of the α-carbon atom of glycine; the corresponding figures for 0·5% dietary glycine were 18–19% and for 0·1% dietary glycine about 11%. On the other hand, the few experiments in which the serine was degraded suggest that the ratio of the radioactivity of the α-carbon atom of glycine to that of the α-carbon atom of serine was not affected by the dietary level of glycine.

DISCUSSION

Quantitative aspects of the endogenous synthesis of glycine and serine

The glycine present in the body proteins of the rats at the end of the experiments is derived from three sources. In the first place, there is the unlabelled glycine which was present in the beginning of the experiments and which has not been replaced. The second fraction comprises the glycine which has been synthesized by the animals during the experiment from various, partly unknown, precursors. This fraction may have a slight radioactivity, since part of the ingested, labelled, glycine is converted into substances which in turn are used for synthesis of new glycine. Thus glycine is converted to some extent into acetate (Sprinson, 1949) and the carbon atoms of the latter are re-utilized for the synthesis of glycine (Arnstein & Neuberger, 1949). However, the dilution factors associated with such reactions are very large and the radioactivity of the glycine fraction which arises by synthesis in the animal can almost certainly be neglected in comparison with the radioactivity of the third fraction, which represents the dietary glycine.

Turnover studies (Shemin & Rittenberg, 1944; Friedberg, Tarver & Greenberg, 1948) indicate that the bulk of the proteins of the viscera of the rat are replaced at rates corresponding to half-life periods

Table 3. *Influence of the level of glycine in the diet on the conversion of the α-carbon atom of glycine into the β-carbon atom of serine*

(All experiments described in this table were done with the mixed proteins of the viscera. Details of the diets and of the isolation, degradation and radioactivity assay are given in the Experimental section and in Table 1. The specific radioactivity of the isolated compounds or carbon atoms is expressed as μc/mole or μc/g. atom and has been calculated for a uniform radioactivity, 1 mc/mole, of the labelled precursor. The values in parentheses in columns 4–6 give the percentage distribution of radioactivity in the three carbon atoms of serine.)

		Specific radioactivity of isolated compounds or carbon atoms				
	Dietary level of		Serine			
Expt. no.	[α-^{14}C]glycine (%)	Glycine (*a*)	β-C (*b*)	α-C	Carboxyl C	Ratio 100*b*/*a*
6	2	374	234	—	—	62
7	2	440	226	—	—	51
8	2	406	239	—	—	59
9	0·5	—	33·6	—	—	—
10	0·5	157	29·7 30·1* (22)	107 (77·5)	0·8 (0·5)	19
11*a*	0·5	—	32·2	—	—	—
11*b*	0·5	179	32·0* (18)	145 (81)	2·0 (1)	18
15	0·1	59·5	6·7* (13)	37·8 (75)	5·7 (11)	11

— Signifies value not determined.

* Value obtained by periodate oxidation of chromatographically pure serine (see Experimental section).

of about 5 days. Thus, after 20 or 39 days about 85 or 99 % respectively of the glycine originally present in the viscera of adult rats should have been replaced by glycine from other sources. In growing animals this 'old' glycine is further diluted owing to a net increase of protein. On the other hand, the mixed proteins of the carcass are replaced more slowly (see Sprinson & Rittenberg, 1949). This is largely due to the relative metabolic inertia of collagen (Neuberger, Perrone & Slack, 1951) which is particularly marked in the adult rat. However, recent experiments (Neuberger & Slack, 1953) have suggested that in the young rat even the glycine of collagen is replaced at an appreciable rate, the turnover rates varying somewhat with different tissues. The results obtained in the present work, involving administration of labelled glycine over relatively long periods, fully support these conclusions which are based on experiments in which the labelled compounds were given over very short periods, and in which the decay of radioactivity was measured. The radioactivity of the glycine obtained from the visceral proteins was relatively constant in all experiments. The lack of effect of the growth rate and of the extension of the feeding period from 20 to 39 days, suggest strongly that almost all the glycine which had been present in the viscera at the beginning of the experiment had been replaced after about 3 weeks. The relative values found for the carcass glycine suggest that only about half of this fraction is replaced during the experimental period and the higher radioactivities found in some experiments can be ascribed to the deposition of new protein associated with growth. The following calculations have therefore been based entirely on the radioactivities of the glycine isolated from the visceral proteins and no correction has been made for 'old' glycine. The error arising from this approximation is likely to be less than 5 % in experiments on animals showing a good growth rate and fed the labelled compound for more than 30 days and to be not more than 10 or at the most 15 % in the less favourable cases.

In order to assess the amounts of glycine synthesized by the rats, the average amount of labelled glycine eaten each day was calculated from the composition of the diet and the observed food consumption (Table 1). The average amount of glycine synthesized (x; in mg./day) was then derived from the equation:

$$x = a(m/n - 1),$$

where a is the average amount of glycine ingested (mg./day) and m and n are the specific radioactivities of the fed and isolated glycine respectively. With the casein diets the values of a and n were corrected for the unlabelled glycine and serine present in the casein. The assumption made that the serine is equivalent to glycine on a molecular basis may not be quite correct but any error thereby introduced is slight. The values thus obtained for different experiments (Table 4) cannot be readily compared owing to differences in final body weights. We have therefore also calculated the average endogenous glycine production y, on a body-weight basis (mg./100 g. body weight/day) with the aid of the following equation:

$$y = 200.x/(\text{initial body weight (g.)} + \text{final body weight (g.)}).$$

In Table 4 the values for y have been expressed in terms of m-moles of glycine. Similar calculations have also been made for serine.

In spite of considerable variations of experimental conditions the values obtained for the daily endogenous glycine synthesis/100 g. body weight are remarkably constant for young rats. The mean value obtained is about 2·5 m-moles/100 g. rat/day, the extreme values being 2·0 and 3·2 m-moles respectively. With the two adult rats the corresponding figure is significantly lower; this may be due to a definite decrease of synthetic capacity with age, but it is probable that the fat content of the bodies of these rats was much higher than that of the younger rats and that the proportion of metabolically active tissue is smaller than the crude body weight would indicate.

Before considering the significance of these findings, it is necessary to examine the validity of an assumption which is implicit in the above calculations. The present treatment assumes that the dietary labelled glycine and the unlabelled glycine synthesized by the rat cannot be distinguished with respect to their metabolic fate. In particular we assume that the probabilities of an ingested amino-acid molecule being irreversibly oxidized or participating in other irreversible or even reversible reactions are equal to those for an amino-acid molecule produced by endogenous synthesis. Assumptions of this type which are made in most metabolic investigations using isotopes are simple and reasonable, provided the amount of labelled substance fed or injected is small. The values for y obtained with diets of a glycine content of 2 and 0·5 % are somewhat higher than those found with diets of glycine content of 0·1 or 0·01 % (Table 4). It is doubtful whether the differences observed, which amount to about 20 %, are statistically significant, but it is possible that the values obtained with the diets containing higher concentrations of glycine are slightly too high. However, the data as a whole, the earlier results on the incorporation of glycine into hippuric acid (Arnstein & Neuberger, 1951) and the observations of Sprinson & Rittenberg (1949) on the rate of ^{15}N excretion after feeding labelled glycine to rats support the assumption that exogenous glycine mixes rapidly with the

Table 4. *Endogenous glycine and serine synthesis calculated from the specific radioactivities of the glycine or serine isolated from the mixed proteins of the viscera and the dietary intake of the labelled amino acid*

(Dietary supplements, initial body weights and average food consumption are given in Table 1; the specific radioactivities of the isolated compounds are given in Table 2. The values in parentheses in column 7 are corrected for the glycine and serine present in casein, making the assumption discussed in the text. The values in the last column are obtained from the following equation: glycine or serine synthesized (mg./day/rat; column 8) × 100/(average body wt. × mol.wt. of glycine or serine) where the average body wt. is obtained by dividing the mean of the aggregate initial and final body weight of each group by the number of animals.)

Expt. no.	Basal diet	Content of diet of labelled glycine or serine (%)	Duration of experiment (days)	Weight change (% of initial)	Average final body wt. (g.)	Labelled amino acid eaten (mg./day/rat)	Calculated amount of glycine or serine synthesized (mg./day/rat)	Calculated amount of glycine or serine synthesized (m-moles/day/100 g. body wt.)
				[α-^{14}C]Glycine; young rats				
1	Casein	2	39	+149	133	178 (238)	198	2·84
3	Casein	2	35	+103	94·5	132 (176)	133	2·52
4	AAD 1	2	21	+39	65·5	90	118	2·79
5	AAD 1	2	23	+45	69·5	94	123	2·79
6	AAD 1	2	39	+28	69·5	90	150	3·24
7	AAD 1	2	39	+72	93·0	114	145	2·63
8	AAD 1	2	39	−14	45·0	70	102	2·80
10	AAD 2	0·5	20	+37	139	47	253	3·09
11*b*	AAD 2	0·5	20	+15	68	25	115	2·41
15	AAD 2	0·1	21	+48	98·5	8	126	2·08
16	AAD 2	0·01	21	+49	99	0·79	121	1·98
				[α-^{14}C]Glycine; adult rats				
2	Casein	2·0	35	+ 4	358	332 (443)	442	1·68
				[β-^{14}C]Serine; young rats				
12	AAD 2	0·7	20	+44	72·5	46·9	281	4·35
13	AAD 2	0·7	20	+95	125·5	63·7	353	3·49
14	AAD 2	0·7	20	+18	71·5	36·1	244	3·52

amino acid already present in the liver and other internal organs. If indeed glycine synthesis occurs exclusively in such tissues, the assumption that mixing is essentially complete before extensive oxidation and other reactions take place would almost certainly be justified. However, a rigorous proof has not yet been produced of the concept that exogenous and endogenous glycine are metabolically equivalent.

The experiments with labelled L-serine (Table 4) were only done at the one dietary level of 0·7 %. However, the results indicate clearly that the amounts of serine synthesized by the rat are about 30 % greater than those of glycine under comparable conditions. This and the finding that, even after prolonged feeding of labelled glycine, the isotope content of the body glycine exceeds that of the serine by 20–35 % suggests that it is primarily serine which is synthesized from nitrogen-free precursors and that most of the synthetic glycine is derived from serine. This amino acid is required for several reactions not involving glycine, such as the formation of ethanolamine and cystathionine, and it is therefore to be expected that its rate of synthesis will exceed that of glycine, if it is to function as the main precursor of the latter.

It follows from the data in Table 4 that the rate of endogenous synthesis of glycine and presumably also that of serine is independent of the glycine or serine content of the diet. The exact amounts of glycine and serine required by the young rat for maximum growth (about 4 g./day) cannot be estimated with great accuracy. The mixed proteins of the rat contain about 10 % of glycine (Dunn, Camien, Malin, Murphy & Reiner, 1949) and approx. 5 % of serine (Sauberlich & Baumann, 1951); for the synthesis of 700–800 mg. of protein/day about 100–110 mg. of glycine and serine are thus needed. If allowance is made for the glycine and serine required for the formation of nucleic acids, porphyrin, ethanolamine, cystine, choline and creatine, the amounts of these amino acids necessary for maximum growth may be estimated to be about 1·5–2·0 m-moles/day. This figure is of the same order of magnitude as that found for endogenous glycine production in the present work. It would thus appear that the quantities of the two amino acids formed by endogenous reactions do not greatly exceed those needed for maximum growth. The effect of benzoate on growth indicates that the removal of glycine by conjugation does not stimulate an increase in the rate of synthesis of this

amino acid. Conversely, an increased supply of glycine in the diet does not lead to a reduction of synthesis.

The values obtained here include both the glycine synthesized from nitrogen-free precursors, probably through serine, and that produced specifically from threonine (Meltzer & Sprinson, 1952). However, the proportion of glycine which can arise from dietary threonine is relatively small. None of the many other amino acids investigated has been found to be an efficient glycine precursor (Åqvist, 1951).

The present experiments do not suggest that cobalamin is concerned with the synthesis of glycine or, particularly, the conversion of serine into glycine, since the rate of endogenous glycine formation is not significantly affected by the presence of cobalamin in the diet. In this respect cobalamin differs from folic acid which has been reported to be involved in glycine formation from serine (Elwyn & Sprinson, 1950). It is possible, however, that none of the rats used in the present experiments was completely deficient in cobalamin, although its absence from the diet affected growth and food consumption under the conditions used.

The conversion of the α-carbon atom of glycine into the β-carbon of serine

It has been known for some time that in the rat the nitrogen and the α- and carboxyl-carbon atoms of serine are in rapid equilibrium with the corresponding atoms of glycine. In the conversion of serine into glycine the β-carbon atom is lost (Shemin, 1946) and in the reverse reaction glycine condenses with a one-carbon fragment, which can originate from labile methyl groups (Sakami, 1949*b*; Siekevitz & Greenberg, 1950), acetone (Sakami 1950), the β-carbon atom of serine, formaldehyde (Siegel & Lafaye, 1950), formate (Sakami, 1948), as well as the α-carbon atom of glycine (Sakami, 1949*a*; Siekevitz & Greenberg, 1949). In the early experiments on the conversion of glycine into serine (Sakami, 1949*a*) rats were fed doses of 5 m-moles [α-^{14}C]glycine/100 g. body weight. Under these conditions, the radioactivity of the β-carbon atom of serine was 82 % of that of the α-carbon atom. Later, Weissbach, Elwyn & Sprinson (1950), investigating the biosynthesis of choline, showed that after feeding [α-^{14}C]glycine (0·4 m-mole/100 g. body weight) the carbinol carbon atom of the ethanolamine moiety contained about 24 % of the radioactivity of the amine carbon atom. Since the ethanolamine moiety of choline is known to be synthesized in the rat by decarboxylation of serine (Arnstein, 1951), it seems likely that the distribution of radioactivity in the β- and α-carbon atoms of serine was similar to that in the corresponding carbon atoms of choline. The above evidence suggested that the extent of the conversion of the α-carbon atom of glycine into the β-carbon atom of serine might be dependent on the amount of glycine fed, although the experimental conditions in these experiments were not strictly comparable and the observed differences might therefore have been due to other factors.

As it is still uncertain which of the possible precursors of the β-carbon atom of serine is quantitatively the most important, it was of interest to determine the extent to which this carbon atom is derived from the α-carbon atom of glycine under normal conditions. The experiments in Table 3 show clearly that this reaction is markedly affected by the level of glycine in the diet. Although the serine in Expts. 6–8 was not degraded, the radioactivity of the serine α-carbon atom could not have exceeded that of the glycine and it may therefore be calculated that the radioactivity of the β-carbon atom of serine was at least 60 % of that of the α-carbon atom. In these experiments glycine was fed at the rate of about 1·8 m-moles/100 g. body weight/day. When the level of glycine in the diet was reduced to 0·5 m-mole (Expts. 10 and 11*b*) and 0·1 m-mole/100 g. body weight/day (Expt. 15) the corresponding radioactivity in the β-carbon atom of serine was about 22 and 17 % of that in the α-carbon atom of glycine. Since reduction of the dietary level of glycine did not increase the rate of endogenous glycine synthesis (see above), the relative decrease in the radioactivity of the β-carbon atom of serine on the low glycine diets must be due to a reduction in the conversion of the α-carbon atom of glycine into the β-carbon atom of serine. It may be deduced from this that endogenous glycine is not an important source of the β-carbon atom of serine and that this conversion, involving formaldehyde or formate as likely intermediates, is mainly used to deal with excessive amounts of dietary glycine.

The effect of the level of a compound in the diet on the rate of its metabolism, similar to that observed in this work, has been noted previously. Thus it has been shown (Mackenzie, Rachele, Cross, Chandler & Du Vigneaud, 1950) that an increase in the amount of L-methionine in the diet from 0·6 to 1·2 % resulted in a ninefold increase in the oxidation of the methyl group to carbon dioxide. Similarly, the rate of transfer of the methyl group of methionine to creatine was also not proportional to the level of methionine in the diet (Cohn, Simmonds, Chandler & Du Vigneaud, 1946).

It has been realized for some time (see e.g. Moss & Schoenheimer, 1940) that interconversion of two compounds occurs in animals, even when adequate amounts of both substances are supplied in the diet. However, most of these observations have been of a qualitative character. The present results on glycine synthesis show clearly that the daily quantity of this amino acid formed by the rat is independent of the amounts supplied in the diet.

On the other hand, the rate of conversion of the α-carbon atom of glycine into the β-carbon atom of serine increases with the glycine content of the diet. It would thus follow that the relatively constant level of glycine in the tissues is maintained mainly by regulation of the rate of disposal of glycine rather than of the rate of formation.

SUMMARY

1. Amino acid or casein diets containing 0·01, 0·1, 0·5 or 2 % [α-^{14}C]glycine or 0·7 % L-[β-^{14}C]serine were fed to groups of rats over periods extending from 20 to 39 days. The glycine was isolated from the proteins of the mixed viscera and of the carcass. The serine was isolated and degraded in some experiments whilst in others the radioactivity of the β-carbon atom was estimated after periodate oxidation without isolation. Food consumption and changes of body weight were measured.

2. Using diets containing 2 % [α-^{14}C]glycine it was shown that the specific radioactivity of the glycine of the visceral proteins, which was about 40 % of that of the fed amino acid, was not affected by the rate of growth nor by variation of the feeding period within the limits mentioned. On the other hand, the radioactivity of the glycine of the carcass increased with increasing growth rate.

3. It was assumed that the amino acid present in the visceral protein at the beginning of the experiment was replaced almost completely and that the dietary glycine or serine and the glycine or serine synthesized by the animal are metabolically indistinguishable. The average daily amount of glycine or serine synthesized was calculated from the food consumption and the radioactivities of the fed and isolated amino acid.

4. The values for glycine synthesis were constant, independent of the level of glycine in the diet, the mean being 2·5 m-moles/100 g. body weight/day. The corresponding figure for serine was 3·5, suggesting that endogenous glycine is largely derived from serine.

5. The conversion of the α-carbon atom of glycine into the β-carbon atom of serine was greatly diminished when the level of glycine in the diet was decreased, indicating that this pathway may be concerned with the disposal of excess glycine.

6. It is concluded that the constant level of glycine in the tissues is maintained by regulation of the rate of degradation and not by variations in the rate of synthesis.

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The Metabolism of DL-[β-^{14}C] and DL-[^{35}S]Cystine by the Rat

BY H. R. V. ARNSTEIN AND J. C. CRAWHALL
National Institute for Medical Research, Mill Hill, London, N.W. 7

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The two amino acids, serine and cystine (or cysteine), show a striking resemblance in some aspects of their intermediary metabolism. They are quantitatively similar in their glycogenic action (Dakin, 1913) and have been shown to be metabolized to pyruvate: cysteine and cystine by liver extracts (Smythe, 1942) and serine by liver extracts (Chargaff & Sprinson, 1943*a*), bacterial suspensions (Gale & Stephenson, 1938; Chargaff & Sprinson, 1943*b*) and probably also *in vivo* by the rat (Arnstein, 1951*a*). Furthermore, the aminoethanethiol moiety of coenzyme A (Lynen, Reichert & Rueff, 1951) may be derived from cysteine by decarboxylation. Such a reaction would be analogous to the biosynthesis of the ethanolamine moiety of choline from serine by the rat (Stetten, 1942; Arnstein, 1951*b*).

It was therefore thought that the β-carbon atom of cystine might be converted in the rat to a one-carbon compound by a reaction similar to that occurring in the formation of glycine from serine, and the present investigation deals with the metabolism of DL-[β-^{14}C]cystine and its possible conversion to a one-carbon precursor of choline and methionine methyl groups. At the same time, a preliminary experiment was carried out with DL-[γ-^{14}C]valine, which seemed another possible methyl-group precursor, but neither amino acid was found to contribute significantly to the biosynthesis of methyl groups. The conversion of cystine to certain other amino acids and the possible incorporation of cystine sulphur into methionine was also investigated in the course of this work.

EXPERIMENTAL

Animals and diet. The animals used in these experiments were albino rats of Institute stock. They were kept on the stock diet previously described (Arnstein, 1951*b*). The isotopically labelled compounds were fed overnight mixed with diet (10 g./rat) containing D-glucose (0·5 g.).

Valine experiment. Two male rats (aggregate body wt. = 479 g.), which had been fasted for 24 hr., were fed DL-[γ-^{14}C]valine (11·7 mg., 16·7 μc) together with unlabelled L-valine (107·0 mg.) mixed with the diet which also contained α-amino-γ-phenylbutyric acid (200 mg.) and *p*-aminobenzoic acid (200 mg.).

Cystine experiments. DL-[β-^{14}C]Cystine (33 mg., 20·8 μc) was fed to two female rats (aggregate body wt. = 512 g.) previously fasted for 24 hr., together with unlabelled L-cystine (50 mg.) in the diet which also contained α-amino-γ-phenylbutyric acid (200 mg.) and *p*-aminobenzoic acid (200 mg.). DL-[^{35}S]Cystine (3·7 mg., 110 μc) was fed to one female rat (body wt. = 215 g.), together with unlabelled L-cystine (25 mg.).

Measurements of radioactivity. The specific radioactivities of all compounds were determined with a bell-shaped helium-filled Geiger-Müller counter having a thin mica window. The measurements were made on solid samples of 'infinite thickness' mounted on polythene disks (Popják, 1950) and compared with a standard consisting of ^{14}C-labelled polymethyl methacrylate supplied by the Radiochemical Centre, Amersham.

Methods of isolation

Hippuric and acetamido acids from the urine. The isolation of hippuric acid and L-α-acetamido-γ-phenylbutyric acid and *p*-acetamidobenzoic acid from the urine was carried out as previously described (Arnstein, 1951*a*).

Choline. Choline was isolated from the carcass and the viscera as the reineckate, which was converted to the chloroplatinate (Du Vigneaud, Cohn, Chandler, Schenck & Simmonds, 1941) for radioactivity assay.

Amino acids from the visceral proteins of rats which had been fed DL-[β-^{14}C]*cystine.* The rats were killed under anaesthesia about 44 hr. after they had been given the food containing the labelled compound. The viscera (liver, kidney, spleen, heart, lungs and gonads) were comminuted and extracted several times with 95% (v/v) ethanol to remove lipids. The residue was extracted three times with 5% (w/v) trichloroacetic acid (TCA), washed with ethanol and ether and dried at 80°. The dried protein (4·5 g.) was hydrolysed with 6N-HCl (45 ml.) at 100° for 24 hr. After removal of the HCl by evaporation the amino acids were purified by precipita-