A Comparison of Alanine and Glucose as Precursors of Serine and Glycine

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The importance of glycine in animal and plant metabolism as a precursor of many nitrogenous compounds, such as creatine, porphyrins, chlorophyll, uric acid and nucleic acid purines is now well established (for a recent review, see Arnstein, 1954). Relatively little is known, however, about the metabolic origin of this amino acid from non-nitrogenous precursors. That glycine can be synthesized by mammals has been recognized for almost half a century (Magnus-Levy, 1907; Wiechowski, 1906), but conclusive proof that it is a nutritionally dispensable amino acid has been obtained only recently in feeding experiments with synthetic diets (Rose, Burr & Sallach, 1952).

The metabolism of glycine is closely linked to that of serine, since both amino acids are readily converted into one another both *in vivo* (Shemin, 1946; Sakami, 1948) and *in vitro* (Blakley, 1954; Kisliuk & Sakami, 1954). The biosynthesis of either serine or glycine from three-carbon or two-carbon compounds in sufficient amounts would therefore be adequate for the animal's requirements, and both mechanisms have in fact been proposed for the biosynthesis of glycine. Thus, the conversion of [2-¹⁴C] and [1-¹³C]pyruvate into glycine *in vivo* has been reported (Anker, 1948), and it was suggested that serine was formed as an intermediate. More recently it has been shown that glycolic acid (Weinhouse & Friedmann, 1951; Chao, Delwiche & Greenberg, 1953) and glyoxylic acid (Weinhouse & Friedmann, 1951; Weissbach & Sprinson, 1953) can be used for the biosynthesis of glycine, presumably by reductive amination of glyoxylic acid. It is not clear, however, whether these C₂ acids are formed in sufficient amounts to account quantitatively for glycine biosynthesis; the available evidence concerning the relative importance of C₃ and C₂ precursors has so far been inconclusive, although it has been shown that, on a diet free of both serine and glycine, the biosynthesis of serine by the rat significantly exceeds that of glycine (Arnstein & Neuberger, 1953b).

The recent observation that the biosynthesis of glycine from non-nitrogenous precursors is markedly reduced in pteroylglutamic acid de-

ficiency (Arnstein & Stanković, 1956) suggested to us that it should be possible to differentiate between these alternative mechanisms by comparing the effect of pteroylglutamic acid deficiency on the biosynthesis of glycine and serine from a non-nitrogenous precursor, such as glucose, since this vitamin deficiency reduces the metabolic inter-conversion of the two amino acids (Elwyn & Sprinson, 1950b; Totter, Kelley, Day & Edwards, 1950). The results of such experiments, which are now reported, show that the biosynthesis of serine from glucose or alanine is independent of the glycine-serine reaction, whilst an important part of glycine must be synthesized from serine. Neither alanine nor pyruvate, which arises from it by transamination, were efficient serine precursors when compared with glucose, and it is suggested that serine is formed from a C₃ precursor which is closely related to an intermediate of glycolysis, such as a triose or glyceric acid. Some of these results have already been reported in a preliminary communication (Arnstein, 1955).

EXPERIMENTAL

Animals and diets

Albino rats of the National Institute for Medical Research (Institute) stock were placed on a 12% casein diet containing 1% of succinylsulphathiazole (Arnstein & Stanković, 1956) soon after weaning for periods varying from 15 to 30 days. The synthetic amino acid diet AAD3 (Arnstein & Stanković, 1956) was then given for 6–15 days, and finally the same diet, but containing [¹⁵N]glycine and either L-[¹⁴C]alanine (0.59%) or D-[¹⁴C]glucose (34%) was fed, usually for either 14 or 20 days, as indicated in Tables 1 and 2. All diets were supplemented with the usual vitamins in the amounts given previously (Arnstein & Stanković, 1956), except that vitamin B₁₂ or pteroylglutamic acid was omitted in the experiments with deficient animals. Full details of the food consumption and changes in body weight of these animals have already been given (Expts. nos. 1–10, Table 1, Arnstein & Stanković, 1956) and the earlier experiment numbers are retained throughout this paper.

Labelled compounds

Uniformly labelled L-[¹⁴C]alanine and D-[¹⁴C]glucose. These compounds were purchased from the Radiochemical Centre, Amersham.

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D-[1-¹⁴C]Glucose. The glucose used in Expt. no. 9 was synthesized following the method of Sowden (1949). The initial conversion of [¹⁴C]methyl iodide into [¹⁴C]nitromethane was carried out in the presence of unlabelled nitromethane (cf. Arnstein & Bentley, 1951). In Expt. no. 5a D-[1-¹⁴C]glucose obtained from the National Bureau of Standards, Washington, U.S.A., was used.

L-[β-¹⁴C]Alanine. L-Alanine was prepared by condensing methyl iodide with the sodium derivative of ethyl benzamidomalonate (Redemann & Dunn, 1939), hydrolysis of the ester and decarboxylation to benzoylalanine, which was resolved as the strychnine salt (Pacsu & Mullen, 1940).

(a) *Trial experiments.* To the sodium derivative of ethyl benzamidomalonate in dry ethanol (7 ml.), prepared from sodium (0.0605 g./2 ml. of ethanol) and ethyl benzamidomalonate (0.734 g./6 ml. of ethanol), methyl iodide (0.355 g.) was added and the solution was heated in a sealed tube at 90–100° for 3 hr. The ethanol was evaporated and the dry residue was dissolved in CHCl₃ (30 ml.) and NaI removed by filtration. The filtrate was evaporated to dryness, giving crude ethyl benzamidomethylmalonate (0.588 g., 80%), which was dissolved in ethanol (8 ml.) and N-NaOH (4 ml.) was added. After 3 hr. at room temperature the ethanol was evaporated and N-HCl (4 ml.) was added. The solution was evaporated to dryness and the residue was decarboxylated by heating for 1 hr. at 120–125° in a stream of N₂. The product was dissolved in CHCl₃ (5 ml.) and purified by partition chromatography on Celite (50 g.), using 0.5N-H₂SO₄ (25 ml.) as the stationary phase and CHCl₃ as the mobile phase. The effluent (30 ml. fractions) was collected in a titration vessel through which CO₂-free N₂ was passed and was titrated with 0.02N-KOH in methanol, using cresol red as indicator. Benzoylalanine was eluted in fractions nos. 5–13 (peak in nos. 6–8; total titre: 60.0 ml. 0.02N-KOH corresponding to a 49% yield based on methyl iodide. In two other experiments with half these quantities the yields of benzoyl-DL-alanine at this stage were 38 and 39%). The fractions were evaporated to dryness, the residue was dissolved in water (25 ml.) and the pH adjusted to 7. The calculated amount of strychnine based on the titration (200 mg.) was added and dissolved by boiling. After treatment with a little charcoal the solution was filtered and kept at 0° for 3 days. Yield of benzoyl-L-alanine strychnine salt: 250 mg. (79%), m.p. 128–129°.

(b) *Radioactive preparation.* The L-[β-¹⁴C]alanine was synthesized from [¹⁴C]methyl iodide (approx. 1.75 mc; 0.75 g.) by an essentially similar procedure. The condensation was, however, much less satisfactory than in the trial experiment, as judged by a comparison of the chemical and radiochemical yields and by the presence of glycine in the final product. It seems possible that the [¹⁴C]methyl iodide may have deteriorated owing to storage for over a year. The following modifications of the trial method were adopted. The crude N-benzoylalanine appeared to be insoluble in CHCl₃. It was dissolved in n-butanol (5 ml.), CHCl₃ (10 ml.) was added and the solution chromatographed on Celite (100 g.), using 2% (v/v) butanol-CHCl₃ as the mobile phase. Fractions 4–19 required 115 ml. of 0.02N-KOH, equivalent to 444 mg. of benzoylalanine (43.5%). These fractions were evaporated to dryness, carrier benzoylalanine (444 mg.) was added and the strychnine salt of benzoyl-L-alanine prepared as above. Yield 877.6 mg. (72%), m.p. 127–129°. The strychnine salt was dissolved in the minimum amount of hot water, N-KOH

(1.7 ml.) was added, the solution cooled and strychnine removed by filtration. N-HCl (1.7 ml.) was added to the filtrate, which was then concentrated to approx. 2 ml., when benzoyl-L-[β-¹⁴C]alanine crystallized. After recrystallization from ethyl acetate-light petroleum (b.p. 60–80°) the product (270 mg., 84%) had m.p. 143–147°; [α]_D²⁰ of K salt 29.9 ± 0.7 in water (c, 2.065). Under the same conditions non-radioactive benzoyl-L-alanine had m.p. 147–148°; [α]_D²⁰ +31.1°. The specific radioactivity of this material was 0.195 μc/mg. (theoretical specific radioactivity, calculated from chemical yields and radioactivity of starting material = 0.84 μc/mg.).

Benzoyl-L-[β-¹⁴C]alanine (250 mg.) was refluxed with 3N-HCl (10 ml.) for 2 hr. The solution was concentrated to dryness, the residue dried *in vacuo* (P₂O₅) and benzoic acid was removed by extraction with light petroleum (b.p. 60–80°). The hydrochloride was dissolved in ethanol (0.9 ml.) and L-[β-¹⁴C]alanine precipitated with pyridine (0.15 ml.). Yield: 69 mg. (60%); [α]_D²⁰ 10.2 ± 1.4 in N-HCl (c, 0.52); specific radioactivity, 0.345 μc/mg. A second crop of L-[β-¹⁴C]alanine was obtained from the mother liquors by adsorption of the amino acid on Zeo-Karb 215 resin (Permutit Co. Ltd.) and elution with 0.02M-ammonia (27 mg. 0.27 μc/mg.). The purity of the L-[β-¹⁴C]alanine was checked by chromatographing 0.25 mg. on Whatman no. 3 paper, using amyl alcohol-pyridine-water (7:7:6, v/v). Both crops contained traces of glycine, which was, however, inactive.

For use in the diets the fractions (39.5 mg. of the main crop, 26.0 mg. of the second crop) were combined and diluted with carrier L-alanine (12.5 g.). The specific radioactivity of the diluted L-[β-¹⁴C]alanine was 1.84 μc/g.

Isolation of amino acids

At the end of the experimental feeding period the animals were killed under anaesthesia and the internal organs (heart, lungs, liver, spleen, reproductive organs and kidneys) were minced, extracted six times with boiling ethanol, three times with trichloroacetic acid (6%, w/v) and again with ethanol and ether, and dried at about 80°. The crude protein was hydrolysed with 6N-HCl (10 ml./g.) at 100° for about 20 hr. in a sealed tube. The required amino acids were isolated by chromatography on Zeo-Karb 225 resin (Permutit Co., Ltd.) from this hydrolysate as described by Arnstein & Neuberger (1953b). In some experiments, serine (Expts. nos. 1, 2 and 4) and glycine (Expt. no. 1) were isolated as the N-benzoyl derivatives by method A of Arnstein & Neuberger (1953b). In the other experiments paper chromatography was used to purify amino acids which had been incompletely separated on the ion-exchange column (Arnstein & Stanković, 1956). The purity of the isolated amino acids was checked by paper chromatography and constant radioactivity after recrystallization.

Degradation of serine, glycine and glutamic acid. Serine was degraded with periodate by the procedure of Sakami (1950), as modified by Arnstein & Neuberger (1953b). Glycine was decarboxylated with ninhydrin (Arnstein, 1951) to give formaldehyde and CO₂, which were counted as the dimedon derivative and BaCO₃ respectively. Glutamic acid was decarboxylated with Chloramine T (Mosbach, Phares & Carson, 1951; Dakin, 1917). CO₂ arising from C₍₁₎ was collected as BaCO₃ and γ-oxobutyric acid from carbon atoms 2–5 was counted as the 2:4-dinitrophenylhydrazone derivative, m.p. 198–199°.

Radioactivity measurements. Samples of 'infinite thickness' were plated on 1 cm.² polythene disks (Popják, 1950) and counted with a bell-shaped helium-filled Geiger-Müller counter. The combined errors due to variations in sample preparation and statistical errors were usually about $\pm 5\%$, but the very low radioactivities of the glycine and serine samples in Expts. nos. 1-4 and no. 10 resulted in errors of about $\pm 10\%$.

RESULTS

In order to compare the results of different experiments the radioactivities of the isolated amino acids have been calculated for a specific radioactivity (= 1 mc/mole) of the labelled alanine or glucose fed in the diet.

The incorporation of ¹⁴C from dietary [¹⁴C]-alanine into serine, glycine, alanine, aspartic acid and glutamic acid isolated from the protein of the mixed viscera is shown in Table 1. The radioactivities of alanine, aspartic acid and glutamic

alanine/serine radioactivity ratios in comparable experiments (Table 1, Expts. nos. 1a-4) suggest that vitamin B₁₂ has little or no effect on the conversion of alanine into either glycine or serine. Pteroylglutamic acid deficiency, however, reduced its conversion into glycine, whilst that into serine appears to be substantially unchanged. The incorporation of alanine into glutamic acid and aspartic acid is also similar in all experiments.

Table 2 shows the conversion of dietary glucose, labelled either in C₆ or in all C atoms ([U-¹⁴C]-glucose), into serine, glycine, alanine, aspartic acid and glutamic acid of the visceral proteins under conditions which are essentially comparable to those of the experiment with uniformly labelled alanine (Expt. no. 10, Table 1). The radioactivities of the isolated alanine were remarkably similar in all experiments, whilst both aspartic acid and glutamic acid contained more radioactivity when [1-¹⁴C]glucose was fed. The conversion of glucose

Table 1. *Conversion of L-[β-¹⁴C]alanine and uniformly labelled [¹⁴C]alanine into serine, glycine and some other amino acids*

The experimental diet contained 0.59% of labelled alanine and was fed for 19 days (Expt. no. 1a), 20 days (Expts. nos. 1-4) or 13 days (Expt. no. 10). The specific radioactivities of the dietary L-[β-¹⁴C]alanine and the uniformly labelled [¹⁴C]alanine were 164 and 991 μc/mole respectively, but all results have been calculated for a specific radioactivity of the fed labelled compounds = 1 mc/mole in order to facilitate comparison of different experiments. For further experimental details see text and Arnstein & Stanković (1956).

Expt. no.	Diet	Labelled compound fed	Radioactivity of isolated amino acids (μc/mole)					Radioactivity ratios	
			Serine	Glycine	Alanine	Aspartic acid	Glutamic acid	Alanine serine	Alanine glycine
1	Normal	L-[β- ¹⁴ C]-alanine	4.1	2.1	—*	8.4	14.1	—	—
1a	Normal		2.8	1.65	10.4	10.7	14.9	3.7	6.3
2	Pteroylglutamic acid-deficient		3.6	1.4	13.5	10.2	16.1	3.7	9.6
3+3a	Vitamin B ₁₂ -deficient		3.1	1.5	9.5	9.6	14.1	3.1	6.3
4	(Pteroylglutamic acid + vitamin B ₁₂)-deficient	Uniformly labelled L-[¹⁴ C]-alanine	3.6	0.94	9.8	10.6	14.2	2.7	10.5
10	Normal		1.5	0.97	10.0	7.9	10.8	6.7	10.3

* Sample lost by accident.

acid are of the same order of magnitude, whilst those of serine and glycine are much lower. When uniformly labelled alanine was fed (Expt. no. 10) this difference between the radioactivities of alanine, aspartic acid and glutamic acid, on the one hand, and serine and glycine, on the other, was even more marked, but it should be noted that the labelled compound was fed for a considerably shorter period in this experiment (13 days compared with 19 or 20 days in Expts. nos. 1-4).

Although the very low radioactivities of the isolated serine and glycine do not permit a very accurate comparison, the alanine/glycine and

into serine was also similar in all experiments and was not affected by pteroylglutamic acid deficiency. Glycine, however, contained much less radioactivity in the absence of pteroylglutamic acid or in the [1-¹⁴C]glucose experiments. It is particularly noteworthy that the difference in radioactivity of the isolated alanine and serine was much less than in the experiments with labelled alanine. Thus, the average radioactivity of serine in Expts. nos. 6-9 was 76% of that of alanine, whilst in the comparable experiment with uniformly labelled alanine serine had only 15% of the radioactivity of the isolated alanine.

The distribution of ^{14}C in the isolated serine, glycine and glutamic acid is given in Table 3. Apart from the radioactivity of the serine carboxyl C in Expt. no. 6 and of the β -C in Expt. 10, which are somewhat low, uniformly labelled glucose or alanine gives rise to a uniform distribution of ^{14}C in serine, glycine and, as far as can be seen from the partial degradation, in glutamic acid. In both experiments with $[1-^{14}\text{C}]\text{glucose}$, on the other hand, significantly more ^{14}C was incorporated into the β -position of serine than into the other carbon atoms, and the α -C of glycine was also somewhat more radioactive than the carboxyl C atom. The absolute radioactivity of the carboxyl carbon atom of the glutamic acid derived from $[1-^{14}\text{C}]\text{glucose}$ (Expt. no. 9) was similar to that in the experiments with uniformly labelled glucose, although in the latter experiments rather more of the total radioactivity was located in this position.

DISCUSSION

Effect of pteroylglutamic acid deficiency on glycine and serine biosynthesis

In normal animals the glycine-serine reaction (Shemin, 1946; Sakami, 1948) leads to the rapid metabolic interconversion of these two amino acids, and it is therefore difficult, if not impossible, to assess whether their biosynthesis from non-nitrogenous precursors takes place primarily by formation of serine from a C_3 precursor (Fig. 1, reaction 1), followed by conversion of serine into glycine (reaction 2) or, alternatively, by the synthesis of glycine from a C_2 precursor (reaction 3) and the subsequent addition of a one-carbon compound to give serine (reaction 2). Both reaction sequences may, of course, occur simultaneously, and it is of interest that glycollic acid (Weinhouse & Friedmann, 1951; Chao *et al.* 1953) and glyoxylic

Table 2. *Conversion of labelled glucose into serine, glycine, alanine, aspartic acid and glutamic acid*

The animals in Expts. nos. 5–7 were litter mates. For details of food consumption and changes in body weight see Arnstein & Stanković (1956). The experimental diets were fed for 14 days in all experiments. The diet in Expts. nos. 5–7 contained 0.59% of non-isotopic L-alanine, which was omitted in Expts. nos. 8 and 9. Results calculated for specific radioactivity of fed glucose = 1 mc/mole. Uniformly labelled D- $[^{14}\text{C}]\text{glucose}$ is indicated by $[\text{U}-^{14}\text{C}]\text{glucose}$.

Expt. no.	Diet	Labelled compound fed	Radioactivity of isolated amino acid ($\mu\text{c}/\text{mole}$)				
			Serine	Glycine	Alanine	Aspartic acid	Glutamic acid
6	Pteroylglutamic acid-deficient	$[\text{U}-^{14}\text{C}]\text{Glucose}$	101	33.6	128	104	140
6a	Pteroylglutamic acid-deficient	$[\text{U}-^{14}\text{C}]\text{Glucose}$	99.4	35.8	123	111	155
5	Normal	$[\text{U}-^{14}\text{C}]\text{Glucose}$	91.7	55.3	134	127	172
7	Normal*	$[\text{U}-^{14}\text{C}]\text{Glucose}$	100	57.2	127	129	168
8	Normal	$[\text{U}-^{14}\text{C}]\text{Glucose}$	119	69.8	131	135	188
5a	Normal	$[\text{U}-^{14}\text{C}]\text{Glucose}$	81.2	29.1	139	178	271
9	Normal	$[\text{U}-^{14}\text{C}]\text{Glucose}$	94.9	27.0	126	173	273

* The animal in this experiment was fed on pteroylglutamic acid-deficient diets (as in Expts. nos. 6 and 6a) for 37 days before the beginning of the experimental period.

Table 3. *Degradation of serine, glycine and glutamic acid isolated from animals fed on labelled glucose or alanine*

For experimental details see text. — Signifies value not determined. $[\text{U}-^{14}\text{C}]\text{Glucose}$ and $[\text{U}-^{14}\text{C}]\text{alanine}$ indicate uniformly labelled D- $[^{14}\text{C}]\text{glucose}$ and L- $[^{14}\text{C}]\text{alanine}$ respectively.

Expt. no.	Diet	Labelled compound fed	Distribution of ^{14}C in isolated amino acids (% of total)					
			Serine			Glycine		Glutamic acid
			β -C	α -C	Carboxyl C	α -C	Carboxyl C	C_{2-5} α -Carboxyl C
5	Normal	$[\text{U}-^{14}\text{C}]\text{Glucose}$	—	—	—	40.2	42.7	—
5a	Normal	$[\text{U}-^{14}\text{C}]\text{Glucose}$	55.6	25.2	16.1	60.2	44.0	—
6	Pteroylglutamic acid-deficient	$[\text{U}-^{14}\text{C}]\text{Glucose}$	37.2	31.8	21.5	49.5	52.1	72.3 20.0
6a	Pteroylglutamic acid-deficient	$[\text{U}-^{14}\text{C}]\text{Glucose}$	—	31.8	32.4	—	—	75.3 19.7
7	Normal*	$[\text{U}-^{14}\text{C}]\text{Glucose}$	37.1	32.1	34.9	—	—	72.6 20.8
8	Normal	$[\text{U}-^{14}\text{C}]\text{Glucose}$	32.1	36.5	31.9	45.5	47.0	76.8 18.1
9	Normal	$[\text{U}-^{14}\text{C}]\text{Glucose}$	65.2	10.7	15.4	55.9	40.9	82.3 13.1
10	Normal	$[\text{U}-^{14}\text{C}]\text{Alanine}$	24.8	39.9	42.6	—	—	—

* See footnote, Table 2.

acid (Weinhouse & Friedmann, 1951; Weissbach & Sprinson, 1953) are available as C_2 precursors of glycine, whilst pyruvate can apparently serve as a C_3 precursor of serine (Anker, 1948). No information is available, however, to indicate which of these reactions predominates.

Fig. 1. Possible alternative metabolic pathways leading to the biosynthesis of glycine and serine.

There is abundant evidence that pteroylglutamic acid is required for the interconversion of serine and glycine both *in vivo* (Elwyn & Sprinson, 1950*b*; Totter *et al.* 1950) and *in vitro* (Blakley, 1954; Kisluk & Sakami, 1954), as well as for glycine biosynthesis (Arnstein & Stanković, 1956), and these observations have now been applied to a study of the metabolic origin of glycine and serine. Since pteroylglutamic acid deficiency had no effect on the biosynthesis of serine from either glucose or alanine it is concluded that the glycine-serine reaction is not essential for serine biosynthesis from non-nitrogenous precursors. On the other hand, the conversion of both alanine and glucose into glycine in pteroylglutamic acid-deficient rats was only about 60 % of that in normal animals. It is particularly noteworthy that both the conversion of dietary [^{15}N]glycine into serine and the dilution of the fed labelled glycine by endogenously synthesized glycine were also reduced to a similar extent (Arnstein & Stanković, 1956). These results suggest that glycine is synthesized largely by the glycine-serine reaction and that serine is an important intermediate in the biosynthesis of glycine from non-nitrogenous precursors.

The β -carbon atom of serine appears to be a major precursor of 'active' formaldehyde, as shown by its importance as a source of methyl groups (Arnstein & Neuberger, 1953*a*). This reaction is also decreased by pteroylglutamic acid deficiency (Stekol, Weiss & Weiss, 1952), and it therefore seems reasonable to suppose that the biosynthesis of glycine and 'active' formaldehyde are linked by the glycine-serine reaction.

It is noteworthy that pteroylglutamic acid deficiency had no effect on the metabolism of glucose to pyruvate in the present experiments.

Utilization of glucose and alanine for the formation of serine and glycine

A comparison of the experiments with uniformly labelled [^{14}C]glucose and [^{14}C]alanine shows that glucose is utilized almost equally efficiently for the biosynthesis of serine, glycine and alanine, whilst alanine, which was used as a metabolic source of pyruvate, was a relatively poor precursor of serine and glycine.

This is not due to a preferential utilization of dietary alanine for other reactions, e.g. protein synthesis, since in all experiments there was extensive metabolism of this amino acid to glutamic acid and aspartic acid, presumably by transamination to pyruvate and participation of the latter in the reactions of the tricarboxylic acid cycle. The relatively better utilization of glucose for serine and glycine biosynthesis would be readily explained by the assumption that the C_3 serine precursor is more closely related to an intermediate of glucose catabolism, such as a triose or glyceric acid, than to pyruvate. The equal utilization of uniformly labelled [^{14}C]glucose and [$1\text{-}^{14}C$]glucose for serine biosynthesis further suggests that this precursor arises by the reactions of the glycolytic pathway, rather than by the oxidative metabolism of glucose to pentose in which carbon atom 1 is lost as carbon dioxide.

Both serine and glycine isolated after feeding uniformly labelled [^{14}C]glucose contained essentially equal amounts of ^{14}C in each carbon atom. After feeding [$1\text{-}^{14}C$]glucose 55–65 % of the total radioactivity in serine was located in the β -position, indicating preferential synthesis of this carbon atom from $C_{(1)}$ of glucose. The radioactivity in the other positions of the serine molecule in this experiment is probably due to redistribution of ^{14}C as a result of the subsequent metabolism of newly formed β -labelled serine. Thus, it has been shown (Elwyn & Sprinson, 1950*a*; Arnstein, 1951) that L-[$\beta\text{-}^{14}C$]serine gives rise to a limited but appreciable extent to labelled glycine, which in turn would be converted into serine labelled in the α - and carboxyl-carbon atoms. In other experiments, however, the β -carbon atom of serine isolated from rats given L-[$\beta\text{-}^{14}C$]serine for 2 days contained 99 % of the total radioactivity, and the glycine was almost inactive (Elwyn & Sprinson, 1954). It would appear, therefore, that the extent of redistribution of ^{14}C from [$\beta\text{-}^{14}C$]serine into other positions is somewhat variable, possibly depending on the animal's requirement for glycine. On the whole, therefore, the equal utilization of uniformly labelled [^{14}C]glucose and [$1\text{-}^{14}C$]glucose and the distribution of ^{14}C in the serine molecule suggests the utilization of carbon atoms 1–3 of glucose after catabolism to an intermediate of the glycolytic pathway.

Incorporation of radioactivity from [^{14}C]glucose into alanine as an indication of the pathway of glucose metabolism

The oxidative pathway of glucose metabolism to pentose results in the preferential loss of carbon atom 1 of glucose as carbon dioxide, and a comparison of the conversion of [^{14}C]glucose, labelled in different positions, e.g. $\text{C}_{(1)}$ and $\text{C}_{(6)}$, into carbon dioxide has been used to estimate the respective contributions of the glycolytic and oxidative pathways (Bloom, Stetten & Stetten, 1953; Bloom & Stetten, 1955). Similarly, pyruvate can arise from carbon atoms 1–3 of glucose only by glycolysis, whilst carbon atoms 4–6 are converted into pyruvate both by glycolysis and by the oxidative pathway. Since alanine is metabolically closely related to pyruvate, the incorporation of radioactivity into this amino acid from glucose labelled in various carbon atoms may also be used as an indication of the relative importance of these two pathways of glucose metabolism.

In the present experiments there was no detectable difference in the radioactivity of alanine isolated from the crude proteins of the internal organs of rats fed on either [$1\text{-}^{14}\text{C}$]glucose or uniformly labelled [^{14}C]glucose, indicating that, in agreement with the results on the oxidation of labelled glucose to carbon dioxide (Bloom *et al.* 1953), the oxidative metabolism of glucose to pyruvate was quantitatively unimportant in the intact rat. One important difference, however, between the present experiments, which are similar to those recently used to estimate the pathways of glucose catabolism in yeast (Blumenthal, Lewis & Weinhouse, 1954), and those based on the conversion of glucose into carbon dioxide, is that only pentose which is catabolized to pyruvate is estimated by the former method, whilst the latter method includes pentose utilized for synthetic reactions such as nucleotide synthesis. It is clear, therefore, that the two methods give minimum and maximum values respectively for the metabolism of glucose by the oxidative pathway.

The absence of any significant contribution of the oxidative pathway to the conversion of glucose into pyruvate in the intact animal suggests that it represents essentially a mechanism for the biosynthesis of the ribose moiety of nucleotides, but is of little importance for the catabolism of glucose.

Conversion of glucose into glutamic and aspartic acid

The greater dilutions of radioactivity during the conversion of uniformly labelled glucose into glutamic acid and aspartic acid compared with those in both [$1\text{-}^{14}\text{C}$]glucose experiments are probably due to the loss of radioactivity originally

present in carbon atoms 3 and 4 during the formation of citrate from pyruvate, in which the carboxyl carbon of pyruvate is converted into carbon dioxide. In the [$1\text{-}^{14}\text{C}$]glucose experiments no radioactivity is lost at this stage. The radioactivity of the newly synthesized citrate would thus be equal to that present originally in $\text{C}_{(1)}$, $\text{C}_{(2)}$, $\text{C}_{(5)}$ and $\text{C}_{(6)}$ of the glucose. Further catabolism of citrate by the reactions of the tricarboxylic acid cycle would result in similar labelling of oxoglutarate and oxaloacetate, and hence glutamic acid and aspartic acid. It is noteworthy that both aspartic acid and glutamic acid show similar differences in radioactivity, whilst the radioactivity of alanine is the same in the experiments with [$1\text{-}^{14}\text{C}$] and uniformly labelled [^{14}C]glucose. It may be deduced from these results that carbon dioxide fixation leading to the direct synthesis of oxaloacetate from pyruvate, which would result in the incorporation of similar radioactivities into alanine and aspartic acid, is relatively unimportant under the conditions of these *in vivo* experiments.

SUMMARY

1. Young rats have been fed on synthetic diets containing 0.59 % of L- $[\beta\text{-}^{14}\text{C}]$ alanine, 0.5 % of uniformly labelled L- $[\text{C}]$ alanine, 34 % of D- $[\text{C}]$ glucose or 34 % of uniformly labelled D- $[\text{C}]$ glucose for periods of 13–20 days under defined conditions in the presence and absence of dietary pteroylglutamic acid and vitamin B_{12} .

2. At the end of the experimental feeding period, alanine, aspartic acid, glutamic acid, serine and glycine were isolated from the protein of the mixed internal organs and their radioactivity was determined.

3. When uniformly labelled glucose was fed all the isolated amino acids had similar radioactivities. Degradation of the serine and glycine showed that the distribution of ^{14}C in these amino acids was essentially uniform. [$1\text{-}^{14}\text{C}$]Glucose gave rise to similar radioactivities in the isolated alanine and serine; the serine contained 55–65 % of the total radioactivity in the β -carbon atom. When labelled alanine was fed serine and glycine were much less radioactive than the isolated alanine, aspartic acid and glutamic acid.

4. Deficiency of pteroylglutamic acid, but not of vitamin B_{12} , specifically reduced the incorporation of radioactivity from labelled alanine or glucose into glycine, whilst it had no effect on the radioactivity of serine.

5. It is concluded that serine is an important intermediate in glycine biosynthesis but that serine biosynthesis from non-nitrogenous sources does not require pteroylglutamic acid or the prior formation of glycine.

6. Serine is synthesized from a non-nitrogenous C_3 precursor which is more closely related to an intermediate of the glycolytic pathway of glucose catabolism, possibly a triose or glyceric acid, than to pyruvate.

7. The conversion of glucose, labelled in $C_{(1)}$ or in all carbon atoms, into alanine has been used to calculate the relative contribution of the glycolytic and oxidative pathways of glucose catabolism. In the intact rat none of the pyruvate appears to arise by the oxidative catabolism of glucose.

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The Effects of Some Inhibitors of Oxidative Phosphorylation on the Morphology and Enzymic Activities of Mitochondria

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It has been shown in previous experiments that uncoupling of oxidative phosphorylation occurs in mitochondria isolated from fatty livers. The same type of enzymic damage has been found to occur in mitochondria from normal livers after treatment with distilled water (Harman & Feigelson, 1952; Dianzani, 1954). Since both water-treated mitochondria and mitochondria from fatty livers are swollen and have a spherical form, it seems probable that this uncoupling of oxidative phosphorylation is intimately connected with the morphological changes. Since adenosine triphosphate (ATP) is necessary for the activation of fatty acids (Lipmann, Jones, Black & Flynn, 1952), the hypothesis that the accumulation of fat within the

cell is the result of the uncoupling of the oxidative phosphorylation has been advanced (Dianzani, 1954). The mechanism of the uncoupling of oxidative phosphorylation occurring in both water-treated and 'fatty' mitochondria is not completely clear, but it seems very likely that the synthetic reaction $ADP + \sim P \rightleftharpoons ATP$ is affected (Dianzani, 1954).

Many substances have been reported to uncouple oxidative phosphorylation *in vitro*. 2:4-Dinitrophenol (DNP) and some compounds commonly used as histological stains are particularly active (Judah & Williams-Ashman, 1951; Lardy & Wellman, 1952; Potter, Siekevitz & Simonson, 1953; Chappell & Perry, 1953). The mechanism of