Some Aspects of the Metabolism of D- and L-Serine by the Rat

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In a previous paper (Arnstein, 1951) L-serine was shown to be a precursor of both the ethanolamine moiety and the methyl groups of choline. It was found that the *in vivo* conversion of L-serine to ethanolamine takes place by loss of the carboxyl group and that the β -carbon atom is incorporated into choline methyl groups, presumably via a one-carbon fragment. Recently, the biosynthesis of choline methyl groups from one-carbon sources has also been reported from several other laboratories (du Vigneaud, Verly & Wilson, 1950; Elwyn & Sprinson, 1950a; Sakami & Welch, 1950; Jonsson & Mosher, 1950).

The conversion of serine to choline is stereochemically specific, as D-serine did not give rise to either the ethanolamine moiety or the methyl groups of choline (Arnstein, 1951). It was of interest, therefore, to compare the D- and L-isomers of serine in other biological reactions, and this paper deals with the conversion of both isomers, labelled with ^{14}C in the β -position, into acetate and glycine. Recently, Elwyn & Sprinson (1950b) showed that only the L-isomer of serine is converted to uric acid, the β -carbon atom giving rise to carbon atoms 2 and 8 of uric acid.

EXPERIMENTAL

Feeding experiments and isolation of metabolites

Animals and diet. The animals used in these experiments were either albino rats of Institute stock or Wistar strain rats supplied by Dr K. Coward, Pharmaceutical Society (P.S. strain).

The animals were kept on Institute stock diet previously described (Arnstein, 1951). The isotopically labelled compounds were fed overnight mixed with diet containing DL- α -amino- γ -phenylbutyric acid and sodium benzoate or *p*-aminobenzoic acid (see Table 1) and the overnight urine was collected.

Isolation of hippuric acid and acetamido-acids from urine. Hippuric acid or *p*-acetamidobenzoic acid (ABA) was separated from L- α -acetamido- γ -phenylbutyric acid (APBA) by a four-stage counter-current extraction (Bush & Densen, 1948) of the acidified urine with CHCl_3 and ethyl acetate (Arnstein & Neuberger, 1949). The urine was extracted four times with CHCl_3 (3–4 vol.), each CHCl_3 layer being subjected to counter-current extraction with water (1 vol.). The aqueous layers were then combined and extracted in a similar fashion with ethyl acetate. The organic solvents were dried and evaporated, hippuric acid or ABA being obtained

from the ethyl acetate and APBA from the CHCl_3 solution. The specific radioactivity of hippuric acid, ABA or APBA obtained by this method and crystallized once from water was unchanged after several recrystallizations.

Isolation of xanthidryl urea from urine. A solution of xanthidryl in methanol (10% w/v; 2 ml.) was added to 3–4 drops of urine dissolved in a mixture of acetic acid (7 ml.) and water (2 ml.). Xanthidryl urea crystallized almost immediately. The product was washed once with acetic acid-water (7:2, v/v) and twice with ether, and dried at 80°.

Location of radioactivity in glycine and acetic acid. The hippuric acid was hydrolysed by refluxing overnight with 6N-HCl. The solution was extracted with ether to remove benzoic acid and evaporated to dryness *in vacuo*. Potassium phosphate buffer (3.5 g. K_3PO_4 + 20 g. KH_2PO_4 /100 ml. water; pH, 5.5) (10–20 ml.) was then added and the glycine was degraded by reaction with ninhydrin at 100°. The formaldehyde was steam-distilled from the reaction mixture and isolated as the dimedone derivative; at the same time the CO_2 arising from the carboxyl group of glycine was collected as BaCO_3 .

The acetamido acids were hydrolysed by refluxing for several hours with 2N- H_2SO_4 . Acetic acid was separated by steam distillation and converted to the lithium salt, preferably by titration with Li dissolved in methanol in order to avoid contamination with carbonate. Lithium acetate was degraded by pyrolysis of the dry salt at 400° *in vacuo*, the acetone thus formed being condensed in a liquid-air trap and further degraded to iodoform. Alternatively, lithium acetate was converted to silver acetate, which was then decarboxylated by reaction with Br_2 in dry CCl_4 (Hunsdiecker & Hunsdiecker, 1942).

Isolation and degradation of serine from urine after feeding D- $[\beta\text{-}^{14}\text{C}]$ -serine. D- $[\beta\text{-}^{14}\text{C}]$ -Serine (0.5252 g.; specific radioactivity, 501 $\mu\text{C.}/\text{mol.}$) mixed with stock diet (5 g.) containing D-glucose (0.5 g.) was fed to four adult female rats of Institute strain (aggregate body weight 1070 g.). The overnight urine was collected (volume of urine and washings, 63 ml.) and chromatographed directly on Zeo-Karb 225 ion-exchange resin (Permutit Co., Ltd.; 20 g.; dimensions of column: 20 $\times$ 1.4 cm. diameter). The amino-acids were eluted with 0.15M-aqueous NH_3 , approximately 5 ml. fractions being collected. Selected fractions were analysed by paper chromatography and the main serine-containing fractions (fractions 80–100) were combined and evaporated to dryness (yield of crude solid, 280 mg.). The crude serine was converted to the *p*-hydroxyazobenzenesulphonate, which was recrystallized from water (specific activity, 396 $\mu\text{C.}/\text{mol.}$). The specific activity of this material was raised slightly by recrystallization, but as insufficient material for repeated crystallizations was available, it was converted to free serine and oxidized with periodate (Rees, 1946). The formaldehyde was isolated as the dimedone derivative

Table 1. Details of administration of isotopically labelled compounds

Details of animals				Details of labelled compounds fed					Diet		
Exp. no.	No. rats	Sex	Aggregate of body wt. (g.)	Strain*	Compound	Wt. (mg.)	Radioactivity (μc.)	Specific radioactivity (μc./mmol.)	Quantity fed (mmol./100 g. body wt.)	Amount of food eaten (g.)	Compounds added/g. diet
1	2	F	600	I.S.	DL- ¹⁴ CH ₂ OH.CHNH ₂ .CO ₂ H	160.2	4.0	2.62	0.254	20.0	{20 mg. α-amino-γ-phenylbutyric acid 20 mg. p-aminobenzoic acid}
2	1	F	300	I.S.	DL- ¹⁴ CH ₂ OH.CHNH ₂ .CO ₂ H	80.0	2.0	2.62	0.254	10.0	{20 mg. α-amino-γ-phenylbutyric acid 20 mg. sodium benzoate}
3	2	F	550	P.S.	L- ¹⁴ CH ₂ OH.CHNH ₂ .CO ₂ H	122.0	10.0	8.60	0.211	10.0	{20 mg. α-amino-γ-phenylbutyric acid 20 mg. sodium benzoate}
4	2	F	508	P.S.	D- ¹⁴ CH ₂ OH.CHNH ₂ .CO ₂ H	142.6	11.7	8.59	0.267	20.0	{20 mg. α-amino-γ-phenylbutyric acid 20 mg. sodium benzoate}
5	2	M	637	I.S.	D- ¹⁴ CH ₂ OH.CHNH ₂ .CO ₂ H	81.3	6.67	8.59	0.122	15.5	{20 mg. α-amino-γ-phenylbutyric acid 20 mg. p-aminobenzoic acid}
6	2	M	692	I.S.	L- ¹⁴ CH ₂ OH.CHNH ₂ .CO ₂ H	100.5	8.25	8.60	0.138	20.0	{20 mg. α-amino-γ-phenylbutyric acid 20 mg. p-aminobenzoic acid}
7	1	F	302	P.S.	¹⁴ CH ₂ NH ₂ .CO ₂ H	9.6	9.6	75.07	0.0423	10.0	{20 mg. α-amino-γ-phenylbutyric acid 20 mg. p-aminobenzoic acid}
8	2	F	542	I.S.	¹⁴ CH ₃ CO ₂ Na	3.07	37.5	1000	0.0275	16.0	{25 mg. α-amino-γ-phenylbutyric acid 18.8 mg. sodium benzoate}

* Institute strain (I.S.) or Pharmaceutical Society strain (P.S.).

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(m.p. 189°, after recrystallization from aqueous ethanol) and counted after dilution with carrier. The calculated specific radioactivity of the undiluted formaldehyde dimedone derivative was 450 μ c./mol.

Synthesis of labelled compounds used in feeding experiments

All melting points are uncorrected.

Sodium [$^{14}\text{COOH}$] acetate. This compound was supplied by the Radiochemical Centre, Amersham.

[α - ^{14}C]-Glycine. [2 - ^{14}C]-Bromoacetic acid, supplied by the Radiochemical Centre, Amersham, was treated with excess aqueous NH_3 (sp.gr. 0.88), essentially as described by Olsen, Hemingway & Nier (1943).

D- and L-[β - ^{14}C]-Serine. Serine labelled in the β -position was prepared by the condensation of ethyl p -nitrobenzamido-malonate (I) with [^{14}C]-formaldehyde. ^{14}C - p -Nitrobenzoyl-DL-[β - ^{14}C]-serine (III), obtained by partial hydrolysis of the condensation product (II), was resolved by preparing the quinine and brucine salts (Fischer & Jacobs, 1906) and hydrolysed to give both optical isomers of serine (Arnstein, 1949).

In the modification of King's synthesis of serine (King, 1947) (p. 441) pyridine was found to be a more convenient catalyst than NaOH for the condensation of formaldehyde with acylamidomalonate esters. An attempt to introduce the isotope at a later stage of the synthesis by condensing [^{14}C]-formaldehyde with the sodium salt of ethyl hydrogen p -nitrobenzamido-malonate was not successful, although the sodium salt of ethyl hydrogen acetamidomalonate was found to react as readily as the diester.

Other syntheses of serine labelled with ^{14}C in the β -position have recently been reported (Levine & Tarver, 1950; Elwyn & Sprinson, 1950b).

Condensation of formaldehyde with diethyl acetamidomalonate, using pyridine as catalyst. Diethyl acetamidomalonate (54.25 g.), aqueous formaldehyde (38.8% (w/w); 21 g.) and pyridine (1 ml.) were shaken together for 20 hr. The homogeneous solution was evaporated to dryness *in vacuo* and dried by azeotropic distillation with benzene. After further drying *in vacuo* crystalline diethyl hydroxymethylacetamidomalonate (60 g., 97%), m.p. 61–66°, was obtained (cf. King, 1947).

The *O*-acetyl derivative was prepared by heating the above diethyl hydroxymethylacetamidomalonate (25 g.) with acetic anhydride (20 ml.) and anhydrous sodium acetate (10 g.) for 1.5 hr. on the steam bath. Water (50 ml.) was then added and after 2 days at 0° the solution was extracted with ether. The ether extract was washed with aqueous NaHCO_3 and water, dried (Na_2SO_4) and evaporated to dryness. The residue was distilled, yielding a colourless, viscous oil, b.p. 140°/0.005 mm. Hg, which crystallized on standing (yield, 18 g.; m.p. 50°). (Found: C, 49.9; H, 6.5; N, 4.8. $\text{C}_{12}\text{H}_{19}\text{O}_7\text{N}$ requires C, 49.8; H, 6.6; N, 4.9%.)

Ethyl hydrogen acetamidomalonate. 1.01 N- NaOH (45.5 ml.) was added to a hot solution of diethyl acetamidomalonate (10 g.) in 50% (v/v) aqueous ethanol (50 ml.). After cooling, 2 N- H_2SO_4 (25 ml.) was added. The solution was saturated with NaCl and extracted twice with an equal volume of ethyl acetate. The extract was washed with a little water, dried (Na_2SO_4) and evaporated *in vacuo*, giving a crystalline residue (7.3 g., 84%), m.p. 129–130°. Two recrystallizations from water afforded the pure compound (prisms), m.p. 131°

(decomp. with evolution of gas). (Found: C, 44.8; H, 5.6; N, 6.9, 7.3. $C_7H_{11}O_5N$ requires C, 44.4; H, 5.9; N, 7.4%.)

Condensation of formaldehyde with the sodium salt of ethyl hydrogen acetamidomalonate. To a solution of the above half-ester (1.89 g.) in N -NaOH (9.9 ml.) was added a slight excess of a dilute aqueous solution of formaldehyde (16.6 ml.;

giving diethyl *p*-nitrobenzamido-malonate (40 g.), m.p. 135–138°; a further crop (15 g.), m.p. 132–135°, was obtained by adding ether to the mother liquors. Two recrystallizations from $CHCl_3$ and benzene respectively afforded the pure compound (needles), m.p. 136°. (Found: C, 52.0; H, 4.8; N, 8.6. $C_{14}H_{16}O_7N_2$ requires C, 51.9; H, 5.0; N, 8.6%.)

20.6 mg./ml.) and pyridine (0.05 ml.). After 4 days at room temperature the solution was evaporated almost to dryness on the steam bath in a stream of air. 1.05 N -HCl (9.5 ml.) was added to the warm solution, when there was an immediate evolution of CO_2 . After 0.5 hr. on the steam bath, 10 N -HCl (5 ml.) was added and the mixture was refluxed for 1.5 hr. The solution was evaporated to dryness, a little water was added and the evaporation repeated. After drying *in vacuo* over KOH, the residue was extracted with 95% ethanol. The solution was filtered and treated with a slight excess of pyridine; after cooling, crystalline serine (0.73 g., 70%) was obtained.

[^{14}C]-Formaldehyde. [^{14}C]-Methanol (32.5 mg., containing approximately 1 mc. of ^{14}C), obtained from the Radiochemical Centre, Amersham, was diluted with non-radioactive methanol (378.9 mg.) and oxidized to formaldehyde in the apparatus, made of Pyrex tubing, shown in Fig. 1. The vessel (A), containing the methanol, was immersed in a bath kept at 20° and dry air free from CO_2 was admitted through the inlet (B) at a rate of 120–150 ml./min. With this rate of flow and temperature, vaporization of the methanol occurred at the required rate, giving a methanol:air ratio of between 1:10 and 1:15 (v/v). When desired, additional air or O_2 could be admitted at B', but this was found to be unnecessary.

Fig. 1. Apparatus for preparation of [^{14}C]-formaldehyde. For details of components, see text. B10 and B19 are standard ground joints.

Diethyl *p*-nitrobenzamido-malonate. Finely powdered *p*-nitrobenzoyl chloride (46.7 g.) was added in small portions to a stirred mixture of diethyl amidomalonate (44.5 g.) and pyridine (40.6 ml.) cooled in ice. Crystals began to separate almost immediately from the warm solution. After cooling, sufficient $CHCl_3$ to dissolve the solid was added. The $CHCl_3$ solution was washed with aqueous $NaHCO_3$ and water, and dried (Na_2SO_4). The solvent was evaporated *in vacuo*,

Thorough mixing and preheating of the reaction mixture was accomplished by passing the gases through a length of heated tube (C) packed with glass beads (4 mm. diameter). The heaters were constructed with 'Nichrome' resistance wire, which was wound on to the glass tubes covered with thin moistened asbestos sheets. After drying, the wire was held in place by the asbestos, which also served as insulating material. The temperature of the heaters was controlled by a

Variac transformer. The catalyst, consisting of molybdenum oxide (90%)–iron oxide (10%) mixture (Meharg & Adkins, 1933) in the form of moderately coarse lumps, was packed into the vertical part of the tube (*D*), which was heated to 350–400°. Condensation of polymer was prevented by warming the exit vapours and by injection of steam at *E*. After cooling by the internal spiral water condenser (*F*), the aqueous solution of formaldehyde was frozen in the liquid-air trap (*G*). A second trap (*H*), cooled in ethanol-solid CO₂, condensed only traces of radioactive material, presumably formaldehyde or unreacted methanol. The exit gases, containing CO, were passed through a heated tube filled with CuO, and the CO₂ formed was collected in two baryta traps (not shown in Fig. 1). The [¹⁴C]-formaldehyde was estimated by titration with I₂ in alkaline solution (Fresenius & Grünhut, 1905) and counted as the dimedone derivative. Yield of aqueous formaldehyde solution: 294 mg. CH₂O (0.545 mc.)/10 ml. BaCO₃ recovered: 273.5 mg. (0.050 mc.).

Condensation of [¹⁴C]-formaldehyde with diethyl p-nitrobenzamidomalonate. The solution of [¹⁴C]-formaldehyde (9.6 ml., 0.524 mc.) was condensed with a slight excess of diethyl p-nitrobenzamidomalonate (3.25 g.) in 50% (v/v) aqueous pyridine (30 ml.), as described above. Hydrolysis of the intermediate, which was not isolated, gave crude *N*-p-nitrobenzoyl-DL-[¹⁴C]-serine, m.p. 167–173° (1.80 g., 0.269 mc.; 51.5%). This material was diluted with carrier p-nitrobenzoyl-DL-serine (5.70 g., m.p. 205°) and recrystallized from water (yield, 6.15 g., 0.217 mc.).

D-[¹⁴C]-Serine. The diluted p-nitrobenzoyl-DL-[¹⁴C]-serine (6.15 g.) and quinine (7.85 g.) were dissolved in 50% (v/v) aqueous ethanol (273 ml.). After 18 hr. at 0° the crystalline quinine salt was filtered off; yield, after drying *in vacuo* over P₂O₅, 7.64 g. After two recrystallizations from 50% (v/v) aqueous ethanol (62.5 ml.), the p-nitrobenzoyl-[¹⁴C]-D-serine quinine salt (5.89 g., 84%) was dissolved in warm 50% aqueous ethanol (56 ml.), N-NaOH (10.03 ml.) was added and the solution cooled at once. The mixture was cooled in ice for about 0.5 hr. and decanted. The supernatant solution was evaporated *in vacuo* to remove the ethanol, the residue was shaken with water (20 ml.) and filtered. The filtrate was acidified with 10N-HCl, when the crude p-nitrobenzoyl-D-[¹⁴C]-serine (1.97 g., 64.1%) crystallized. The crude product was refluxed for 5 hr. with 5N-HCl (50 ml.). The solution was evaporated to dryness *in vacuo*, water (20 ml.) was added and the evaporation repeated. After the addition of hot water (50 ml.) the solution was cooled overnight at 0°, filtered and evaporated to dryness. The residue was dissolved in aqueous ethanol (95%, v/v; 20 ml.), treated with pyridine (2 ml.) in ethanol (5 ml.), and cooled, giving

crystalline D-[¹⁴C]-serine (0.62 g., 48.3%). After one recrystallization from water-ethanol the D-serine had a specific radioactivity 8.59 μc./mmol. and $[\alpha]_D^{25} = +6.60^\circ \pm 0.2^\circ$, $c = 10.66$ in water, 1 dm. tube. This material was diluted with non-radioactive D-serine and oxidized with periodate (Rees, 1946); the radioactivity was located entirely in the β-position, within the limits of the counting error ($\pm 1.5\%$).

L-[¹⁴C]-Serine. The mother liquors from the preparation of p-nitrobenzoyl-D-[¹⁴C]-serine quinine salt were evaporated *in vacuo* to remove the ethanol. The residue was dissolved in warm water (110 ml.) and N-NaOH (13.35 ml.) was added, the solution being cooled at once in ice. After several hours at 0° the solution was filtered, concentrated to about 10 ml. and acidified with 5N-HCl (5 ml.). The crude p-nitrobenzoyl-L-[¹⁴C]-serine (2.54 g., 83%) and brucine (3.96 g.) were dissolved in water (20.3 ml.). On cooling, the brucine salt separated and was recrystallized from water (12.5 ml.). The brucine salt (4.53 g.) was then dissolved in hot water (40 ml.), 1.01N-NaOH (7.08 ml.) was added and the solution was cooled at once in ice. A further quantity of water (20 ml.) was added and after 18 hr. at 0° the crystalline precipitate was filtered off and washed with water (100 ml.). The filtrate was extracted three times with ether (100 ml.), acidified to litmus with 2N-HCl (1 ml.) and concentrated *in vacuo* to approximately 5 ml. at a bath temperature of 60°. The solution was then acidified to congo red with 2N-HCl, when p-nitrobenzoyl-L-[¹⁴C]-serine (1.59 g., 51.7%) was obtained. The crude product was refluxed for 5 hr. with 3N-HCl (40 ml.). The solution was cooled, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in 95% ethanol (20 ml.), treated with pyridine (1 ml.) in ethanol (10 ml.) and cooled, giving crystalline L-[¹⁴C]-serine (0.413 g., 32.5%). After one recrystallization from water-ethanol the L-serine had a specific radioactivity 8.60 μc./mmol.; $[\alpha]_D^{20} = -6.59^\circ \pm 0.4^\circ$, $c = 7.685$ in water, 1 dm. tube. This material was diluted with non-radioactive DL-serine and oxidized with periodate (Rees, 1946); the radioactivity was located entirely in the β-position, within the limits of the counting error ($\pm 1.5\%$).

RESULTS

The specific radioactivities of the xanthidryl urea and the acylamino-acids isolated from urine after feeding both stereoisomers of [¹⁴C]-serine are given in Table 2. In order to compare the different experiments the dilutions of isotope have been calculated (Table 3) and are expressed as the relative

Table 2. *Specific radioactivities of isolated compounds*

(The radioactivity measurements were carried out with a Geiger counter, using infinite thickness samples.)

Exp. no.	Labelled compound fed	Specific radioactivity of isolated compound (μc./mol.)			
		Xanthidryl urea	Hippuric acid	p-Acetamido-benzoic acid	L-α-Acetamido-γ-phenylbutyric acid
1	DL- ¹⁴ CH ₂ OH·CHNH ₂ ·CO ₂ H	—	—	5.74	19.4
2	DL- ¹⁴ CH ₂ OH·CHNH ₂ ·CO ₂ H	—	10.0	—	16.7
3	L- ¹⁴ CH ₂ OH·CHNH ₂ ·CO ₂ H	5.26	2.95	—	4.32
4	D- ¹⁴ CH ₂ OH·CHNH ₂ ·CO ₂ H	9.46	4.74	—	10.0
5	D- ¹⁴ CH ₂ OH·CHNH ₂ ·CO ₂ H	2.69	—	20.02	81.38
6	L- ¹⁴ CH ₂ OH·CHNH ₂ ·CO ₂ H	3.74	—	4.85	7.26
7	¹⁴ CH ₂ NH ₂ ·CO ₂ H	6.07	—	20.24	9.92
8	¹⁴ CH ₃ CO ₂ Na	—	14.1	—	376.0

Table 3. *Dilution of isotopic tracer in isolated compounds*

(The dilutions of tracer are expressed as the relative isotope coefficient (RIC) and the concentration coefficient (CC), as defined by Anker, 1948.)

Exp. no.	Labelled compound fed	Compounds isolated							
		Xanthidryl urea		Hippuric acid		<i>p</i> -Acetamido-benzoic acid		<i>L</i> - α -Acetamido- γ -phenylbutyric acid	
		RIC ($\times 10^{-3}$)	CC ($\times 10^{-3}$)	RIC ($\times 10^{-3}$)	CC ($\times 10^{-3}$)	RIC ($\times 10^{-3}$)	CC ($\times 10^{-3}$)	RIC ($\times 10^{-3}$)	CC ($\times 10^{-3}$)
1, 2	DL- $^{14}\text{CH}_2\text{OH} \cdot \text{CHNH}_2 \cdot \text{CO}_2\text{H}$	—	—	3.82	29.4	2.19	17.2	7.05	53.0
3	L- $^{14}\text{CH}_2\text{OH} \cdot \text{CHNH}_2 \cdot \text{CO}_2\text{H}$	0.612	2.89	0.343	1.62	—	—	0.502	2.38
4	D- $^{14}\text{CH}_2\text{OH} \cdot \text{CHNH}_2 \cdot \text{CO}_2\text{H}$	1.10	4.11	0.552	2.06	—	—	1.16	4.35
5	D- $^{14}\text{CH}_2\text{OH} \cdot \text{CHNH}_2 \cdot \text{CO}_2\text{H}$	0.313	2.57	—	—	2.33	19.1	9.48	77.6
6	L- $^{14}\text{CH}_2\text{OH} \cdot \text{CHNH}_2 \cdot \text{CO}_2\text{H}$	0.435	3.15	—	—	0.564	4.09	0.844	6.12
7	$^{14}\text{CH}_2\text{NH}_2 \cdot \text{CO}_2\text{H}$	0.081	1.91	—	—	0.270	6.34	0.132	3.11

Table 4. *Dilution factors of isotopic tracer in isolated compounds*

(The dilution factor is the reciprocal of the concentration coefficient as defined by Anker (1948). It is the dilution of isotope which would have been observed if exactly 1 mmol./100 g. body wt. had been fed; Arnstein, 1951.)

Exp. no.	Strain of rat*	Labelled compound fed	Dilution factor			
			Urea	Glycine (of hippuric acid)	<i>p</i> -Acetamido-benzoic acid	<i>L</i> - α -Acetamido- γ -phenylbutyric acid
1, 2	I.S.	DL- $^{14}\text{CH}_2\text{OH} \cdot \text{CHNH}_2 \cdot \text{CO}_2\text{H}$	—	34.0	58.2	18.9
3	P.S.	L- $^{14}\text{CH}_2\text{OH} \cdot \text{CHNH}_2 \cdot \text{CO}_2\text{H}$	346	618	—	420
4	P.S.	D- $^{14}\text{CH}_2\text{OH} \cdot \text{CHNH}_2 \cdot \text{CO}_2\text{H}$	243	485	—	230
5	I.S.	D- $^{14}\text{CH}_2\text{OH} \cdot \text{CHNH}_2 \cdot \text{CO}_2\text{H}$	389	—	52.4	12.9
6	I.S.	L- $^{14}\text{CH}_2\text{OH} \cdot \text{CHNH}_2 \cdot \text{CO}_2\text{H}$	318	—	245	163.5
7	P.S.	$^{14}\text{CH}_2\text{NH}_2 \cdot \text{CO}_2\text{H}$	524	—	158	322

* Institute strain (I.S.) or Pharmaceutical Society strain (P.S.).

Table 5. *Distribution of isotopic tracer in the acetyl groups of excreted acetamido-acids*

Exp. no.	Labelled compound fed	Compound isolated	Distribution of radioactivity in acetate		Degradation method
			Methyl carbon atom (%)	Carboxyl carbon atom (%)	
1, 2	DL- $^{14}\text{CH}_2\text{OH} \cdot \text{CHNH}_2 \cdot \text{CO}_2\text{H}$	<i>L</i> - α -Acetamido- γ -phenylbutyric acid	88.8	11.2	Pyrolysis of lithium acetate
5	D- $^{14}\text{CH}_2\text{OH} \cdot \text{CHNH}_2 \cdot \text{CO}_2\text{H}$	<i>L</i> - α -Acetamido- γ -phenylbutyric acid	90.9	9.1*	Br ₂ /silver salt
		<i>p</i> -Acetamidobenzoic acid	87.1	12.9*	Br ₂ /silver salt
6	L- $^{14}\text{CH}_2\text{OH} \cdot \text{CHNH}_2 \cdot \text{CO}_2\text{H}$	<i>L</i> - α -Acetamido- γ -phenylbutyric acid	90.7	9.3*	Br ₂ /silver salt
		<i>p</i> -Acetamidobenzoic acid	70.2	29.8*	Br ₂ /silver salt
8	$^{14}\text{CH}_3\text{CO}_2\text{Na}$	<i>L</i> - α -Acetamido- γ -phenylbutyric acid	99.0	1.0	Pyrolysis of lithium acetate
			98.4	1.6	Pyrolysis of lithium acetate

* Radioactivity calculated by difference.

isotope coefficient (RIC) and the concentration coefficient (CC) as defined by Anker (1948). The reciprocal of the concentration coefficient, called the dilution factor (Arnstein, 1951), then gives the dilution of isotope which would have been observed if exactly 1 mmol. labelled compound/100 g. body weight had been fed (see Table 4).

The results given in Tables 2–4 demonstrate that both D- and L-[β - ^{14}C]-serine give rise to labelled acetyl groups, the efficiency of the conversion depending on the stereochemical configuration of the compound fed and the strain of rat used.

The distribution of isotopic tracer in the acetyl groups of the excreted acetamido acids is given in

Table 5. Pyrolysis of lithium acetate labelled only in the methyl carbon atom always gave rise to barium carbonate containing about 1.5% of the original radioactivity; the results obtained by this method have therefore been corrected to allow for this error.

DISCUSSION

DL-Serine is deaminated both by bacterial suspensions (Gale & Stephenson, 1938) and by liver extracts (Chargaff & Sprinson, 1943*a*), the reaction taking place under either aerobic or anaerobic conditions. L-Serine appears to be converted to pyruvate by *Escherichia coli* suspensions at the same rate as the

APBA. It was suggested by Anker that alanine is first deaminated to pyruvate, which after decarboxylation would acetylate both the aliphatic and aromatic amino-acids. The D-isomer (IV) was supposed to react first with a keto-acid (V) to give a hypothetical intermediate such as (VI) which is then converted to the acetamido acid (VII). Such an acetamido acid would be expected to transfer its acetyl group preferentially to the α -amino- γ -phenylbutyric acid, as it has been shown that acetyl-glycine as well as other acetamido acids, labelled in the acetyl group, give rise to a higher isotope content in the aliphatic than in the aromatic acetamido group (Bloch & Rittenberg, 1947).

DL-compound (Chargaff & Sprinson, 1943*b*), but the D-isomer has not been investigated.

The incorporation of the β -carbon atom of serine into acetyl groups

In the present investigation the *in vivo* formation of L- α -acetamido- γ -phenylbutyric acid (APBA) and *p*-acetamidobenzoic acid (ABA) from serine was studied by feeding simultaneously [β - ^{14}C]-serine, DL- α -amino- γ -phenylbutyric acid and *p*-aminobenzoic acid to adult rats and isolating the acetylated amino-acids from the urine. Pyruvic acid is known to acetylate α -amino- γ -phenylbutyric acid, but not *p*-aminobenzoic acid, whereas acetate is able to acetylate both *p*-aminobenzoic acid and α -amino- γ -phenylbutyric acid, i.e. aromatic as well as aliphatic amines (Bloch & Rittenberg, 1945).

After feeding D- or L-[β - ^{14}C]-serine the isotopic tracer was found in both the excreted acetamido acids. However, quantitative differences in the conversion of the two stereoisomers to APBA and ABA were observed, especially in experiments with rats of the Institute strain, which appeared to be able to deaminate serine more readily than the P.S. strain. The specific radioactivity of the ABA was less than that of the APBA, the difference being particularly marked when D-serine was fed. In this case the specific radioactivity of the APBA was about four times that of the ABA; after feeding L-serine the radioactivity of the APBA was only 50% greater than that of the ABA.

Similar differences in acetylating capacity between the two stereoisomeric forms of alanine have been observed (Anker, 1950*a*). L-Alanine was found to give identical isotope contents in both acetamido acids, thus behaving like pyruvate (Anker, 1948), but the D-isomer gave a higher isotope content in the

The above mechanism would explain satisfactorily the difference in the ratio of the radioactivity of the aromatic and aliphatic acetamido acids after feeding D- and L-serine (see Table 4). However, degradation of the acetic acid obtained from the isolated acetyl derivatives showed that the carboxyl carbon atom of the acetic acid derived from D-[β - ^{14}C]-serine contained about 10% of the radioactivity of the acetyl group. After administration of methyl-labelled acetate the carboxyl carbon atom of the acetate obtained from the excreted APBA contained only about 1% of the radioactivity. The above distribution of isotopic tracer in the acetic acid derived from D-[β - ^{14}C]-serine could, therefore, hardly arise by the exclusive conversion of D-serine to an acetamido acid followed by transacetylation, as in this case all the radioactivity would be expected to be located in the methyl carbon of the acetate. Recently, it has been suggested that both stereoisomers of alanine acetylate amino-acids by the same mechanism and that differences in isotope content of the acetamido acids are due to different sites of acetylation of *p*-aminobenzoic acid and α -amino- γ -phenylbutyric acid (Anker, 1950*b*). It is pointed out that sulphanilamide (and presumably other aromatic amines, such as *p*-aminobenzoic acid) is acetylated in the liver only, but that α -amino- γ -phenylbutyric acid is acetylated also in the kidney. The kidney is known to contain high concentrations of D-amino-acid oxidase, which converts D-alanine to pyruvate, and an enzyme system which is capable of converting pyruvate to acetate. The high isotope content in APBA after feeding D-alanine is thus ascribed to the conversion of D-alanine to acetate of high isotope content and its utilization for acetylating α -amino- γ -phenylbutyric acid in the kidney. Since the acetylation of *p*-aminobenzoic acid takes place in the liver, further

dilution of the labelled acetate by the large hepatic acetic acid pool would account for the lower isotope content in ABA. Since D-serine is also oxidized to pyruvate, presumably via hydroxypyruvate (Sprinson & Chargaff, 1946) by D-amino-acid oxidase, a similar argument can be applied to the acetylation reactions of D- and L-serine. However, the almost identical distribution of isotope within the acetyl groups of both APBA and ABA after feeding D-serine (Table 5) would appear to be evidence against such an explanation, since it would be expected that the two acetylation reactions, taking place in different organs and at presumably different rates, would result in differences in the distribution of isotope in the two acetamido acids.

The present findings may be satisfactorily explained by assuming that only a part of the D-serine is converted to a C₂-fragment which acts as an acetylating agent, possibly by transacetylation, while the remainder is converted to pyruvate. L-Serine, on the other hand, is converted wholly to pyruvate, though probably not through hydroxypyruvic acid (Sprinson & Chargaff, 1946). Since pyruvate can be converted to oxaloacetate by the Wood-Werkman reaction and thence to the symmetrical dicarboxylic acids of the tricarboxylic acid cycle, any isotope originally in the methyl carbon atom of pyruvate will become partially redistributed. Hence acetate derived from methyl-labelled pyruvate will contain some isotopic label in the carboxyl group. It is probable that the tracer found in the carboxyl carbon atom of acetate after feeding [β -¹⁴C]-serine arises by this mechanism.

The incorporation of the β -carbon atom of serine into glycine

The postulate that D- as well as L-serine can give rise to pyruvate is supported by the conversion of both stereoisomers, labelled in the β -position, to glycine by rats of the P.S. strain. In another experiment, using DL-[β -¹⁴C]-serine and Institute strain rats the glycine, isolated as hippuric acid, had a higher isotope content (see Table 4) than in the experiments with P.S. rats. Degradation of the glycine from this experiment showed that 67% of the radioactivity was located in the α -carbon atom. Similar results have recently been quoted by Elwyn & Sprinson (1950c) in references to unpublished work. This finding excludes amidomalonic acid as an intermediate, as in this case only the carboxyl carbon

atom of glycine would be labelled. The conversion of the β -carbon atom of serine to glycine must therefore involve deamination, randomization of the isotopic label mainly between the α - and β -carbon atoms, and reamination. Carbon atoms 1 and 2 of pyruvate are known to be precursors of glycine (probably via serine) (Anker, 1948) and the tricarboxylic acid cycle provides a mechanism for the randomization of tracer between carbon atoms 2 and 3 of pyruvate, as well as accounting for the fixation of isotope in the carboxyl carbon atom by the Wood-Werkman reaction followed by conversion to a symmetrical dicarboxylic acid, as already discussed. Furthermore, the tricarboxylic acid cycle accounts satisfactorily for the distribution of isotope in glycine after feeding labelled acetate to rats (Arnstein & Neuberger, 1949). It is probable, therefore, that the β -carbon atom of serine is converted to the α -carbon atom of glycine by deamination of serine to pyruvate, participation in the tricarboxylic acid cycle and reconversion to serine, the glycine then arising by loss of the β -carbon atom (Shemin, 1946) from the α , β -labelled serine.

After feeding β -labelled L-serine the isotope content of the excreted hippuric acid and both the acetamido acids was lower than after giving the D-isomer. This is ascribed to the dilution of the L-serine fed by endogenous L-serine. D-Serine does not appear to be synthesized by the animal to any significant extent, as the specific radioactivity of the β -carbon atom of the excreted serine was found to be the same as that of the fed D-serine.

SUMMARY

1. A synthesis of [β -¹⁴C]-serine from [¹⁴C]-methanol is described.

2. The conversion of serine to glycine and acetate has been studied by feeding both enantiomorphs, labelled in the β -carbon atom with ¹⁴C, to adult rats and isolating hippuric acid, L- α -acetamido- γ -phenylbutyric acid and p-acetamidobenzoic acid from the urine.

3. It is concluded that both enantiomorphs of serine are precursors of acetyl groups *in vivo*.

4. The mechanism of this conversion is discussed.

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The Action of a Hydrolytic Enzyme System from *Helianthus tuberosus* L. on Carbohydrates Present in the Tubers

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Green (1887) described an 'inulin-ferment' (later named inulase: Green, 1893) which was present in glycerol extracts made from sprouting tubers of the Jerusalem artichoke and which liberated from inulin reducing sugar and uncharacterized intermediate products. Wolff & Geslin (1918) made from chicory roots extracts in which reducing sugar was slowly liberated, and Colin (1919) reported a similar observation with extracts of artichoke tubers; in both cases the liberation of reducing sugar was correlated with a change of optical rotation in the negative direction. Pringsheim & Kohn (1924) attempted to prepare enzymes from dahlia tubers, but considered their efforts unsuccessful. Thaysen, Bakes & Green (1929) were led to suspect the presence of an inulase in artichoke-tuber extract, but could not confirm this experimentally. These various findings have led to the general conclusion that higher plants are not good sources of enzymes attacking inulin (Pringsheim & Leibowitz, 1929; cf. Lemoigne, 1942).

However, Vadas (1934) suggested the use of a short incubation of mashed tubers as a preliminary to ethanolic fermentation of the carbohydrates, and Asai (1938) noted that as much as 50% of the carbohydrate in the tubers was converted to reducing sugar when the crushed material was incubated for 20 hr. at 37° and pH 3.9.

Physiological studies of the artichoke plant (cf. Colin, 1919; Belval, 1946) indicate that enzyme systems exist concerned with the transformation of the tuber carbohydrates. The latter consist of a mixture of related oligo- and poly-saccharides as

well as inulin itself. In a previous paper (Bacon & Edelman, 1951) we have described the application of paper partition chromatography to these substances.

During that investigation we re-examined the possibility of preparing from the tubers extracts with enzymic activity towards inulin and related substances. The results presented here demonstrate the existence in tuber extracts of a hydrolytic enzyme system, which liberates fructose from inulin and related substances, but has relatively little action upon sucrose.

Part of this work was communicated to the Biochemical Society at Leeds on 23 September 1949 (Edelman & Bacon, 1949).

MATERIALS AND METHODS

Except where otherwise stated, the methods of analysis used were those described by Bacon & Edelman (1951), as also were the batches of artichoke tubers. Reducing substances (RS) are expressed in terms of hexose and by 'total RS' is meant RS after hydrolysis with 0.5% (w/v) oxalic acid for 30 min. at 100°. The sample of commercial inulin used was obtained from Messrs Thomas Kerfoot and Co., Vale of Bardsley, Ashton-under-Lyne, who kindly informed us that it was prepared from the root of the dandelion, *Taraxacum officinale*. All samples of inulin were purified by filtering the hot aqueous solution, freezing the filtrate, dehydrating the resulting precipitate with ethanol and acetone, washing it with light petroleum and drying it in a vacuum desiccator.

The lead acetate and sodium oxalate solutions used throughout were: 30% (w/v) $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$ and 3% (w/v) $\text{Na}_2\text{C}_2\text{O}_4$. All dialysis was carried out in Visking synthetic cellulose casing (John Crampton and Co. Ltd., Wythenshawe, Manchester).

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