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The Metabolism of DL- $[\beta\text{-}^{14}\text{C}]$ and DL- $[\text{S}\text{-}^{35}\text{S}]$ 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- $[\beta\text{-}^{14}\text{C}]$ 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- $[\gamma\text{-}^{14}\text{C}]$ 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- $[\gamma\text{-}^{14}\text{C}]$ 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- $[\beta\text{-}^{14}\text{C}]$ 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- $[\text{S}\text{-}^{35}\text{S}]$ 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- $[\beta\text{-}^{14}\text{C}]$ 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-

tion as the Hg complexes (Campbell & Work, 1952; Neuberg & Kerb, 1912). The mixture was regenerated by H_2S and chromatographed on a column (50 cm. $\times$ 4.5 cm. diam.) of Zeo-Karb 225 resin, using 1.5N-, 2.5N- and 4N-HCl successively as eluants, essentially as described for Dowex 50 resin (Stein & Moore, 1950; Ehrensward, Reio, Saluste & Stjernholm, 1951), and individual amino acids were isolated as described below.

Aspartic and glutamic acids were isolated by evaporating the appropriate fractions to dryness and recrystallizing samples of the residue from water until their radioactivity remained unchanged.

Serine, threonine, glycine and alanine were isolated as the *N*-benzoyl derivatives, which were separated chromatographically and recrystallized to constant radioactivity (Arnstein & Neuberger, 1953*a*).

Valine and leucine were obtained as the hydrochlorides by evaporating to dryness the fractions containing these amino acids. The crude solids were assayed directly for ^{14}C without further purification. Fractions containing leucine + methionine were similarly assayed and as the radioactivities of these fractions were very low these amino acids were not further purified.

Cystine was isolated by evaporating the appropriate fractions to dryness, dissolving the residue in water and adjusting the pH of the filtered solution to about 5 by adding pyridine. The material which separated was filtered off on to filter paper mounted on a perforated disk (Popják, 1950) and assayed for ^{14}C after drying. Owing to the small amount of material available further purification was not practicable.

The separation of the amino acids was checked by paper chromatography of the isolated compounds.

Assay of ^{35}S from methionine and cystine of the visceral protein of a rat which had received DL- ^{35}S cystine

In this experiment the animal was killed approx. 36 hr. after being fed the labelled amino acid. The viscera were comminuted, extracted with ethanol and TCA and hydrolysed as before. The amino acids were again purified by precipitation as the Hg complexes and regenerated with H_2S . The amino-acid mixture thus obtained from 200 mg. protein was chromatographed on paper (Whatman no. 3, 22.5 $\times$ 18.25 in.). The mixture was applied in solution as evenly as possible along the base line (31 cm.) and methionine and cystine markers were run on either side of the mixture. The chromatogram was eluted (18 hr.) with phenol-water (5:2, v/v) in the presence of NH_3 . The position of the markers was detected by spraying the ends of the paper with ninhydrin. Methionine was located at 25–29 cm. from the base line, cystine at 7–13 cm., the solvent front being at 30 cm. Methionine and cystine were then eluted from the appropriate central portions of the paper with water and 2N-HCl respectively. The methionine fraction was rechromatographed on another paper, using amyl alcohol:pyridine:water (3.5:3.5:3, v/v/v) as eluant. The methionine was eluted from the appropriate section of the paper with water, and the solution was evaporated to dryness. A two-dimensional chromatogram (amyl alcohol:pyridine and phenol:water/ NH_3 , as before) showed that this material contained no cystine, although amino acids other than methionine were still present. The methionine sulphur was therefore converted to sulphate by digesting the mixture with Pirie's reagent (Pirie, 1932). The sulphate was isolated

as benzidine sulphate, which was assayed for ^{35}S on a filter paper mounted on a 1 cm.² perforated metal disk, the amount of material assayed being estimated subsequently by titration with 0.01N-NaOH (cf. Simpson & Tarver, 1950).

The cystine fraction was treated with Br_2 water and the mixture was rechromatographed on paper using phenol-water/ NH_3 as before. The cysteic acid, which was well separated from the other amino acids, was eluted with water, converted to sulphate and assayed as benzidine sulphate as described above.

Degradation of the isolated amino acids and of the acetic acid from the acetamido acids

Serine. This amino acid was degraded by periodate oxidation, using the modification of the procedure of Sakami (1950) described earlier (Arnstein & Neuberger, 1953*a*).

Alanine. Benzoylalanine (0.5 m-mole) which had been isolated chromatographically, was hydrolysed by refluxing with 4N-HCl (25 ml.) for 6 hr. The solution was evaporated to dryness and the solid was extracted with ether. The residue was dissolved in 0.5M phosphate buffer (pH 5.8), the solution was refluxed in a stream of CO_2 -free N_2 to expel CO_2 , ninhydrin (0.5 g.) was added and the solution was again refluxed. Acetaldehyde was trapped in 50% (v/v) aqueous methanol cooled in freezing mixture (-10 to -15°) and CO_2 was absorbed in $Ba(OH)_2$. The acetaldehyde was immediately further degraded to iodoform by adding 2N-NaOH (10 ml.) followed by a mixture of iodine (0.5 g.) KI (0.5 g.), 2N-NaOH (10 ml.) and 50% (v/v) aq. methanol (20 ml.).

Glutamic acid. Glutamic acid (85 mg.) was dissolved in water (5 ml.) and after addition of 0.5M phosphate buffer (pH 5.8) the solution was refluxed in a stream of CO_2 -free N_2 . Ninhydrin (0.3 g.) was then added, the solution was again refluxed and the CO_2 , which was derived from the α -carboxyl group, was collected as $BaCO_3$.

Acetic acid. Silver acetate, obtained by hydrolysis of α -acetamido- γ -phenylbutyric acid or *p*-acetamidobenzoic acid, was degraded by decarboxylation with Br_2 in dry CCl_4 (Arnstein, 1951*a*).

Synthesis of labelled compounds

DL- $[\gamma-^{14}C]$ Valine. This amino acid was obtained from the Radiochemical Centre, Amersham.

DL- ^{35}S Cystine. DL- ^{35}S Cystine was synthesized by Mr P. T. Grant by condensation of ^{35}S thioacetic acid (ethanethiolic acid), obtained from the Radiochemical Centre, with α -acetamidoacrylic acid; the product, *N*- ^{35}S -diacetylcysteine, was hydrolysed and converted to DL- ^{35}S cystine by oxidation with air.

DL- $[\beta-^{14}C]$ Cystine. The synthesis of this compound is based on the method described by Crawhall & Elliott (1951). In the modification described here, no transfer of radioactive material is required until the last stage. $^{14}CH_3OH$ (approx. 150 mg., 0.9 mc) was converted to an aqueous solution of $^{14}CH_3O$ (approx. 104 mg./5 ml.) (Arnstein, 1951*a*), which was added to a 10% excess of ethyl *N*-benzylthiocarbonylamino malonate (1.30 g.) in pyridine (10 ml.). The mixture was kept at room temp. overnight, 10 mg. unlabelled formaldehyde (in dil. aq. solution) was added, and after a further 7 hr. the solvent was evaporated *in vacuo* at $<60^\circ$. The residue was dried *in vacuo* over conc. H_2SO_4 overnight. Anhydrous ether (5 ml.) was added to the viscous oil and the solution was cooled in an ice-salt bath.

Purified thionyl chloride (10 ml.) (Martin & Fieser, 1943), also previously cooled, was added dropwise to the ether solution at a slow rate. The mixture was allowed to stand in the cold for 1 hr. after the addition of the thionyl chloride; as much of the thionyl chloride as possible was then removed by evaporation *in vacuo* at $<20^{\circ}$. 5N-NaOH in ethanol was then added to the residual oil till the solution was alkaline to litmus. After 20 min. the alkaline solution was neutralized with 3N-HCl and the ethanol removed under reduced pressure. Excess 3N-HCl was added and the solution was refluxed for 2 hr. After removal of the HCl under reduced pressure, water (25 ml.) followed by N-NaOH (25 ml.) was added to give a strongly alkaline solution which was allowed to stand for 30 min. with occasional stirring. After addition of conc. HCl (50 ml.) the solution was refluxed for 24 hr. The HCl and some benzoyl mercaptan were removed by evaporation *in vacuo*, water being added to assist the removal of the last traces. Water (50 ml.) was added and the solution was twice extracted with ether, the ether layer being removed with a pipette. After evaporation of the aqueous layer, the residue was extracted with ethanol (10 ml.). The insoluble material was filtered off and washed with more ethanol. The filtrate (40 ml.) was diluted with an equal volume of water and the solution was oxidized with 0.2N-iodine (required: 10 ml., 52% yield) using starch as an external indicator. Excess iodine was removed with a drop of sodium thiosulphate solution and the pH of the solution was adjusted to 5 by adding ammonia (sp.gr. 0.880) till alkaline, followed by acetic acid.

After 20 hr. at 0° the $[\beta\text{-}^{14}\text{C}]$ cystine was filtered off and dried (yield: 0.2193 g., 47.5% based on ethyl N-benzylthiothiocarbonylamino malonate; specific radioactivity = 0.63 $\mu\text{C}/\text{mg.}$). The radioactivity of this material was unchanged after recrystallization from water.

RESULTS

Conversion of cystine and valine to choline. The choline isolated from the carcass and viscera from

the rats which had been fed DL- $[\beta\text{-}^{14}\text{C}]$ cystine had a specific radioactivity of 0.24 $\mu\text{C}/\text{mole}$; that isolated after feeding DL- $[\gamma\text{-}^{14}\text{C}]$ valine was completely non-radioactive.

Comparison of the conversion of cystine and valine to 'acetate'. The incorporation of radioactivity into the acetyl derivative of α -amino- γ -phenylbutyric acid (Table 1) shows that DL- $[\beta\text{-}^{14}\text{C}]$ cystine and DL- $[\gamma\text{-}^{14}\text{C}]$ valine are converted by the rat to 'acetate'. In the valine experiments the acetyl derivative of *p*-aminobenzoic acid was also radioactive. The dilution factors for the conversion of both amino acids and dietary acetate to α -acetamido- γ -phenylbutyric acid are of the same order of magnitude. Degradation of the acetic acid obtained by hydrolysis of the α -acetamido- γ -phenylbutyric acid excreted by animals which had been fed DL- $[\beta\text{-}^{14}\text{C}]$ cystine indicated that 95% of the radioactivity was located in the methyl carbon atom. This distribution of isotope is very similar to that found in the acetyl carbon atoms of α -acetamido- γ -phenylbutyric acid after feeding $^{14}\text{CH}_3\text{CO}_2\text{Na}$ when 98–99% of the ^{14}C was located in the methyl carbon atom (Arnstein, 1951b).

Conversion of cystine to glycine. The radioactivity of the hippuric acid excreted after feeding DL- $[\beta\text{-}^{14}\text{C}]$ cystine (Table 1) was very much lower than that of the α -acetamido- γ -phenylbutyric acid, indicating that $[\beta\text{-}^{14}\text{C}]$ cystine is converted to glycine only to a small extent, which is also shown by the correspondingly high dilution factor. Similarly, the radioactivity of the glycine isolated from the protein of the mixed viscera (Table 2) was low, being only about 0.7% of that of the cystine from the same source.

Table 1. *Specific radioactivities of compounds isolated from the urine of rats which had received DL- $[\beta\text{-}^{14}\text{C}]$ cystine, DL- $[\gamma\text{-}^{14}\text{C}]$ valine or sodium $[\alpha\text{-}^{14}\text{C}]$ acetate in the diet*

Details of labelled compound fed			Specific radioactivity of isolated compounds ($\mu\text{C}/\text{mole}$) and dilution factors of isotopic tracer (in brackets)		
Compound	Specific radioactivity ($\mu\text{C}/\text{m-mole}$)	Amount given (m-mole/100 g. body wt.)	Hippuric acid	<i>p</i> -Acetamido-benzoic acid	L- α -Acetamido- γ -phenylbutyric acid
DL- $\text{S-}^{14}\text{CH}_2\text{.CHNH}_2\text{.CO}_2\text{H}$	31.6*	0.13*	7.1 (580)	—	112† (36.0)
DL- $\text{S-}^{14}\text{CH}_2\text{.CHNH}_2\text{.CO}_2\text{H}$					
DL- $\text{CH}_3\text{CH}(\text{CH}_3)\text{.CHNH}_2\text{.CO}_2\text{H}$	16.7	0.21	—	281 (13.1)	186 (19.8)
$^{14}\text{CH}_3\text{CO}_2\text{Na}$	1000	0.0069	14.1 (490)	—	376 (18.4)

—, Value not determined.

* Calc. as cysteine.

† Degradation of the acetic acid obtained by hydrolysis showed that 95% of the radioactivity was located in the methyl carbon atom of the acetate.

Conversion of cystine to other amino acids. In Table 2 the specific radioactivity of cystine isolated from the visceral protein of rats fed DL- $[\beta\text{-}^{14}\text{C}]$ -cystine is compared with the radioactivities of glutamic and aspartic acids, alanine, serine, leucine, valine and threonine. The radioactivity of a mixture of leucine and methionine was also determined and found to be equal to that of the leucine fraction, indicating that the radioactivity of the methionine is approximately the same as that of the leucine.

Table 2. *Specific radioactivities of amino acids isolated from the protein of the mixed viscera of rats which had been fed DL- $[\beta\text{-}^{14}\text{C}]$ cystine*

(Details concerning the administration of the labelled cystine and the isolation, degradation and radioactivity assay of the amino acids are given in the Experimental section. For comparison, the specific radioactivity is expressed as $\mu\text{C}/\text{mole}$ or $\mu\text{C}/\text{g. atom}$. The cystine fed had a radioactivity of $30 \mu\text{C}/\text{m-mole}$ cysteine.)

Amino acid	Specific radioactivity ($\mu\text{C}/\text{mole}$ or $\mu\text{C}/\text{g. atom}$)	Radioactivity in individual carbon atoms (%)
Cystine	224.4*	—
Glutamic acid:		
Total	26.0	—
α -Carboxyl-carbon atom	3.4	13
Alanine:		
Total	22.4	—
β -Carbon atom	15.8	71
α -Carbon atom	5.6†	25
Carboxyl-carbon atom	1.0	4
Aspartic acid	16.4	—
Serine:		
Total	5.2	—
β -Carbon atom	3.4	60
α -Carbon atom	1.5	26
Carboxyl-carbon atom	0.8	14
Glycine	1.6	—
Methionine + leucine	1‡	—
Leucine	1‡	—
Valine	0.5‡	—
Threonine	<0.06	—

* Calc. as cysteine.

† Calc. by difference.

‡ Approx. values, based on radioactivity of crude amino-acid fractions (see Experimental section).

The specific radioactivity of the cystine isolated from the visceral protein was only about 0.8 % of that of the fed amino acid. Glutamic acid had about 0.1 % of the specific radioactivity of the fed cystine, which is higher than that of the other amino acids isolated with the exception of cystine itself. Aspartic acid and alanine contained almost as much radioactivity as the glutamic acid, but serine was much less radioactive. Glycine contained approximately as much ^{14}C as the α - and carboxyl-carbon atoms of serine, which are known to be in equilibrium with glycine (see, for example, Arnstein & Neu-

berger, 1953a). Methionine, leucine and valine appear to be weakly radioactive, but these amino acids were not isolated in a pure state and it is possible that their activity was due to small amounts of impurities. The radioactivity of threonine was too low to be measured accurately and it seems likely that this amino acid was in fact non-radioactive.

Table 3. *Specific radioactivity of the sulphur of cystine and methionine from the mixed visceral proteins of a rat which had been fed DL- $[\text{S}^{35}]$ cystine*

(Details of the administration of the labelled cystine and the isolation methods are given in the Experimental section. The radioactivity of the sulphur was determined after conversion to benzidine sulphate, which was estimated by titration with 0.01N-NaOH. Using this value, the observed counts/min. were converted to counts/min./mg. S. In order to facilitate comparison with the fed cystine, the radioactivity is also expressed as $\mu\text{C}/\text{m-mole}$, which was obtained by comparing the observed counting rate with that of a standard of known radioactivity. The term 'dilution factor' is defined in Table 1. The value in brackets in column 4 is the dilution factor for the cystine isolated from the visceral protein of rats fed DL- $[\beta\text{-}^{14}\text{C}]$ -cystine (Tables 1 and 2) and has been included for comparison.)

Compound	Specific radioactivity		Dilution factor
	Counts/min./mg. S	$\mu\text{C}/\text{m-mole}$	
Cystine	12 050	2.06	39 (19)
Methionine	80	0.014	5500

Table 3 shows that after feeding $[\text{S}^{35}]$ cystine the methionine of the visceral protein has only 0.7 % of the specific radioactivity of the cystine from the same source. It appears therefore that cystine sulphur is not incorporated into methionine to any significant extent.

DISCUSSION

Recent experiments on the synthesis of the methyl groups of methionine and choline by rats fed diets deficient in or devoid of labile methyl groups have shown that the β -carbon atom of serine is an important methyl-group precursor, which can give rise to about 70 % of the newly synthesized methyl groups (Arnstein & Neuberger, 1953b). It seemed likely that the remaining 30 % of the newly synthesized methyl groups are derived from other constituents of the diet, although the possibility that methyl groups already present in the body at the beginning of the experiment were re-utilized to some extent, could not be excluded. It is known that histidine (Reid & Landefeld, 1951; Soucy & Bouthillier, 1951; Toporek, Miller & Bale, 1952) and tryptophan (Knox & Mehler, 1950) can give rise to one-carbon fragments which are subsequently converted to methyl groups. Cysteine appeared to be another possible methyl-group precursor if the

β -carbon atom of this amino acid were split off to a one-carbon compound by a similar reaction to that occurring during the conversion of serine to glycine. Alternatively, reversal of the formation of cysteine from methionine (or homocysteine) and serine and cleavage of the intermediate, cystathionine, into glycine and a homocystine derivative, such as *S*-formylhomocysteine, might lead to the formation of a one-carbon fragment from the β -carbon atom of cysteine. Such a reaction would not be expected to be a major pathway of cysteine metabolism as it is known from experiments with rats that cysteine cannot replace methionine nutritionally, even in the presence of choline and homoserine (Armstrong & Binkley, 1948) or α -aminobutyric acid (Fromageot & Clauser, 1949), which might serve as sources of methyl groups and the carbon chain of homocysteine respectively. Furthermore, L-cystathionine, fed together with choline or cobalamin, also does not support the growth of rats on a diet lacking methionine (Stekol & Weiss, 1950; Anslow, Simmonds & Du Vigneaud, 1946), although homocysteine is effective under these conditions. This indicates that the sulphur of cysteine or cystathionine is not available for the synthesis of a sufficient amount of methionine. On the other hand, L-allocystathionine appears to be effective in replacing methionine to a small but definite extent (Anslow *et al.* 1946), and it seemed possible, therefore, that the results of the above nutritional studies might be due to an insufficiently fast conversion of cysteine to methionine rather than to a complete absence of such a reaction. This possibility was investigated in the present work with [^{35}S]cystine. It was found that cystine sulphur is incorporated into methionine only to a very small extent, which may indicate a low degree of reversibility of the conversion of methionine sulphur into cystine. However, methionine was not isolated in this experiment and it is therefore possible that the observed radioactivity in the methionine fraction was due to an impurity, e.g. a cysteine peptide, which was not completely separated.

The experiments with [β - ^{14}C]cystine indicate some conversion of cystine into serine. However, only 60 % of the total radioactivity in the serine was located in the β -carbon atom and the incorporation of ^{14}C into serine must therefore have taken place by an indirect pathway involving a redistribution of the isotope. The relative specific radioactivities of serine and alanine and the similar distribution of ^{14}C in the two amino acids are compatible with the formation of serine from alanine or pyruvate, with which alanine is known to be in rapid equilibrium by transamination. The conversion of cysteine to pyruvate has been demonstrated with extracts of rat liver (Smythe, 1942) and pyruvate is known to be converted into glycine by the rat, serine being

a likely intermediate in this reaction (Anker, 1948). The distribution of ^{14}C in the alanine and serine, is satisfactorily explained by the participation of pyruvate in the reactions of the tricarboxylic acid cycle, which results in the isotope being distributed at random between the α - and β -carbon atoms of pyruvate and incorporation of ^{14}C into the carboxyl carbon atom by carbon dioxide fixation.

In agreement with the nutritional evidence described above, it is concluded from the present experiments that in the rat the utilization of serine (Stetten, 1942) and the sulphur of methionine (Du Vigneaud, Kilmer, Rachele & Cohn, 1944) for cysteine biosynthesis is not reversible, but it has not been established whether this is due to the lack of cystathionine synthesis from cysteine or to the absence of cleavage of cystathionine to serine and homocysteine. It is of interest in this connexion that cysteine is known to be a precursor of serine and methionine in *Neurospora* (Horowitz, 1947).

The extremely low radioactivity in choline and methionine after feeding [β - ^{14}C]cystine shows that the β -carbon atom of cystine does not give rise to a one-carbon compound, such as formaldehyde or formate, which would be a precursor of methyl groups.

The major pathway of cysteine metabolism in the rat appears to involve the formation of pyruvate, which results in a high radioactivity in the alanine of the visceral protein and in the acetyl-carbon atoms of the excreted α -acetamido- γ -phenylbutyric acid. A direct conversion of cysteine to alanine rather than pyruvate is not ruled out by the present results, but it has recently been shown that pyruvate is formed enzymically from cysteine via cysteine-sulphinic and β -sulphinopyruvic acids (Kearney & Singer, 1952). The formation of pyruvate from cystine is also indicated by the extensive incorporation of ^{14}C into glutamic and aspartic acids, since these two amino acids are in equilibrium with α -ketoglutarate and oxaloacetate respectively, which are metabolically closely related to pyruvate by the reactions of the tricarboxylic acid cycle. Pyruvate is known to acetylate α -amino- γ -phenylbutyric acid (Anker, 1948, 1950) and its quantitative importance in cysteine metabolism may therefore be assessed by comparing the efficiency of cystine as a precursor of the acetyl group of α -amino- γ -phenylbutyric acid with that of compounds known to be converted to pyruvate *in vivo* or with that of sodium acetate which is a very effective precursor of acetyl groups (Anker, 1948, 1950). Such a comparison shows that DL-cystine is only slightly less effective as a source of acetyl groups than DL-valine, which is known to be extensively converted to 3-carbon acids, probably propionate and pyruvate (Peterson, Fones & White, 1952; Fones, Waalkes & White, 1951), or sodium acetate (cf. Table 3) or DL-serine (Arnstein, 1951a).

In the present experiments the labelled cystine and valine were fed as the DL-amino acids and it is possible that some of the above conclusions are not strictly applicable to the metabolism of the optically active enantiomorphs. However, it has been shown that in the rat the overall metabolism of D- and L-valine (Rose, Johnson & Haines, 1942) and the rate of oxidation of the sulphur of L- and DL-cystine (Lawrie, 1932) are similar. Furthermore, the rat is able to utilize D-valine for growth (White, Fones & Sober, 1952). D-Cystine is not utilized by the rat for growth (Du Vigneaud, Dorfmann & Loring, 1932), but this is probably due to the fact that the corresponding keto acid is destroyed faster than it is converted to L-cysteine (Stekol, 1948) and not to different metabolic pathways of the two enantiomorphs. It seems likely therefore that the intermediary metabolism of L-cystine and L-valine is at least qualitatively similar to that of the corresponding DL-amino acids.

SUMMARY

1. The metabolism of DL-cystine by the rat has been studied by feeding DL-[³⁵S]cystine and DL-[β-¹⁴C]cystine.

2. The relatively poor conversion of the carbon-

labelled cystine into serine and the almost complete absence of radioactivity in methionine after feeding sulphur-labelled cystine indicates that the formation of cystine from methionine and serine via cystathionine is not reversible to any significant extent.

3. There was no appreciable conversion of the β-carbon atom of cystine to the methyl groups of choline or methionine. A similar experiment with DL-[γ-¹⁴C]valine showed that this amino acid also was not a precursor of methyl groups.

4. The major pathway of cysteine metabolism appears to be to pyruvate as shown by the good incorporation of ¹⁴C from dietary DL-[β-¹⁴C]cystine into alanine, aspartic acid and glutamic acid of the visceral protein and into the acetyl group of α-acetamido-γ-phenylbutyric acid excreted in the urine.

5. A synthesis of DL-[β-¹⁴C]cystine from ¹⁴CH₃OH is described.

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