The Biosynthesis of Penicillin

1. THE INCORPORATION OF SOME AMINO ACIDS INTO PENICILLIN

By H. R. V. ARNSTEIN AND P. T. GRANT

National Institute for Medical Research, Mill Hill, London, N.W. 7

(Received 28 October 1953)

Although many useful antibiotics have been discovered in the last decade, penicillin appears still to be unique in combining extremely high antibacterial potency with negligible toxicity. The penicillin produced commercially is almost entirely benzylpenicillin (I, $R = C_6H_5 \cdot CH_2-$),

which is effective mainly against Gram-positive bacteria. Since structurally modified penicillins might show enhanced activity against other micro-organisms, many penicillin analogues have been prepared. These compounds, which differ only in the acyl group, $RCO-$ (I), were obtained by biosynthetic methods (Behrens, 1949) or by chemical modifications of natural penicillins (Coghill, Stodola & Wachtel, 1949). Detailed information concerning the antibacterial activity and the therapeutic effectiveness of these penicillin derivatives is apparently not available (see, for example, Coghill *et al.* 1949), but it seems that none of them possesses properties greatly superior to those of benzylpenicillin. Indeed, the antibacterial activity of these modified penicillins against a benzylpenicillin-resistant strain of *Staphylococcus aureus* was not significantly greater than that of benzylpenicillin itself (Behrens & Kingkade, 1948). This evidence suggests that variation of the side-chain (R) does not markedly affect the antibacterial activity of penicillin.

The only other known analogue which still retains the fused β -lactam-thiazolidine rings of penicillin is a methyl penicillinate (II) synthesized by Sheehan, Henery-Logan & Johnson (1953). The fact that this compound is inactive against penicillin-sensitive bacteria suggests that the penicillin amide group is essential for biological potency. On this basis, the only remaining possibility of obtaining active penicillin analogues would be by substitution in the β -lactam-thiazolidine ring

system. The preparation of such penicillins by biosynthesis is rendered difficult by an almost complete lack of knowledge of the metabolic pathways involved in penicillin biosynthesis.

Earlier studies of penicillin precursors (e.g. Behrens, 1949; Süss, 1950) were based on the assumption that the addition of a precursor to a penicillin-producing mould culture would increase the yield of penicillin. It is evident, however, that a precursor might not be effective in stimulating penicillin formation if it participated only in steps which are not rate-limiting under the particular experimental conditions. The problem of penicillin biosynthesis has therefore been re-investigated by the isotopic tracer technique. This method is particularly suited to such work, since considerations of rate-limiting steps are not essential to the interpretation of results. The present paper deals with the probable role of some amino acids in the biosynthesis of penicillin, and the following paper (Arnstein & Grant, 1954) describes experiments indicating that cysteine is a precursor of penicillin. Most of this work has been briefly reported in a preliminary communication (Arnstein & Grant, 1953).

EXPERIMENTAL

Fermentation methods

Preparation of spore inoculum. The organism used in all experiments was *Penicillium chrysogenum* WIS 48-701 (Johnson, 1952), which was obtained from Dr M. Lumb of Boots Pure Drug Co. Ltd., Nottingham. Spores were suspended in heat-treated horse serum and freeze-dried in ampoules which were then sealed and stored at 4°. From these master cultures, a spore inoculum was prepared essentially as described by Perret (1953). Before use, the inoculum was diluted to contain approx. 6×10^6 spores/ml., as determined by a direct count with a haemocytometer cell.

Method. Penicillin was degraded chemically in order to locate the distribution of radioactivity in the penicillamine and penilloaldehyde moieties of the molecule. In most experiments, the penilloaldehyde was degraded further and the radioactivity in carbon atoms 5-7 was determined separately. Most of the chemical reactions involved in this method have been outlined by Clarke, Johnson & Robinson (1949). The following is a typical degradation.

Benzylpenicillin *N*-ethylpiperidine salt (394 mg., 0.88 m-mole) in water (5 ml.) was cooled to 0° and the free acid extracted into ether (2 × 10 ml.) at pH 2.5. The ether solution was shaken with ice-cold 0.1M sodium phosphate buffer (2 × 5 ml., pH 6.9) and the aqueous solution of sodium benzylpenicillin separated, made 0.1N with respect to H₂SO₄ and heated for 3 hr. at 100° in a current of CO₂-free N₂. The evolved CO₂ was absorbed in sat. aqueous Ba(OH)₂; the precipitated BaCO₃ was washed with water, ethanol and ether and dried (124 mg., 78%). The radioactivity of the BaCO₃ represents the amount of ¹⁴C at C₍₇₎ of penicillin.

The above solution of penilloic acid was adjusted to pH 6.5 with 2N-NaOH and 10 ml. sat. aqueous HgCl₂ were slowly added with stirring. The dense white ppt. of penicillamine mercaptide was coagulated by stirring at 40° for a few minutes, centrifuged and washed repeatedly with sat. aqueous HgCl₂ till the supernatant ceased to give a ppt. with 2:4-dinitrophenylhydrazine. After being washed with ethanol and ether, it was dried (yield of crude material 195 mg., 90%). The mercaptide was suspended in 0.1N-HCl (15 ml.) and treated with H₂S. HgS was filtered off and the filtrate was evaporated to dryness *in vacuo*. The residue was boiled for 5 min. with dry acetone (10 ml.) containing 1 drop of conc. HCl. The hot solution was filtered and the flask was washed with more acetone (5 ml.). The filtrate was concentrated to 2 ml. when isopropylidenepenicillamine hydrochloride (79 mg., 40%) crystallized. It was recrystallized from hot acetone or from acetone-ligroin, giving material of constant radioactivity, m.p. 192°.

The supernatant solution remaining after precipitation of the penicillamine mercaptide was immediately cooled in ice and treated with H₂S. HgS was filtered off and H₂S was removed from the filtrate with a stream of air. A portion of the filtrate (20% of the total volume) was added to a saturated solution of 2:4-dinitrophenylhydrazine in 2N-HCl, yielding crude benzylpenilloaldehyde 2:4-dinitrophenylhydrazone (31 mg., 49%; m.p. 171°), which was recrystallized from ethanol to give the pure product, m.p. 192-193°. The bulk of the above solution containing penilloaldehyde was cooled to 0° and a fivefold excess of freshly prepared ammoniacal Ag₂O solution (from 1.16 g. Ag₂O and 6 ml. conc. ammonia, sp.gr. 0.88) was added. The mixture was shaken for 12 hr. at 4°, acidified with conc. HCl and filtered. The filtrate was evaporated to dryness *in vacuo*, the residue was dissolved in 6N-HCl (10 ml.) and the solution was heated for 12 hr. at 110° in a sealed tube. After evaporation to dryness *in vacuo* the residue was extracted with ether to remove phenylacetic acid. The remaining material was dissolved in ethanol, the solution was filtered and pyridine was added to the filtrate giving glycine (18 mg., 27%), which was shown to be free from other amino acids by chromatography on paper. The glycine was further degraded with ninhydrin to formaldehyde, which was isolated as the dimedone derivative (m.p. 189°), and CO₂, which was converted into BaCO₃ (Arnstein, 1951). The radioactivities of the BaCO₃ and formaldehyde dimedone corresponds to the amount of ¹⁴C in C₍₅₎ and C₍₆₎ of penicillin, respectively.

RESULTS

The utilization of glycine, valine, serine and cystine for penicillin biosynthesis. The penicillin isolated from fermentations to which ¹⁴C-labelled glycine, valine, serine or cystine had been added contained significant amounts of ¹⁴C in all cases (Table 1). Of

Table 1. Conversion of [α -¹⁴C]glycine, DL-[γ -¹⁴C]valine, DL-[β -¹⁴C]serine and DL-[β -¹⁴C]cystine into penicillin

With the exception of Expt. no. 3, fermentations were carried out in duplicate. In Expts. nos. 1, 2 and 4, the amino acids were added at zero time, 18, 41, 65, 90 and 114 hr. as equimolar solutions (0.5 ml., approx. 0.4 μ mole/ml. broth/addition). Expt. no. 3 was a comparable large-scale fermentation (1.5 l.) as described by Arnstein & Grant (1954). Cystine was added in N-HCl, followed by an equivalent amount of N-KOH, the other amino acids were added as aqueous solutions; all solutions were sterilized by Seitz filtration. The pure sodium benzylpenicillin used as standard assayed at 1650 units/mg. For other experimental details see text.

Expt. no.	Amino acid added		Yield of penicillin (units)	Radioactivity of penicillin		Specific radioactivity (μ C/m-mole)		Dilution of molar radioactivity (A/B)	
						Amino acid added (A)	Penicillin (B)		
	mg.	μ C		μ C*	% of added 14 C				
1	[α - 14 C]Glycine	4.92	1.82	4 800	0.019	1.0	27.8	2.30	12.1
				6 650	0.019	1.0	27.8	1.68	16.6
2	DL-[γ - 14 C]Valine	7.02	1.56	3 700	0.026	1.7	26.1	4.16	6.3
				2 750	0.023	1.5	26.1	4.85	5.4
3	DL-[β - 14 C]Serine	378.5	1.96	300 000	0.054	2.8	0.543	0.105	5.2
4	DL-[β - 14 C]Cystine	7.47†	3.08	2 200	0.057	1.8	56.5‡	15.2	3.7
				3 600	0.106	3.4	56.5‡	17.2	3.3

* Calculated from the specific radioactivity and the total amount of penicillin produced.

† 4.89 mg. (3.08 μ C) DL-[β -¹⁴C]cystine + 2.58 mg. non-radioactive L-cystine.

‡ Calculated as μ C/m-mole cystine. The figure is an average of the specific radioactivity of the L- and D-cystine, which was 37.2 and 76.2 μ C/m-mole cystine, respectively.

the four amino acids investigated, cystine appears to be the best penicillin precursor, as shown by the relatively high incorporation of ^{14}C into penicillin and by the low dilution of the molar radioactivity. Serine and valine were utilized with approximately similar dilutions, which are, however, somewhat higher than those in the cystine experiments. Glycine is the least efficient precursor, the dilution of molar radioactivity in this case being about 4 times that in the cystine experiments. Table 1 also shows the penicillin yields obtained in these fermentations. In control experiments with micro-fermentations, titres of about 150 units/ml. were usually obtained and it seems, therefore, that the addition of glycine or DL-valine had little effect on the penicillin yield. DL-Cystine may have caused a slight decrease in penicillin formation, but in view of the small number of experiments this observation may not be statistically significant. The serine experiment was a large-scale fermentation (Arnstein & Grant, 1954) in which somewhat higher penicillin titres (200–400 units/ml.) were usually obtained.

Location of radioactivity in biosynthetic penicillin.
The distribution of ^{14}C in the penicillin derived from

$[\alpha\text{-}^{14}\text{C}]$ glycine, DL- $[\gamma\text{-}^{14}\text{C}]$ valine and DL- $[\beta\text{-}^{14}\text{C}]$ cystine (Table 2) indicates clearly that valine is incorporated only into the penicillamine moiety of penicillin and that glycine and cystine are converted into the β -lactam portion of the molecule. The α -labelled glycine gave rise to penicillin containing ^{14}C chiefly at $\text{C}_{(6)}$, although about 3% of the total radioactivity was located at $\text{C}_{(5)}$. In this experiment, glycine and serine of the mycelial protein were isolated as the 2:4-dinitrophenyl derivatives. It was found that the specific radioactivity of the serine was about one-half that of the glycine.

$[\beta\text{-}^{14}\text{C}]$ Cystine gave rise to penicillin containing 95% of the total radioactivity at $\text{C}_{(6)}$, which is the carbon atom common to the thiazolidine and β -lactam rings.

In all experiments, about 95% of the radioactivity present in the penicillin has been accounted for in the degradation products; the remaining 5% was not assignable to any individual carbon atom, but in view of the difficulty of measuring very low radioactivity it is possible that some or all of the positions designated as 'inactive' in Table 2 did in

Table 2. *Distribution of radioactivity in penicillin obtained from fermentations with labelled amino acids*

Details of the fermentations are given in Table 1. The penicillin from duplicate fermentations was combined, diluted with carrier and degraded as described in the Experimental section. The percentage radioactivity in separate carbon atoms of penicillin = $100 a/b$ where a is the molar radioactivity of the appropriate degradation product and b the molar radioactivity of penicillin after dilution with carrier.

Degradation product	Atom number in thiazolidine- β -lactam rings of penicillin*	Distribution of radioactivity in penicillin carbon atoms (%)		
		Glycine expt.	Valine expt.	Cystine expt.
BaCO_3 from acid-labile CO_2	7	Inactive	Inactive	Inactive
Isopropylidenepenicillamine HCl	(2 + 3)†	Inactive	95.2	Inactive
Benzylpenilloaldehyde 2:4-dinitrophenylhydrazone	5 + 6	—	Inactive	94.5
BaCO_3 from glycine carboxyl carbon	5	2.6	—	96.1
Formaldehyde dimedone from glycine α -carbon	6	94.1	—	Inactive

* See Experimental section for numbering of penicillin.

† Includes also the *gem*-dimethyl groups at $\text{C}_{(3)}$ and the carboxyl group at $\text{C}_{(3)}$.

— Signifies not determined.

Table 3. *Conversion of the optical enantiomorphs of $[\beta\text{-}^{14}\text{C}]$ cystine into penicillin, respiratory carbon dioxide and mycelium*

In duplicate experiments, D- or L- $[\beta\text{-}^{14}\text{C}]$ cystine was added daily in equal amounts (40 $\mu\text{g.}/\text{ml.}$ broth, i.e. 1 mg./flask/day) starting at zero time. The amino acids were added as Seitz-filtered solutions in N-HCl , each addition being followed by an equal amount of N-KOH . CO_2 in the effluent air was absorbed in 40% NaOH as described in the Experimental section and counted as BaCO_3 . At the end of the fermentation, the mycelium was filtered off, washed with water and dried. After extraction with ether, it was powdered and assayed directly for ^{14}C .

Amino acid added	Radioactivity (μC)			Specific radioactivity ($\mu\text{C}/\text{m-mole}$)		Dilution of molar radioactivity (A/B)
	Respiratory CO_2	Fat-free mycelium	Penicillin	Cystine added* (A)	Penicillin (B)	
L-Cystine	0.08	0.068	0.0029	3.05	1.38	2.21
	0.07	0.071	0.0036	3.05	0.88	3.46
D-Cystine	0.024	0.022	0.00046	3.28	0.22	14.9
	0.022	0.025	0.00145	3.28	0.25	13.1

* Calculated as $\mu\text{C}/\text{m-mole}$ cysteine.

fact contain traces of ^{14}C . It is unlikely, however, that more than 1–2 % of the total radioactivity was present in any one of these carbon atoms.

The stereochemical specificity of the conversion of cystine into penicillin. In view of the relatively efficient incorporation of DL-cystine into penicillin, it was of interest to investigate the stereochemical specificity of this reaction. Carbon-atom 6 of penicillin has been shown to have the same configuration as L-alanine (Kaczka & Folkers, 1949). It was expected that L-cystine would therefore be a better penicillin precursor than the D-enantiomorph. The results given in Table 3 show clearly that this was indeed the case, L-cystine being about 5 times more efficient as a precursor than D-cystine. It is of interest that the general metabolism of the two enantiomorphs, such as their conversion into respiratory carbon dioxide or into the mycelia protein, appears to correspond fairly closely to their utilization for penicillin biosynthesis. These results are compatible with the possibility that D-cystine is not metabolized as such by the mould but is converted into the L-enantiomorph, although only to a limited extent.

Incorporation of DL-cystine into penicillin when added at various times during the fermentation. In previous experiments, the labelled compounds were added at intervals throughout the fermentation in order to obtain penicillin with a relatively high specific radioactivity. Although this was achieved, only about 1–3 % of the total amount of precursor was converted into penicillin (Table 1). It was thought that a more favourable utilization of the precursor might take place if a single addition of a smaller quantity were made at the most suitable stage of the fermentation, i.e. at the peak of penicillin biosynthesis. DL- $[\beta\text{-}^{14}\text{C}]$ Cystine was therefore added at various times to a series of similar fermentations (Table 4). It can be seen that the best incorporation was obtained when the precursor was added at 23 or 47 hr. It may be significant that this time appears to coincide with the beginning of

active amino acid metabolism, as shown by the extensive conversion of glycine, valine or cystine into respiratory carbon dioxide (Fig. 2). The decline in the incorporation of cystine into penicillin during later stages of the fermentation was less marked than expected, and a significant utilization of

Fig. 2. Conversion of glycine, valine and cystine into respiratory carbon dioxide in penicillin fermentations. $[\alpha\text{-}^{14}\text{C}]$ Glycine, DL- $[\gamma\text{-}^{14}\text{C}]$ valine and DL- $[\beta\text{-}^{14}\text{C}]$ cystine were added to fermentations as described in Table 1, each addition being 0.303, 0.260 and 0.513 μC , respectively. Respiratory carbon dioxide was collected over various time intervals (0–18, 18–41, 41–65, 65–90, 90–114 and 114–142 hr.) during the fermentation and converted into BaCO_3 , which was weighed and assayed for ^{14}C . The times at which the labelled amino acids were added are indicated by arrows. The amount of added amino acid converted into carbon dioxide (x) at various periods of the fermentation was derived from the formula, $x = 100 a/b$, where a is the amount of ^{14}C in all the BaCO_3 fractions collected from the beginning of the fermentation till the stated time, and b is the amount of ^{14}C in the additions of labelled amino acid made before the stated time. $\square$ — $\square$, Glycine expt.; $\triangle$ — $\triangle$, valine expt.; $\circ$ — $\circ$, cystine expt.

Table 4. Utilization of DL- $[\beta\text{-}^{14}\text{C}]$ cystine for penicillin biosynthesis when added at various times during the fermentation

DL- $[\beta\text{-}^{14}\text{C}]$ Cystine (0.716 mg., 0.452 μC) in 0.1 ml. N-HCl was added to fermentations at the times shown below. At the end of each fermentation (121 hr.) the penicillin was isolated after dilution with carrier as described in the Experimental section.

Time of addition of DL- $[\beta\text{-}^{14}\text{C}]$ -cystine (hr.)	Radioactivity in penicillin		Specific radioactivity ($\mu\text{C}/\text{m-mole}$)		Dilution of molar radioactivity (A/B)
	μC	% of added ^{14}C	Cystine (A)	Penicillin (B)	
0	0.012	2.7	76.2*	1.72	44.3
23	0.018	4.0		2.88	26.4
47	0.019	4.2		2.66	28.6
71	0.014	3.1		2.07	36.8
95	0.008	1.8		1.29	59.0

* Calculated as $\mu\text{C}/\text{m-mole}$ cysteine.

precursor was observed even when the amino acid was added at 95 hr., i.e. 26 hr. before the end of the fermentation (Table 4).

DISCUSSION

Early speculations concerning the biosynthesis of penicillin postulated the participation of three intermediates, phenylacetic acid, aminomalon-semialdehyde and penicillamine (Behrens, 1949). Phenylacetic acid may be formed from phenylalanine, since both compounds stimulate penicillin biosynthesis. Aminomalonsemialdehyde could presumably originate from serine, although this reaction has never been demonstrated. It is noteworthy that the presence of penicillamine in penicillin fermentations has also not been shown and no work on the biosynthesis of this amino acid has been reported. An alternative mechanism of penicillin biosynthesis was proposed later by Hockenhull, Ramachandran & Walker (1949) who suggested a condensation of D- β -hydroxyvaline, L-cysteine and a carboxylic acid, such as phenylacetic acid in the case of benzylpenicillin. However, no experimental work supporting this hypothesis was reported by these authors and until recently there has been no evidence concerning the origin of the thiazolidine- β -lactam rings of penicillin or the participation of amino acids in the biosynthesis. On the contrary, an experiment with deuteriophenylacetyl-DL-[^{15}N]valine appeared to indicate that valine was not used for penicillin biosynthesis (Behrens, 1949; see also Behrens *et al.* 1948). In other experiments, it was found that the phenylacetyl derivatives of DL-valine, DL-serine, DL-cysteine and glycine, as well as those of other amino acids, were not superior to phenylacetic acid or phenylacetamide in increasing penicillin production. Since hydrolysis of the phenylacetyl derivatives must have occurred, the above evidence indirectly suggests that glycine, serine, valine or cystine do not stimulate penicillin formation. Similar results were obtained in experiments using serine, valine or cystine (Behrens, 1949).

The present experiments demonstrate that glycine, serine, valine and cystine participate in the biosynthesis of the penicillin molecule, although they have little or no effect on the amount of penicillin formed. Thus the daily addition of the above amino acids, labelled with ^{14}C , to penicillin fermentations resulted in the incorporation of about 1–3.5% of the added radioactivity into the penicillin (Table 1). The apparently low incorporation of radioactivity into penicillin is probably due to the fact that the amount of penicillin formed is quantitatively insignificant when compared with the amount of mycelial protein. In agreement with this explanation, it was found that the radioactivity

in the mycelium was about 20 to 50 times that in the penicillin (Table 3). Moreover, a large proportion of the added amino acids is converted into respiratory carbon dioxide (Fig. 2 and Table 3), and the amount of amino acid available for penicillin formation is therefore considerably less than that added to the medium.

The efficiency of incorporation of precursor also depends on the time of its addition to the fermentation. Thus the utilization of cystine was found to be optimum at 23–47 hr. after inoculation of the medium (Table 4). At these times a single dose was used somewhat more efficiently for penicillin biosynthesis than a larger amount added throughout the fermentation (Table 1). The total amount of ^{14}C incorporated into penicillin is therefore not a suitable criterion of precursor efficiency. On the other hand, the dilution of the molar radioactivity of the precursors indicates their relative importance, since, in a series of reactions $A \rightarrow B \rightarrow C \rightarrow \text{product}$, the specific radioactivity of the most immediate precursor, C, will be diluted least, provided of course that the experimental conditions, such as the amount of precursor added and the times of addition, are comparable. On this basis, cystine appears to be a better penicillin precursor than valine, serine or glycine (Table 1).

Degradation of the penicillin obtained in the valine, glycine and cystine experiments showed clearly that valine is a source of the penicillamine moiety of penicillin, whereas the other two amino acids contribute to the biosynthesis of the β -lactam portion (Table 2). It is likely that glycine and serine are incorporated into penicillin only after conversion into cystine. Glycine is known to be a serine precursor both in intact rats (Sakami, 1948) and in suspensions of rat liver (Winnick, Moring-Claesson & Greenberg, 1948). A similar reaction evidently takes place in the mould used in the present work, since radioactive serine was isolated from the mycelium of the fermentation to which [α - ^{14}C]glycine had been added. The formation of cystine from serine has been shown to occur in rat liver tissue (Binkley & du Vigneaud, 1942) and probably also in the intact rat (Stekol, Weiss & Weiss, 1950).

Evidence indicating that cystine, or a close derivative of this amino acid, may be used intact for penicillin biosynthesis is provided by the difference in utilization of the D- and L-enantiomorphs (Table 3). L-Cystine, which has the same configuration as the relevant carbon atom (position 6) of penicillin (Kaczka & Folkers, 1949) was converted into penicillin with an average dilution of molar radioactivity of only 2.8 compared with 14.0 for the corresponding value in the D-cystine experiment.

Recently, it has been reported that [^{14}C]formate is incorporated into penicillin, although the position of the radioactive atoms in the penicillin molecule

was not determined (Martin *et al.* 1953). It seems likely that formate condenses with glycine to give serine and thence cysteine, and that this pathway accounts for the conversion of formate into penicillin.

It has also been shown that the addition of L-cysteine or methionine (which can serve as a source of cystine sulphur as demonstrated both in the animal organism (Tarver & Schmidt, 1939) and in the mould *Aspergillus nidulans* (Hockenhull, 1949)) to fermentations containing radioactive sulphate reduced the amount of radioactivity found in the penicillin without affecting the penicillin yield (Stevens, Vohra, Inamine & Roholt, 1953). These experiments indicate, in general agreement with the present work, that cysteine is a more direct penicillin precursor than sulphate.

It is concluded that the fused thiazolidine- β -lactam portion of penicillin is probably synthesized by a condensation of cysteine, or a close derivative, with valine, or a related amino acid such as hydroxy- or dehydro-valine. More conclusive evidence showing that cysteine is incorporated intact into penicillin and that the observed dilution of the specific radioactivity of the added labelled cysteine is due to endogenous cysteine synthesis by the mould is presented in the following paper (Arnstein & Grant, 1954).

SUMMARY

1. The conversion of [α - 14 C]glycine, DL-[β - 14 C]-serine, DL-, D- and L-[β - 14 C]cystine and DL-[γ - 14 C]-valine into penicillin has been studied by adding the labelled amino acids to fermentations of *Penicillium chrysogenum* WIS 48-701.

2. Valine was found to be a precursor of the penicillamine moiety of penicillin, whereas cystine and to a lesser extent glycine were incorporated into the β -lactam ring of the molecule.

3. Penicillin biosynthesis from cystine appears to be stereochemically specific, L-cystine being utilized to a far greater extent than the D enantiomorph.

4. The highest incorporation of radioactivity from cystine into penicillin was obtained when the amino acid was added to the fermentation at 23-47 hr. after inoculation.

5. The slight dilution of the specific radioactivity of cystine during its conversion into penicillin suggests that cysteine may be an important and direct precursor of penicillin.

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