ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1972, p. 315-322

Vol. 1, No. 4
Printed in U.S.A.

Effect of Exogenous Penicillin on Penicillin Biosynthesis

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Received for publication 2 November 1971

The addition of phenoxymethylpenicillin (10 mg/ml) at any time during the penicillin fermentation inhibited further accumulation of the antibiotic in broth but had no effect on growth. Benzylpenicillin, 6-aminopenicillanic acid (6-APA), and some semisynthetic penicillins also showed this effect, but penicillin N, penicilloic acid, cephalosporin C, and 7-aminocephalosporanic acid did not limit penicillin accretion. Incorporation of radioactive precursors (cysteine, valine, and sodium phenoxyacetate) into penicillin in the presence of inhibitory concentrations of the antibiotic indicated that penicillin synthesis continued despite the lack of accretion of the antibiotic in broth. The rates of penicillin synthesis in a 48-hr and a 136-hr culture were compared by short-term exposure to $Na_2{}^{35}SO_4$, and no significant difference in the biosynthetic rate was observed. Exogenous penicillin in the range of 1 to 15 mg/ml of culture broth had no effect on utilization of acetate or glucose by *Penicillium chrysogenum*. The antibiotic-synthesizing capacity of the organism was not irreversibly inhibited by exogenous penicillin. The degradation of penicillin during the fermentation was also studied. Penicillin V was stable in broth filtrate. Catabolic enzymes such as penicillinase and penicillin-acylase were not demonstrated in whole broth, nor was the accumulation of 6-APA, penicilloic acid, or other degradation products detected. An examination of the intracellular penicillin concentration and the amount of penicillin associated with the mycelium revealed that cells contained significantly more penicillin late in the fermentation than earlier in the cycle. This cell-associated antibiotic may be a regulatory factor in further penicillin synthesis.

The penicillin fermentation, like the synthesis of many other antibiotics and secondary metabolites, is characterized by two phases. After a period of rapid growth, strains of *Penicillium chrysogenum* steadily synthesize penicillin. The cultures, nutrients, and physical factors influencing penicillin production have been reviewed extensively (1, 4, 7–9, 13, 14). The cause of the termination of penicillin accumulation in spite of the maintenance of optimal conditions during fermentation has not been fully determined.

This investigation was undertaken to ascertain whether penicillin itself acts as an inhibitor or regulator of penicillin biosynthesis.

MATERIALS AND METHODS

Antibiotics. Potassium benzylpenicillin (penicillin G), potassium phenoxymethylpenicillin (penicillin V), *p*-methylpenicillin V, phenoxypropylpenicillin, penicillin N, 6-aminopenicillanic acid (6-APA), and cephalosporin C were products of Eli Lilly & Co., Antibiotic Manufacturing and Development. Methylpenicillin and 7-aminodeacetoxycephalosporanic acid were prepared at the Lilly Research Laboratories. α-Aminobenzylpenicillin (ampicillin), sodium 2,6-dimethoxyphenylpenicillin trihydrate (methicillin), and sodium 5-methyl-3-phenyl-4-isoxazolylpenicillin (oxacillin) were products of Bristol Laboratories, Syracuse, N.Y. Potassium phenoxyethylpenicillin (phenethicillin) was obtained from Pfizer, Inc., New York, N.Y., and 6-(2-ethoxy-1-naphthamide) penicillin (nafcillin) was obtained from Wyeth Laboratories, Inc., Philadelphia, Pa. Preparation of phenoxymethylpenicilloic acid was achieved by alkaline hydrolysis of potassium penicillin V. ^{35}S-penicillin V was prepared by fermentation with $Na_2{}^{35}SO_4$, acid extraction into butyl acetate, and crystallization as the potassium salt.

Cultures and fermentation conditions. A strain of *P. chrysogenum*, mutant E-15 (10), was used throughout this investigation with the exception of one study with *P. chrysogenum* Q176 and NRRL 1951. Spore inocula were prepared by growing cultures at 25 C on the agar medium of Somerson, Demain, and Nunheimer (19). Spores were removed from the surface of a slant suspended in 10 ml of nutrient broth, and were used to inoculate a 2-liter Erlenmeyer flask containing 1 liter of the vegetative medium described

by Vanderhaeghe (24) except that $Na_2S_2O_3 \cdot 5H_2O$ was omitted. The flasks were incubated at 25 C on a rotary shaker operating at 250 rev/min. After 2 days, 10 ml of germinated culture was used to inoculate each 500-ml Erlenmeyer flask containing 100 ml of one of the following: (i) the chemically defined medium of Tardrew and Johnson (21); (ii) a lactose-corn steep liquor fermentation medium (15); or (iii) the same medium as ii above except that the lactose was omitted and 0.5% glucose was added to the medium during makeup (sterile glucose, 0.7 ml of a 70% glucose solution, was added aseptically with a syringe at 8-hr intervals starting at 16 hr after inoculation and continuing until 8 hr prior to harvest).

Unless indicated, 0.3% and 0.5% sodium phenoxyacetate were added to the synthetic and complex fermentation media, respectively. Growth conditions were the same as described above, with the fermentation terminated at 6 days. At harvest, 10 ml of whole broth was centrifuged for 10 min at 2,000 × *g*, and from the volume of the wet pellet growth was calcalculated as the percentage of solids. All flasks were closed with two fiber-filter discs (Kendall Co., Walpole, Mass.) and were autoclaved at 120 C for 20 min.

Cell extracts. Mycelium was obtained from fermentation broths by centrifugation and was washed three times with distilled water. Cells were suspended in 2 volumes of buffer (potassium phosphate, 0.05 M, *p*H 7.0) and passed through a French pressure cell (American Instrument Co.) at 10,000 psi. The resulting extract was centrifuged at 30,000 × *g* for 15 min, and the supernatant fluid was decanted.

Antibiotic assays. Culture filtrates or cell extracts were assayed for β-lactam-containing antibiotics by a modification of the hydroxylamine reaction (6) for the Technicon AutoAnalyzer. This assay has precision error of ± 1.2%. Penicillin potencies in the presence of added cephalosporin antibiotic were determined as the difference between untreated and penicillinase (Riker Laboratories, Northridge, Calif.)-treated broths. Penicillin was measured biologically by a turbidimetric method with *Staphylococcus aureus* as the test organism. This assay has an error of about ± 10%. Unless indicated, all samples were assayed by both the chemical and the biological method. The 6-APA content of broths was estimated from the difference between chemical and biological assays for penicillin. Penicilloic acid was determined chemically (16).

Chromatography. Penicillins and 6-APA were identified by applying samples and appropriate standards to Whatman no. 1 paper, which underwent descending development overnight in the upper phase of *n*-butanol-ethanol-water (4:1:5). After phenylacetylation (5) and drying, chromatograms were placed on agar plates seeded with *Bacillus subtilis*. After 15-min contact, chromatograms were removed and the plates were incubated overnight at 37 C. Duplicate chromatograms were prepared in those studies in which isotopic labeling was used. The position of radioactive compounds was detected with a Vanguard strip scanner (model 880), and the R_F of radioactive peaks was compared with the R_F of biologically active zones. The R_F values of 6-APA, penicilloic acid, and penicillin V were 0.19, 0.27, and 0.63, respectively, under these conditions. Penicilloic acid and other biologically inactive penicillins were detected by spraying chromatograms with iodine as described by Thomas (22).

Respiration studies. Respiration of *P. chrysogenum* E-15 was measured by standard manometric techniques (23). Cells were removed from 48- and 72-hr fermentation broths by centrifugation and were washed twice with 0.05 M phosphate buffer, *p*H 7.0. In addition to the cells, Warburg flasks contained glucose or acetate (5 mg), buffer (0.05 M) in a total volume of 3.0 ml, and potassium hydroxide (0.2 ml, 20%) in the center well. Flasks were shaken for 1 hr in a water bath at 30 C.

Incorporation of radioactive tracers. Radioactive precursors, sterilized by filtration, were added to 48-hr *Penicillium* broths at a concentration of 0.05 μCi/ml. Cultures were incubated an additional 4 days under control growth conditions, unless otherwise indicated. At harvest, whole broth was filtered through filter aid on a Büchner funnel. Filtrates were chilled, adjusted to *p*H 2.0, and extracted with one-half volume cold *n*-butyl acetate. Penicillin was crystallized from the organic phase by the addition of 80% potassium acetate. Crystals were collected on a Büchner funnel, washed with isopropanol, and dried under vacuum overnight. Samples were weighed for assay. The samples were dissolved in water and prepared for counting as described by Hall and Cocking (12). Radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrophotometer, model 3375, with the use of an external standard. The amount (micromoles) of radioactive tracer incorporated into penicillin was calculated from the total disintegrations in the purified penicillin multiplied by the specific activity of the precursor. Purity of penicillin was based on milligrams of biological activity per milligram (dry weight) of crystallized material. Evidence for the presence of radioactivity as ^{14}C- or ^{35}S-penicillin was obtained by comparing biologically active and radioactive zones on chromatographic strips. Radioactivity of cells exposed to ^{35}S-penicillin V was determined by liquid scintillation counting of washed-cell samples hydrolyzed in Soluene 100 (Packard Instrument Co.).

RESULTS

The effect of penicillin on growth of *P. chrysogenum* E-15 and on net antibiotic synthesis was evaluated by adding various levels of penicillin V throughout the fermentation. The results in Table 1 show that the addition of 10 mg of penicillin V/ml at inoculation (0 hr) or at 66 hr postinoculation did not inhibit growth but exerted a limiting effect on net penicillin synthesis. The addition of penicillin in increments more closely resembling the accretion of penicillin in a fermentation did not lessen the inhibition. Penicillin G added to penicillin fermentations had a similar effect on antibiotic production without inhibiting growth (Table 2).

The penicillin nucleus, 6-APA, at concentra-

TABLE 1. *Effect of exogenous penicillin V on growth and penicillin synthesis*[a]

Penicillin added	Growth (% of control)	Penicillin synthesis	
		Net amt (mg/ml)	Percentage of control
None	100	3.44	100
At 0 hr			
1 mg/ml	110	3.17	90
5 mg/ml	100	0.74	21
10 mg/ml	100	0.63	19
At 66 hr			
1 mg/ml	115	1.72	50
5 mg/ml	110	0.34	10
10 mg/ml	100	0.31	9
15 mg/ml	110	0.00	0
10 mg/ml added gradually throughout fermentation	100	0.02	0

[a] Fermentation carried out in glucose medium.

TABLE 2. *Effect of exogenous penicillin G on growth and penicillin synthesis*[a]

Penicillin added	Growth (% of control)	Penicillin synthesis	
		Net amt (mg/ml)	Percentage of control
None	100	3.85	100
At 0 hr			
1 mg/ml	105	3.34	87
5 mg/ml	100	0.88	23
10 mg/ml	100	0.65	17
At 66 hr			
1 mg/ml	110	3.95	103
5 mg/ml	100	1.50	39
10 mg/ml	110	0.96	25
15 mg/ml	100	0.03	0
10 mg/ml added gradually throughout fermentation	100	0.72	19

[a] Fermentation carried out in glucose medium.

tions of 10 mg/ml completely inhibited penicillin accumulation when added to fermentations either initially or throughout the synthetic phase (Table 3). The acyl precursor of penicillin V, sodium phenoxyacetate, at levels beyond that normally incorporated into the medium, had no effect on growth or subsequent synthesis of penicillin V.

Cysteine and valine, which have been shown to be precursors of the penicillin nucleus (2, 3), were added to fermentations but did not limit penicillin synthesis at concentrations equivalent to 10 mg of penicillin V/ml.

The effect of other penicillins and cephalosporins on penicillin V accumulation was examined (Table 4). The effect of these ranged from no inhibition with *p*-methylpenicillin V, penicillin N, cephalosporin C, and 7-aminodeacetoxycephalosporanic acid to 100% inhibition with methicillin and penicillin V. The uptake of these compounds by the synthesizing organism was not studied.

The specificity of the intact β-lactam ring as an inhibitor of penicillin synthesis was further ex-

TABLE 3. *Effect of exogenous 6-aminopenicillanic acid (6-APA) and sodium phenoxyacetate on penicillin synthesis*[a] *and growth*

Addition	Growth (% of control)	Penicillin synthesis	
		Net amt (mg/ml)	Percentage of control
None	100	2.50	100
6-APA at 0 hr			
5 mg/ml	120	1.63	65
10 mg/ml	100	0.12	0
20 mg/ml	96	0.01	0
6-APA added gradually throughout fermentation			
5 mg/ml	110	0.90	37
10 mg/ml	100	0.04	0
Na phenoxyacetate			
4.3 mg/ml	100	2.49	100
12 mg/ml	95	2.37	92
30 mg/ml	95	2.80	110

[a] Fermentation was carried out in glucose medium.

TABLE 4. *Inhibition of penicillin V synthesis by semisynthetic penicillins*[a]

Penicillin added	Net penicillin synthesis (mg/ml)	Per cent inhibition of synthesis
None	3.10	0
p-Methylpenicillin V	3.15	0
Ampicillin	2.10	31
Phenethicillin	1.05	65
Oxacillin	0.84	72
Methylpenicillin	0.74	76
Phenoxypropylpenicillin	0.71	78
Nafcillin	0.21	93
Methicillin	0.00	100
Penicillin V	0.00	100
Penicillin N	2.95	5
Cephalosporin C	3.07	1
7-Aminodeacetoxycephalosporanic acid	3.04	0

[a] Fermentation was carried out in lactose medium. Sterile penicillins (10 mg/ml) were added aseptically at 48 hr.

TABLE 5. *Effect of penicilloic acid on synthesis of penicillin V*[a]

Addition	Inhibition of penicillin synthesis %
None	—
Penicilloic acid	
5 mg/ml	19
10 mg/ml	10
20 mg/ml	12
Penicillin V	
10 mg/ml	100

[a] Fermentation was carried out in lactose medium. Sterile penicilloic acid and penicillin V were added at 48 hr.

TABLE 6. *Penicillin V (Pen V) inhibition of penicillin synthesis by NRRL 1951 and Q176 strains*[a]

Strain	Pen V added (μg/ml)	Net Pen V synthesis[b] (μg/ml)
Q176	None	420
	500	210
	1,000	210
	2,000	−300
NRRL 1951	None	125
	100	120
	200	−30
	400	−100

[a] Fermentation was carried out in lactose medium. Penicillin V was added aseptically at the time of inoculation.

[b] Bioactivity.

amined by the addition of phenoxymethylpenicilloic acid to penicillin fermentations (Table 5). No inhibition of penicillin synthesis in the presence of penicilloic acid was observed, whereas the equivalent amount of penicillin V completely blocked antibiotic accumulation.

Penicillin V as an inhibitor of penicillin synthesis was investigated by use of strains of *P. chrysogenum* with varying capacity to synthesize antibiotic. The addition of penicillin V at concentrations equal to or exceeding that accumulated by each culture under control conditions inhibited subsequent penicillin synthesis (Table 6). When less than total inhibitory concentrations of penicillin were added, the amount of penicillin ultimately synthesized usually equaled the difference between the concentration originally in the medium and that which would have been produced in its absence.

Exogenous penicillin V and 6-APA at levels of 10 mg/ml were each capable of preventing the accumulation of 6-APA in a fermentation from which the acyl precursor had been omitted (Table 7). This inhibitory concentration was considerably greater than the amount of 6-APA normally produced in the absence of precursor.

TABLE 7. *Inhibition of 6-aminopenicillanic acid (6-APA) synthesis by exogenous penicillin V or 6-APA*[a]

Addition	6-APA synthesis	
	Net amt (mg/ml)	Percentage of control
None	2.24	100
Penicillin 4		
5 mg/ml	1.29	58
10 mg/ml	−0.16	0
20 mg/ml	−0.62	0
6-APA		
4 mg/ml	2.06	92
8 mg/ml	0.85	38

[a] Fermentation was carried out in lactose medium with no added sodium phenoxyacetate. Penicillin V and 6-APA were added aseptically at 48 hr.

TABLE 8. *Effect of added penicillin V on the utilization of glucose and acetate by Penicillium chrysogenum*

Age of culture (hr)	Substrate[a]	Added penicillin V concn (mg/ml)	O_2 taken up[b]
48	Glucose	0	1.94
		1	1.97
		2.5	2.23
		5	1.66
		10	1.49
		15	2.08
72	Glucose	0	1.82
		1	1.51
		2.5	1.47
		5	1.54
		10	1.88
		15	1.51
48	Acetate	0	1.51
		1	1.56
		2.5	1.80
		5	1.22
		10	1.57
		15	1.44

[a] The amount of substrate was 5 mg/flask.

[b] Expressed as microliters per minute per milliliter.

The metabolism of *P. chrysogenum* E-15 with the use of glucose and acetate in the presence of various levels of penicillin V was measured in a Warburg respirometer. The rates of oxygen consumption, $Q(O_2)$, recorded in Table 8 showed little effect of penicillin on the utilization of these substrates.

The antibiotic-synthesizing capacity of the organism was not irreversibly inhibited by exogenous penicillin. Cells (72 and 96 hr) that were washed and transferred to 48-hr spent medium produced control levels of penicillin. In addition, *Penicillium* cells were removed from fermentation broth at 72 and 96 hr, washed, and transferred to 48-hr sterile penicillin broth. One sample of 72-hr cells was exposed to 10 mg of penicillin V/ml for 2 hr prior to transfer. The cell suspensions were incubated for 3 to 4 days, during which time penicillin synthesis was monitored. Penicillin production by all cell suspensions was comparable to that of the control in fresh lactose medium during the same period (Fig. 1).

The incorporation of radioactive precursors into penicillin V was examined in the presence of exogenous penicillin V and 6-APA. DL-Cystine-*1*-^{14}C, L-valine-*U*-^{14}C, and phenoxyacetic-*1*-^{14}C acid were added to penicillin fermentations at 48 hr along with 10 mg of 6-APA or penicillin V per ml. Penicillin extracted and crystallized from harvested broth filtrates was assayed for purity and radioactivity. Net penicillin synthesis was determined. The micromoles of labeled precursor incorporated into penicillin in the presence and absence of inhibitory concentrations of 6-APA and penicillin V are given in Table 9. Although net antibiotic synthesis was limited by exogenous penicillin and 6-APA, the incorporation of DL-cystine-*1*-^{14}C was not equally inhibited, L-valine-*U*-^{14}C incorporation was unaffected, and phenoxyacetic-*1*-^{14}C acid incorporation was enhanced.

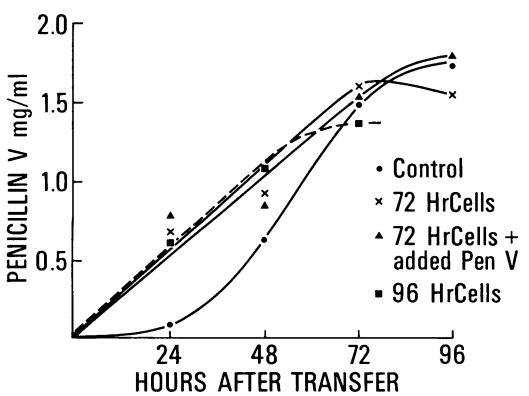

FIG. 1. *Effect of transferring Penicillium cells (72- and 96-hr, and 72-hr cells exposed to 10 mg of penicillin/ml) to sterile 48-hr broth. Lactose medium was used.*

TABLE 9. *Incorporation of ^{14}C-cystine, -valine, and -phenoxyacetic acid into crystalline penicillin V in presence of added penicillin*

Precursor	Addition[a]	Inhibition of synthesis (%)	Precursor incorporated into penicillin (*μmoles*)
DL-Cystine-*1*-^{14}C	None	0	4.03×10^{-2}
	Pen V	68	3.28×10^{-2}
	6-APA	93	0.97×10^{-2}
L-Valine-*U*-^{14}C	None	0	0.14×10^{-2}
	Pen V	49	0.18×10^{-2}
	6-APA	66	0.11×10^{-2}
Phenoxyacetic-*1*-^{14}C acid	None	0	3.84×10^{-2}
	Pen V	73	7.08×10^{-2}
	6-APA	43	9.90×10^{-2}

[a] Penicillin V (Pen V) and 6-aminopenicillanic acid (6-APA) were added at 10 mg/ml.

TABLE 10. *Incorporation of $Na_2{}^{35}SO_4$ into penicillin V by 48- and 136-hr cultures of Penicillium chrysogenum*

Age of whole broth (hr)	$Na_2{}^{35}SO_4$ incorporated[a]
48	0.133×10^{-3}
48[b]	0.162×10^{-3}
136	0.130×10^{-3}

[a] Expressed as micromoles incorporated per flask in 2 hr.

[b] Penicillin (10 mg/ml) was added at 48 hr.

The rates of penicillin synthesis early and late in the fermentation cycle were compared. Penicillin was extracted from 48- and 136-hr broths after 2 hr of incubation with $Na_2{}^{35}SO_4$. The specific activity of the crystallized penicillin indicated no difference in rate of penicillin synthesis at these times. The addition of penicillin to a 48-hr fermentation did not significantly affect the incorporation of $Na_2{}^{35}SO_4$ into penicillin (Table 10).

Degradation of penicillin. The significant incorporation of ^{14}C-labeled precursors into penicillin with little or no net increase in antibiotic accumulation suggested product turnover in the penicillin fermentation. Thus, the stability of penicillin in fermentation broth of various ages was examined by adding penicillin V to broths that had been sterilized by filtration. After 48 hr of incubation at 25 C, penicillin potencies dropped less than 10%.

Washed mycelium, cell extracts, and dialyzed broth filtrates were examined for penicillin acylase activity. No 6-APA was formed when these were incubated with penicillin V for 6 hr at *p*H 7.0 and 30 C.

Penicilloic acid was measured in broths to which penicillin had been added, as well as in control fermentations, and was found to be negligible. Four days after the addition of ^{35}S-penicillin V to 48-hr fermentation broths, no 8-hydroxypenillic acid, penicic acid, nor other ^{35}S-containing degradation products of penicillin were detected by chromatographic techniques. All ^{35}S activity was associated with penicillin V on the chromatographs, and the total radioactivity added initially was accounted for in the broth and mycelium.

Intracellular and cell-bound penicillin. The possibility that accumulation of penicillin on or within the cell during the fermentation caused apparent inhibition of penicillin synthesis was considered. Extracts of cells from a control penicillin fermentation and from one to which penicillin had been added were assayed for penicillin content. The data (Table 11) showed that the intracellular penicillin concentration increased during the middle of the fermentation cycle, but decreased toward the end. The penicillin content of control cells was lower than that of cells exposed to added penicillin. The amount of penicillin associated with older mycelium was twice that of younger cells.

TABLE 11. *Intracellular penicillin concentration of cells during penicillin V fermentation cycle*

Fermentation	Time (hr)	Penicillin V	
		Extracellular (mg/ml of broth)	Intracellular (μg/g[a] of cells)
Control	49	—	126
	67	5.44	645
	139	8.78	300
Penicillin V (10 mg/ml) added at 43 hr (A)	43	2.32	225
	43A	11.1	—
	91	14.6	975
	139	14.9	660

[a] Wet weight.

TABLE 12. *Penicillin extracted from washed Penicillium cells*

Age of cells (hr)	Penicillin/mg (dry wt) of cells (μg)	
	Eluted with buffer	Extracted with acetone
67	8	12
90	4	4
113	10	13
137	27	28

TABLE 13. *Adsorption of ^{35}S-penicillin V onto Penicillium chrysogenum cells*

Concn ^{35}S-penicillin V (mg/ml)	Time incubated (hr)	^{35}S-penicillin V/ml of cells (μg)
1	1	11
5	1	32
10	1	64
1	3	18
5	3	80
10	3	183

An "equilibrium" between extracellular and intracellular penicillin in fermentation broths with high penicillin potencies was investigated. After a 2-hr exposure to ^{35}S-penicillin V, cells were harvested from 48- and 136-hr whole broth and washed; cell-free extracts were prepared. Radioactivity of the concentrated extracts was determined and chromatographically shown to be associated with penicillin. Extracts obtained from cells late in the fermentation cycle contained about three times the ^{35}S-penicillin V found in extracts of younger cells, whereas the radioactivity of the particulate fractions was equivalent.

Little antibiotic could be extracted or eluted from washed *Penicillium* cells taken at different times throughout a control fermentation (Table 12). However, the amount of penicillin associated with 137-hr cells was considerably higher than with any other cells throughout the fermentation.

The adsorption of penicillin by mycelium in the presence of increasing levels of the antibiotic was estimated by incubating a constant quantity of washed cells with ^{35}S-penicillin V. The amount of ^{35}S-penicillin V adsorbed by intact cells was proportional to the increased exogenous penicillin concentration but was too small to account for the loss of penicillin late in the fermentation (Table 13).

DISCUSSION

The results of this investigation show that the cessation of penicillin accumulation during fermentation may be related to the end product itself. Exogenous penicillin added at any time throughout the fermentation cycle limited subsequent antibiotic accumulation without affecting growth.

Termination of penicillin production has been attributed to cessation of growth (17). High con

centrations of accumulated penicillin in fermentation broths would not have been a factor in chemostat cultures or low-potency penicillin broth. The present studies show that net penicillin accumulation was limited by exogenous penicillin before growth was complete.

The amount of penicillin or 6-APA that inhibited penicillin synthesis depended upon the synthesizing capacity of the *Penicillium* strain, suggesting that the antibiotic-producing potential of a strain may be determined by a genetically regulated mechanism of feedback inhibition. Perhaps higher penicillin yields would result from mutants with an altered regulation or sensitivity of a penicillin biosynthetic enzyme.

Although 6-APA and penicillins having various groups in the side chain were each capable of inhibiting subsequent accumulation of penicillin V, some specificity was noted. Penicilloic acid, penicillin N, cephalosporin C, and 7-aminocephalosporanic acid did not inhibit penicillin synthesis. An enzyme that catalyzes the exchange of side chains between certain penicillins has been isolated from cell extracts of *P. chrysogenum* (18). Spencer (20) showed that purified preparations of penicillin acyltransferase directly acylate 6-APA with activated acyl groups. However, penicilloic acid, penicillin N, and cephalosporin C do not participate in the acyltransferase reaction, nor does 7-aminocephalosporanic acid substitute for 6-APA as the receptor. Similarities in specificity between this enzyme and inhibitors of penicillin synthesis suggest that the inhibition of penicillin synthesis by penicillin may be due to end-product or feedback inhibition of such an enzyme as penicillin acyltransferase.

An active acyltransferase may account for the incorporation of phenoxyacetic-*1*-^{14}C acid into penicillin in the presence of inhibitory concentrations of exogenous 6-APA and penicillin observed in this investigation. Penicillin V and 6-APA limited the accretion of nucleus in medium from which precursor was omitted. If acyltransferase is actively involved in penicillin biosynthesis and subject to penicillin feedback inhibition, the accumulation of 6-APA, isopenicillin N, or some other β-lactam intermediate would be anticipated; however, no intracellular intermediates were detected in this study.

The termination of penicillin production was not due to loss or injury of the penicillin-synthesizing mechanism in *Penicillium*, since cells exposed to inhibitory concentrations of antibiotic produced control levels of penicillin when transferred to fresh medium. Penicillin associated with the mycelium in high-potency broths may influence net antibiotic synthesis. The ratio of extracellular to intracellular penicillin concentrations showed no abnormal accumulation of penicillin by cells in high-potency broth. However, cell extracts obtained from mycelium late in the fermentation contained more penicillin per milligram of mycelium than extracts obtained early in the fermentation. More penicillin was also eluted from a constant quantity of older cells. The fact that ^{35}S-penicillin V penetrated cells to a greater extent late in the fermentation cycle suggests either a change in mycelium permeability or a penicillin gradient effect in high-potency broths. Whether cell-associated penicillin plays any role in regulation of antibiotic synthesis is unknown at this time.

The incorporation of radioactive precursors into penicillin while net antibiotic synthesis was limited suggests penicillin to be an unstable fermentation product that may undergo constant turnover. The studies reported here give no evidence for hydrolysis of penicillin to 6-APA and the respective acyl compound, although penicillin acylase activity has been reported in strains of *P. chrysogenum* (11). No other degradative enzymes or products of penicillin were demonstrated in this investigation.

The role of penicillin as a regulator of its own synthesis may not be known until the mechanism of penicillin biosynthesis itself is completely defined.

ACKNOWLEDGMENTS

We are indebted to D. W. Dennen for penicilloic acid assays, to Donald R. Croley for assistance in purification and crystallization of penicillin, and to Dallas Farr for excellent technical assistance.

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