peared to have no effect. The activity range and periodic fluctuation of SGOT in normal animals must be ascertained before proper evaluation of these changes following drug administration can be made.

Summary. Using 20 mg of BSP per kg of body weight and determining the percent retention after 20 minutes, it was demonstrated that daily administration of 10 mg/kg for 6 days of methyltestosterone and norethandrolone to rabbits significantly impaired the removal of the dye from the blood stream. Less frequently and to a lesser degree the SGOT activity appeared to increase with drug administration.

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Specificity of Penicillin Amidases. (28559)

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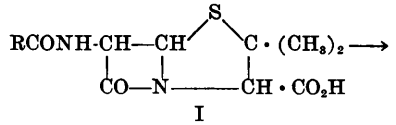
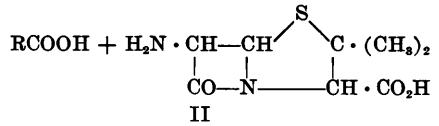
Numerous workers have reported the presence of a penicillin amidase or acylase of the following type among fungi, yeasts, actinomycetes, and bacteria(1-9). This enzyme catalyzes the hydrolysis of penicillins (I) into 6-aminopenicillanic acid (II) and acid corresponding to the side chain.



I



II

The substrates for these enzyme studies have been, for the most part, naturally occurring penicillins.

Rolinson *et al.*(5) considered that 2 distinct types of enzyme activities were encountered, differing primarily in rates of splitting of particular types of penicillins. One type, widely distributed among the actinomycetes and filamentous fungi, readily hydrolyzes penicillins K, dihydro F and V but splits penicillin G very slowly. The second type, found in certain bacteria, splits penicillin G readily and penicillin V at a much slower rate.

Erickson and Bennett(8), using cells of *Penicillium chrysogenum,* and Cole and Rolinson(10), using cells of *Emericellopsis minima,* found activity of the first type in that penicillin V was split much more readily than G. Claridge *et al.*(1), Rolinson *et al.*(5) and Huang *et al.*(2) reported on penicillin amidases from bacteria which would fall into the second category in that penicillin G was split more readily than penicillins V, K, O, or phenethicillin. Recently Huang *et al.*(11) tested the hydrolysis of a series of biosyn-

thetic and semisynthetic penicillins by freeze-dried cells of a strain of *Nocardia* and of *Proteus*. Penicillin G was the preferred substrate; all departures from the benzylpenicillin side-chain structure led to reduction of substrate activity.

With the preparation of a large number of synthetic penicillins, we were able to examine in more detail the specificities of the amidases from various sources. *P. chrysogenum* and *Cephalosporium* CMI 49137 were selected as cultures having amidases of the first type while *Alcaligenes faecalis* and 2 strains of *Escherichia coli* were chosen as sources of the second amidase type.

Methods. The bacteria were grown 18 hours at 37°C in Difco heart infusion broth. *P. chrysogenum* cells were obtained as a sample from a penicillin production fermentation harvested at 120 hours. The *Cephalosporium* cells were harvested after growing 120 hours at 21°C in shake flasks containing a complex medium of starch hydrolyzate, cotton seed meal, yeast extract, and fish meal.

To the bacterial cells obtained by centrifuging 10 ml of broth was added sufficient penicillin substrate in 0.2 M potassium phosphate buffer (pH 7.0) to give 0.005 M concentration in a total volume of 5 ml. For *Penicillium* and *Cephalosporium* cultures, 100 ml of whole broth were first mixed in a Waring blendor for 30 seconds. The mycelial fragments were then thoroughly washed in 1% NaCl, to remove any residual antibiotic, and reconstituted to original volume. The cells from 4 ml of broth were used in each reaction mixture.

The reaction mixtures were placed in a 37°C water bath and samples at 4 and 24 hours were assayed for presence of 6-aminopenicillanic acid (6-APA) by means of paper chromatography. To do this, cells were removed by centrifugation, 15 μl of the supernatant spotted on ½ inch strips of S and S 589 white ribbon paper and the strips then developed by downward solvent flow at 4°C for 20 hours in a system consisting of 1-butanol, glacial acetic acid and water (60:15:25). Duplicates of all samples were prepared, one strip being sprayed after development with a phenylacetyl chloride solution to convert any 6-APA formed to penicillin G. Both strips were then placed on large agar plates seeded with *Bacillus subtilis* and incubated overnight at 28°C. Less than 0.1 μg of 6-APA can be detected by this method.

The presence of 6-APA was taken as evidence of splitting of the penicillin. In the solvent system used, 6-APA has an R_f of 0.45, the penicillins approximately 0.85, cephalosporin N 0.3, and cephalosporin C 0.2.

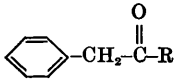
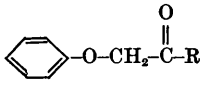
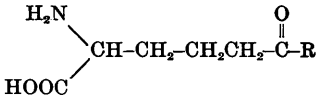
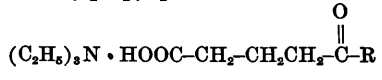
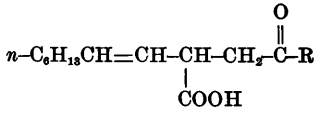
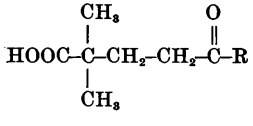
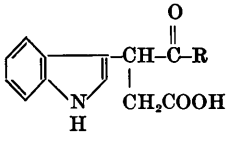
Results. The results obtained are summarized in Table I. The degree of splitting is scored from + to 4+ based on amount of 6-APA formed. A negative indicates no 6-APA was detectable.

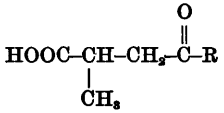
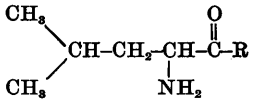
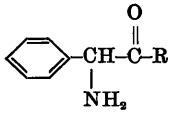
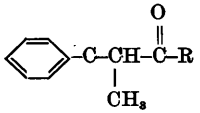
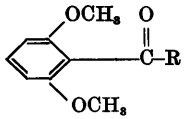
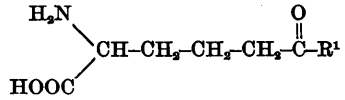
It can be seen, as reported by Rolinson *et al.*(5), that the fungi show greater splitting of penicillin V than penicillin G, whereas with the bacteria this condition is reversed. It is interesting to note that the bacterial amidase was found in only one of the two strains of *E. coli* tested.

The enzyme found in the *Penicillium* has a much broader spectrum of activity, forming 6-APA from all the compounds tested except methicillin and cephalosporin N. In our earlier paper(1), we reported that we were unable to detect the formation of 6-APA from penicillin G by cells of *P. chrysogenum*. We now believe that our inability to measure 6-APA production was caused by a lack of sensitivity in the assay which has been overcome by the use of the paper strip method to separate the 6-APA formed from the residual penicillin.

The bacterial amidases produced by the XG-3 strain of *E. coli* and the *A. faecalis* strain were unable to split any of the synthetic penicillins tested that contained a carboxyl group on the side chain. Absence of a carboxyl group on the side chain did not ensure that the penicillin would be split by this bacterial enzyme as evidenced by the lack of activity against methicillin. In contrast to the bacterial amidase, the *Penicillium* preparation was able to hydrolyze side chains having carboxyl groups. Its inability to hydrolyze cephalosporin N is probably due to the combined effect of the amino and carboxyl groups.

TABLE I. Specificity of Penicillin Amidases.

Penicillin side chain	Enzyme source: *Penicillium chrysogenum* A-9342	*Cephalosporium* CMI 49137	*Alcaligenes faecalis* A-9424	*Escherichia coli* ATCC 9637	*Escherichia coli* XG-3 A-9455
Natural Penicillins					
Penicillin G 	+	—	4+	—	4+
Penicillin V 	3+	+	2+	—	3+
Cephalosporin N 	—	—	—	—	—
Carboxy-Penicillins					
3-carboxy propyl penicillin 	2+	—	—	—	—
2-carboxy, 3-decenyl penicillin 	+	—	—	—	—
3-carboxy, 3-methyl butyl penicillin 	3+	—	—	—	—
2-carboxy, 1-indolyl ethyl penicillin 	+	—	—	—	—

Penicillin side chain	Enzyme source: *Penicillium chrysogenum* A-9342	*Cephalosporium* CMI 49137	*Alcaligenes faecalis* A-9424	*Escherichia coli* ATCC 9637	*Escherichia coli* XG-3 A-9455
2-carboxy propyl penicillin 	+	—	—	—	—
Amino Penicillins					
1-amino, 3-methyl butyl penicillin 	3+	—	2+	—	—
ampicillin 	2+	—	3+	—	3+
Miscellaneous Penicillins					
phenethicillin 	+	—	+	—	+
methicillin 	—	—	—	—	—
cephalosporin C 	—	—	—	—	—

R = 6-aminopenicillanic acid residue.

R^1 = 7-aminocephalosporanic acid residue(15).

None of the preparations was able to split methicillin, a penicillin resistant to the action of penicillinase(12). Cephalosporin C was also found to be completely resistant to the action of the amidases used. It is interesting to note that both cephalosporin C and N have the same side chain and both are completely resistant to hydrolysis. They are reported, however, to differ in susceptibility to penicillinase(13).

Enzyme nomenclature. The names penicillin amidase or penicillin acylase are treated here as synonymous trivial names for the enzyme hydrolyzing the acyl group from penicillins. The Report of the Commission on Enzymes of the International Union of Biochemistry(14) lists the systematic name for penicillin amidase as penicillin amidohydrolase (number 3.5.1.11). However, the trivially named penicillinase, hydrolyzing the β-lactam of penicillins is also named penicillin amidohydrolase (number 3.5.2.6). This confusion could perhaps be clarified by specifying penicillinase as penicillin lactam amidohydrolase or penicillin lactam-hydrolase. The enzyme hydrolyzing the side chain from penicillins would retain the systematic name penicillin amidohydrolase and also the trivial name penicillin amidase(4), which has priority over the name penicillin acylase. A further distinction should be made in the systematic name of penicillin amidase in order to designate the origin of the enzyme. The fungal penicillin amidohydrolase shows a different specificity from the bacterial amidohydrolase in that the former splits penicillin V and some of the synthetic penicillins more readily than penicillin G, whereas the latter shows greatest activity against penicillin G.

Summary. A series of penicillins have been tested for splitting by amidases from several sources. The two distinct types as postulated by Rolinson *et al.*(5) were demonstrated. In addition, the amidases of bacterial origin showed a further specificity in that synthetic penicillins containing a carboxyl group on the side chain were not hydrolyzed. Methicillin, cephalosporin N, and cephalosporin C were not split by any of the amidases tested. In the case of the latter two antibiotics, the common side chain may account for the lack of activity.

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