A Preliminary Report on a New Enzyme, "Penicillin-amidase"

Sir :

The authors have found in the mycelium of *Penicillium chrysogenum* Q 176 a new enzyme "penicillin-amidase" which splits penicillin G into its components, i. e. phenyl acetic acid and a residual portion of penicillin G, or "penicin". The reaction catalized by the enzyme may be as follows :

A remarkable decrease of the antibiotic potency, measured by the cup method, and an increase in the amount of amino nitrogen in the solution of penicillin G have been recognized only in the presence of washed fresh mycelium of Q 176 added with toluene, while in the case of control tests such as with heated mycelium or in absence of any mycelial substance such changes in antibiotic potency and amino-nitrogen content have not been recognized. From the reaction mixture phenyl acetic acid and a substance which we presume "penicin" have been isolated, the latter substance being in the form of hygroscopic crystalline needles giving m.p. 158~159°C. This substance, which itself does not give ninhydrin reaction, develops an intense blue violet coloration by ninhydrin reagent after it was acted with penicillinase. It is suggested therefor that by penicillinase it has been decomposed to a new α -amino-dicarboxylic acid which we propose to call "penicic acid". The estimation of "penicic acid" can be carried out by ninhydrin reaction through which the activity of "penicillin-amidase" can also be determined. The increase of free amino nitrogen, as shown above, may be as well used for the estimation of the enzyme activity.

By the use of the purified enzyme preparation obtained by fractional precipitations with ethanol and acetone, it has been shown that the new enzyme is distinctly different from the proteolytic enzymes, asparaginase and hippuricase of the mould. The optimal activity ranges are at 35~38°C and pH 7.6~8.0. The enzyme has also been found to be present in Taka-diastase and in the micelium of *Aspergillus oryzae*.

It has not yet been made quite clear why penicillin potencies drop so rapidly in fermenting tanks after the production of penicillin has reached its "peak". The main cause of the rapid drop may be indicated by the fact that the formation of the new enzyme takes place in fermenting broth near the "peak" of its potency.

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