Zusammenfassung. Die Aktivität von Leucinoaminopeptidase in der Prostata und den Samenbläschen der Ratten wurde mit histochemischen und quantitativen Methoden nach verschiedenen Hormonbehandlungen bestimmt. Es wurde gefunden, dass alle androlytischen Behandlungen, die eine Atrophie in diesen Organen verursachten, die enzymatische Aktivität erhöhten. Nach allen Behandlungen, die aber andromimetisch wirkten, war sie immer erniedrigt.

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Isolation and Purification of the Penicillinase from Mycobacteria

Woodruff and Foster[1] described in 1945 that a rapidly growing avirulent strain of *Mycobacterium tuberculosis* destroys penicillin. A few years later, Iland and Baines[2] found that a species of *Mycobacterium tuberculosis* produces an extracellular penicillinase after a long incubation period. Ribeiro[3] described penicillinase production in a strain of BCG, and Soltys[4] reported penicillinase as a constitutional enzyme in *Mycobacterium phlei* and BCG strains, and as an inductive penicillinase in human and bovine *Mycobacteria tuberculosis*. He was not able to find penicillinase activity in avian type of *Mycobacteria tuberculosis*. Investigating a great number of species and strains of genus Mycobacteria, Bönicke and Dittmar[5] demonstrated penicillinase as endocellular enzyme in almost all the strains examined. The concentration of extracellular enzyme in liquid media is, according to these authors, proportional to the autolysis rate. By use of 'lysis inducing media' with low concentration of nitrogen substances, they were able to demonstrate that the concentration of extracellular penicillinase depends on the autolysis rate. *Mycobacteria tuberculosis* of the avian type, without apparent autolysis, produced endocellular penicillinase after cellwall disruption by ultrasonic treatment.

Mycobacterial penicillinase activity in various phases of isolation

Preparation	Penicillinase arbitrary units
1. Culture fluid centrifuged until cell free	2.5 PAU/ml
2. Supernatant fluid after benzoic acid precipitation	0
3. Distilled water washing of precipitation	0
4. *M*/1 Tris buffer pH 9.0 solution of butanol precipitate	150 PAU/ml
5. Approximate units per mg of dissolved precipitate	200 PAU/mg
6. Ethanol-phosphoric acid precipitate	500 PAU/mg
7. Protein fraction in supernatant after precipitation by PAS	800 PAU/mg

The arbitrary penicillinase units correspond to the amount of enzyme destroying 50% of sodium penicillin units in a batch of 2000 units in water bath at 37°C, after 10 min, at a pH 7.0 = PAU.

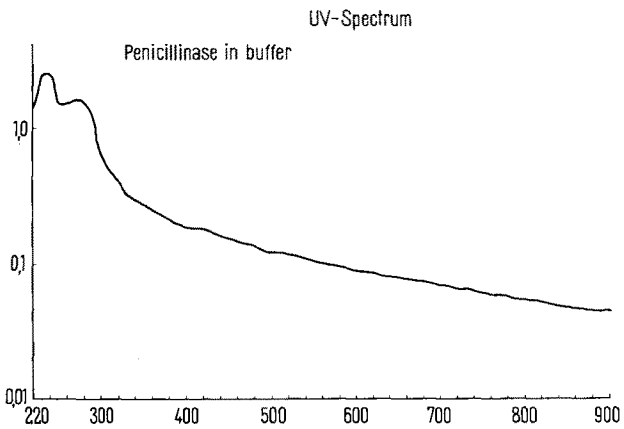

Fig. 1. Penicillinase 0.1 mg/ml of Tris buffer 2 *M* of pH 8.7. Zeiss Spectrophotometer.

Isolation and purification of the penicillinase from mycobacteria

For this purpose, we used *Mycobacterium smegmatis* (Borstel strain SN 2), which shows a great tendency to autolysis and at the same time produces a high yield of extracellular penicillinase. The enzyme was isolated according to the method of Gough[7] as modified by Herrmann[8], who used this method for isolation of high active tuberculins.

Mycobacterium smegmatis was obtained from Löwenstein-Jennsen medium, and inoculated by an enrichment carried out in Lockeman liquid medium. After 21 days of incubation at room temperature, cultures were filtrated through Glass filter (Schott G 5 M) and 5% of sodium benzoate dissolved in the filtrate. The solution was cooled to 0°C, and the benzoic acid was precipitated with 3 *n* HCl up to a pH 3.8. The precipitate was separated by filtration on Whatmann paper No. 3, and washed several times with distilled water saturated with benzoic acid, until the filtrate was free from HCl. All filtrates are free of proteins and contain nearly all the phosphate content of the original liquid medium. The filter cake was dried in vacuum over concentrated sulphuric acid, and then dissolved in *n*-butanol. The butanol suspension was filtered, and the thin filter cake was re-suspended in *n*-butanol and mixed with an equal volume of distilled water. Penicillinase was present in the insoluble protein fraction between the two liquid phases.

After discarding butanol and water, the insoluble fraction was washed by centrifuging with distilled water

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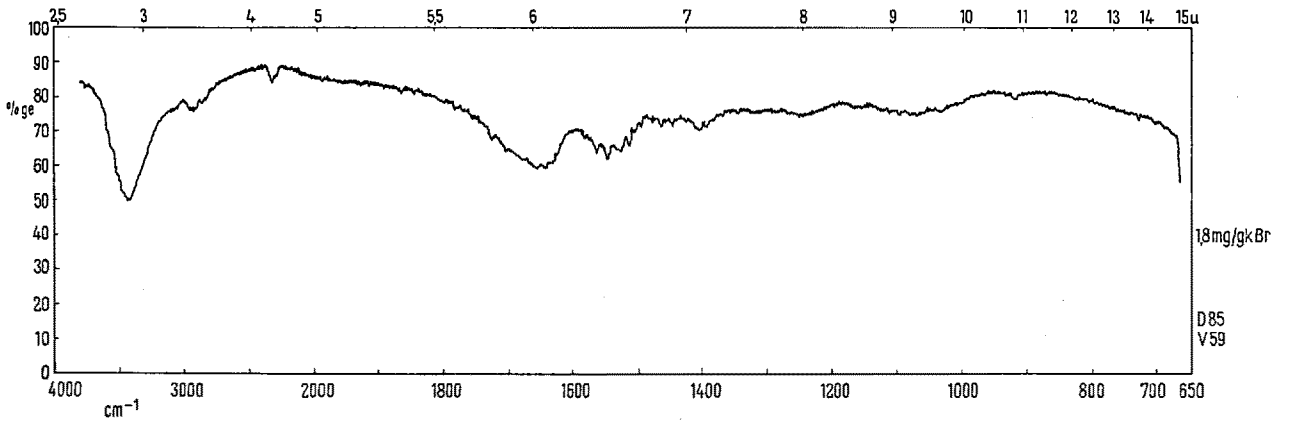

Fig. 2. Penicillinase 1.8 mg/g of KBr. Infrared spectrum of 2.4 min/500 e. 100 cm^{-1}. Vector (Spaltprogramm): 3. Elcctrical Data: D 8.5; V 5.9. Prisma NaCl (Ernst Leitz GmbH, Wetzlar).

several times. The sediment was finally dissolved in $M/1$ Tris buffer of pH 9.0. Very little material remains insoluble with no activity. The solution was therefore mixed with ethanol until the concentration reached 20%. At this point, penicillinase was precipitated by $2n$ phosphoric acid till pH 5.4. After several washings with distilled water, the precipitate was dried in vacuum over phosphorus pentoxyde. The preparation was brownish, hygroscopic, insoluble in water, acetate, phosphate, veronal, and citrate buffers. Only Tris-buffer of high ionic strength was able to dissolve it completely. 1 mg of this substance is equivalent to 500 penicillinase arbitrary units (PAU). Mycobacterial penicillinase is soluble in liquid medium, and remains active at pH between 4.5 and 7.83. Its present insolubility can only be explained by the possibility that the molecule of penicillinase was to some extent denaturated by the organic solvent used for the removal of benzoic acid. This possibility is shown when using other solvents such as acetone, ethanol, benzol, benzyl alcohol, toluol, terc. butanol, sec. butanol etc. All those solvents diminish enzyme activity, and at the same time lower its solubility in Tris-buffer. Most conservative was n-butanol. In view of the fact that the IR spectrum of the enzyme preparations indicated the presence of polysaccharides, further purification was necessary. To a solution of enzyme (in Tris buffer) 5% sodium p-aminosalicylate was dissolved, and PAS precipitated by $3n$ HCl till pH 5.8. The precipitate dissolved in methanol contained only a trace of proteins without activity, and the rest insoluble polysaccharides. Supernatant solution contained protein with an equivalent of 800 PAU/mg. IR spectrum shows no trace of polysaccharides. The preparation is water-insoluble being soluble only in $M/1$ phosphate buffer and $2M$ acetate buffer at pH between 6.8 and 7.8. It is no longer hygroscopic and looks like whitish powder.

Crystallisation of mycobacterial penicillinase was done in the form of mixed crystals. Penicillinase was dissolved in phosphate buffer, in which ammonium phosphate (t) was dissolved to 19% concentration. It is necessary that the amount of buffer is small. The solution was cooled to 4°C, and mixed with 4 Vol of cold (— 20°C) acetone. Very rapidly a crystalline precipitate of phosphates containing all the enzyme in a hybride crystalline form was formed. The precipitate was washed several times with acetone and centrifuged. This crystalline preparation is not water-soluble, but it remains soluble in all the aforementioned buffers. Paper electrophoresis of penicillinase in a $M/15$ phosphate buffer of pH 6.0, and $0.2M$ veronal buffer of pH 8.6 shows only one individual band, which moves very slowly towards anode after 16 h. UV spectar shows two maximal adsorption peaks at 240 and 280 μ, with a linear deflection after 340 μ. IR spectrum of the preparation shows absence of nucleic acids, lipids and polysaccharides, with characteristic waves of carbonyl-groups, suggesting a piperazine structure in the enzyme molecule. After acid hydrolysis this carbonyl-groups wave disappears, fortifying our convinction that the sensibility of mycobacterial penicillinase depends upon the preservation of piperazine linkages[9].

Résumé. La pénicillinase des mycobacteries est précipitée par l'acide benzoïque. L'élution d'acide se fait par n-butanol. L'élimination des polysaccharides étant donné par la précipitation avec PAS la substance devienne mieux soluble. La crystallisation de l'enzyme est obtenue en forme de crysteaux hybrides avec du phosphate d'ammoniaque. L'analyse spectrophotométrique prouve l'absence d'impuretés, l'électrophorèse revèle une bande individuelle d'une motilité ralentie.

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An Anthozoan Chitin[1]

From a study of the organization of the skeleton of the Hawaiian reef coral, *Pocillopora damicornis* L., evidence is presented here that the major constituent by weigth of the organic component of the skeleton is chitin (poly-acetylglucosamine). This is the first record of chitin in the class Anthozoa[2].

[1] Contribution No. 147 from the Hawaii Marine Laboratory.

[2] L. E. R. PICKEN, *The Organization of the Cell and other Organisms* (Clarendon Press, Oxford 1960).