edly administered in relatively large doses is able to exert a considerable degree of protection against the toxicity of sterile meningococcal endotoxin as measured by its lethal action in mice and rabbits. No evidence was obtained of detoxifying action *in vitro*.

Whether this therapeutic effect is due to penicillin itself or to some impurity in the commercial preparations available to us is a question not necessarily answered by our experiments with inactivated penicillin inasmuch as penicillinase may have denatured the substance responsible for this effect in addition to the penicillin itself. Further work on this problem is in progress.[6]

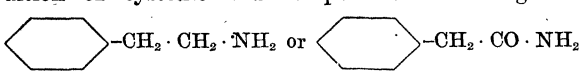
ALDEN K. BOOR
C. PHILLIP MILLER
THE UNIVERSITY OF CHICAGO

PHYSIOLOGICAL COMPARISON OF TWO STRAINS OF PENICILLIUM

THIS note is to direct attention to an outstanding difference in the capacities of two commercially important strains of Penicillium for biosynthesis of penicillin on different media.

In our laboratory *Penicillium chrysogenum* X1612 consistently produces about 100 Oxford units of penicillin per ml on the following synthetic medium in shaken cultures (100 ml medium/500 ml Erlenmeyer flask making 240 oscillations/minute through a distance of 8 cm). Quantities are in Gm/L: Starch, 5; lactose, 25; glucose, crude, 5; acetic acid, glacial, 6; Na_2HPO_4, 1.6; K_3PO_4, 2; NH_4NO_3, 4; $(NH_4)_2SO_4$, 1; KNO_3, 1; $MgSO_4 \cdot 7H_2O$, 0.25; $FeSO_4 \cdot 7H_2O$, 0.2; $ZnSO_4 \cdot 7H_2O$, 0.04; $CuSO_4 \cdot 5H_2O$, 0.005; Cr (from $K_2Cr_2O_7$), 3 gamma. Additions of numerous adjuvants singly and combined (except phenylacetic or phenaceturic acids or esters of same) produced little, if any, increase in yield.

Under similar conditions *Penicillium* sp., NRRL 1984–A produced less than 20 units of penicillin/ml. Combined additions of indole acetic acid (5 ppm) and of naphthylene acetic acid (0.1 ppm) to this medium approximately doubled the yields. Further additions of cysteine hydrochloride (50 mg/L) and various other adjuvants lacking the phenyl radical increased the yields to about 60 units/ml while addition of cysteine and compounds containing the



linkages produced yields in the neighborhood of 130 to 140 units per ml. This is set forth diagrammatically in Fig. 1.

Addition of sulfite waste liquor (25 ml/L) alone or combined with different adjuvants produced slight additional increases (Fig. 1), the maximum potency attained being about 150 units/ml.

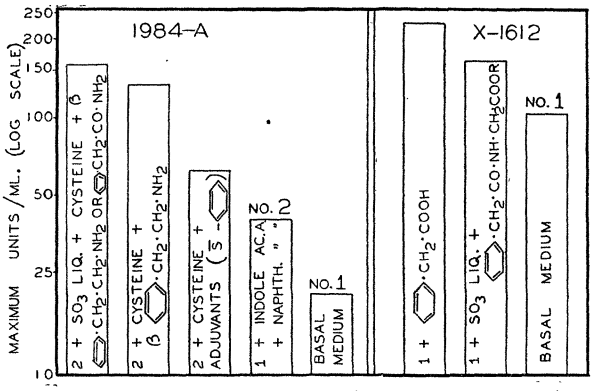


FIG. 1. Maximum yields of penicillin with two strains of penicillium in synthetic media and without various adjuvants.

To summarize, *Penicillium* sp., NRRL 1984–A yields 40 to 50 units penicillin/ml on a purely synthetic medium under the conditions of our experiments if growth factors are present as indole acetic acid and/or naphthylene acetic acid. In such a synthetic medium, adjuvants enhance considerably production of penicillin, as the following are concomitantly made available:

1. Cystein (or cystine in presence of a suitable reducing agent such as sulfite waste liquor)
2. The –C–C–N– chain with the proper linkage at each end (O, H)
3. The phenyl ring, or preferably 2 and 3 combined as phenylaceturates, α-phenylacetamide or β-phenylethylamine.

Penicillium chrysogenum, X1612, on the other hand, appears to be capable of effecting total synthesis of the penicillin molecule in reasonable quantities on a much less complex medium, although again here furnishing a suitable phenyl linkage is beneficial (Fig. 1). Addition of phenylacetic acid, 3.3 Gm/L, to the basal synthetic medium gave maximum yields of 225 u/ml.

In other experiments the influence of addition of sulfite waste liquor and of different adjuvants on the yield of penicillin in a corn steep medium was studied. The standard solution contained corn steep solids, 20 Gm/L; lactose, 30 Gm/L; KH_2PO_4, 0.004 M; $MgSO_4 \cdot 7H_2O$, 0.001 M and $NaNO_3$, 0.035 M. Numerous variations of this solution were studied also in which the total salt concentration (exclusive of those furnished by steep liquor) was uniform but in which the molecular proportions of the three salts were varied. In all, thirty-six combinations of salts and adjuvants were tested with and without sulfite waste with each strain of mold. In every combination that was studied addition of sulfite waste liquor

[6] The authors are grateful for the technical assistance of Mary Bogie and Lois Nelson.

caused a decrease in yield with X1612 approximately as follows:

5 ml sulfite/L depression = 12–20 per cent.
15 ml sulfite/L depression = 20–35 per cent.
30 ml sulfite/L depression = 35–50 per cent.

but in nineteen of the combinations, addition of sulfite waste improved the yields with 1984–A. The range of increases with this strain was from 8 to 73 per cent. The mean increase was 37 per cent.

These experiments emphasized again the inherent differences in the two strains of mold for synthesis of penicillin and point to the necessity of furnishing 1984–A with more complex and partially linked precursors of the penicillin molecule.

The potencies reported in this paper were determined by standard cylinder plate assays using *Staphylococcus aureus,* NRRL 313 (F.D.A. strain 209P) as the test organism and a standard of calcium penicillin G.

ROBERTSON PRATT[1, 2]
JEAN DUFRENOY[1]
DIVISION OF MICROBIOLOGICAL RESEARCH,
THE CUTTER LABORATORIES,
BERKELEY, CALIF.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE HISTOCHEMICAL LOCALIZATION OF ADENOSINETRIPHOSPHATASE IN PLANT AND ANIMAL TISSUES

ADENOSINETRIPHOSPHATASE (ATPase) has become a focus of interest to research workers in widely diverse fields of biological and medical sciences because of its critical role in intermediary metabolism. The principle of Gomori's technique for the localization of alkaline[1] and acid,[2] glycerophosphatases in animal tissues by histochemical reactions has been adapted to the demonstration of ATPase; the various modifications that the glycerophosphatase procedures have undergone have been summarized in a recent review.[3] The principle of Gomori's technique is the precipitation *in situ* of the phosphate, liberated enzymatically, by calcium in alkaline media, and lead in acid media, and the conversion of the insoluble phosphate to the more easily visualized black lead sulfide.

The pH-optimum for ATPase in animal tissues[4, 5] occurs at about 9.0. Mouse heart and wheat were chosen to represent the two types of tissue, and since Booth[6] showed that other phosphatases in wheat have optimum activity at a pH of 5.1–5.2, this range of hydrogen ion concentration was tried for the ATPase and it was found to be suitable.

One innate difficulty with the method is the fact that the presence of free phosphate at a given histological or cytological location may obscure the phosphatase activity at this site. The difficulty is appreciably reduced when paraffin, rather than frozen, sections are employed, since much of the free phosphate is removed in the preparation of the paraffin sections. Of course, the tissue infiltrated with paraffin has undergone some loss in enzyme activity; however, this may be compensated by employing longer digestion periods.

The limited availability of ATP made it imperative that some modification of the usual procedure, employing a staining dish or Coplin jar full of substrate solution, be introduced. Accordingly a simple hanging-drop technique was devised that requires only one drop of the substrate solution for each tissue section. This technique is generally applicable to all cases where it is desirable to use a minimum of substrate or other reagent in histochemical tests.

The acid and alkaline substrate media are prepared in the following manner:

Substrate solution: Dissolve 5 mg of the Ba salt of ATP in 0.5 cc of 0.1 M HCl, add 0.1 cc of a Na_2SO_4 soln. to precipitate the Ba (1.11 per cent. Na_2SO_4 was required by our sample of ATP[7]), centrifuge and neutralize the supernatant liquid with 0.1 M NaOH.
Acid substrate medium: Combine in the following order: 0.5 cc of 0.1 M acetic acid, 0.25 cc of 0.1 M Pb $(NO_3)_2$, 0.25 cc of neutral substrate soln., 0.10 cc of 0.1 M $CaCl_2$, and 1.5 cc of 0.1 M sodium acetate. Centrifuge before use to remove turbidity.
Alkaline substrate medium: Combine the following: 0.75 cc of neutral substrate soln., 0.5 cc of pH 9.0 buffer (9.36 cc 0.1 M sodium barbital + 0.64 cc 0.1 M HCl), and 0.05 cc of 0.1 M $CaCl_2$. Centrifuge before use.
Control media: Replace the neutral substrate soln. by distilled water.

The enzyme activity is demonstrated as follows: Fix heart tissue in acetone for the preparation of both frozen and paraffin sections. Soften the wheat kernels by soaking in water for 4 to 6 hours before sectioning on the freezing microtome, or for 7 hours before fixing in absolute alcohol for the preparation of paraffin sections. Cover a frozen section (15 μ

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[1] With the assistance of Ruth Birch, Marjorie Decker, Iola Dunkle and Patricia Streator.
[2] Currently on leave from the University of California College of Pharmacy.
[7] The ATP and mouse hearts were obtained through the courtesy of Professor M. B. Visscher, Dr. H. G. Wood and Dr. M. F. Utter, of the Physiology Department of the University of Minnesota Medical School.