THE EFFECT OF PENICILLIN ON THE LETHAL ACTION OF MENINGOCOCCAL ENDOTOXIN IN EXPERIMENTAL ANIMALS[1]

NUMEROUS reports have appeared in the literature concerning the bacteriostatic and bactericidal action of penicillin, but its influence on the toxic effect of bacteria and their products has been given little study. Neter and Will[2] did not find that penicillin prevented the pathogenic action of tetanus toxin in mice.

This is a preliminary report of a study of the effect of penicillin on the toxins of the pathogenic Neisseria.

Endotoxin was prepared from 6 strains of type I meningococcus and 1 strain of type II meningococcus from 18-hour growths on casein-digest agar[3] and washed twice or thrice by centrifugation of saline suspensions. The microorganisms were resuspended in water, brought to pH 8.0, kept 14 hours in the refrigerator, neutralized and sterilized by heating for 45 minutes at 60° C. in the water bath; sterility was proved by culture. The concentration of endotoxin was adjusted so that 0.3–0.5 cc killed at least four fifths of the mice within 30 hours after intraperitoneal injection. Our experiments, however, were not concluded before 90 hours to insure inclusion of all casualties. Preparations were always used before appreciable deterioration had occurred.

The penicillin (sodium salt) used was the ordinary, therapeutic product of several manufacturers.[4] It was administered to mice by subcutaneous injection in doses of 1,000 units contained in 0.1 cc of water.

In one series of experiments on mice, endotoxin was injected intraperitoneally in sufficient amount to kill most or all of the controls and the test animals were treated by courses of injections of penicillin beginning before and continuing after the endotoxin. In some the endotoxin had been mixed with penicillin before injection. Although several different combinations of treatment were tried the experiments have been grouped into two categories (B and C in Table 1) because the striking difference between them was the failure of penicillin acting on endotoxin *in vitro* to reduce its toxicity to any significant degree. On the other hand, all of the experiments in which the mice received several injections of penicillin showed a considerable reduction in mortality as compared with the controls.

TABLE 1

EFFECT OF PENICILLIN ON THE MORTALITY OF MICE INJECTED WITH MENINGOCOCCAL ENDOTOXIN. SUMMARIZED RESULTS OF EXPERIMENTS WITH EQUIVALENT QUANTITIES OF ENDOTOXIN

	Number of mice	Number died	Per cent. died
A. Endotoxin alone (controls)	179	159	89
B. Penicillin mixed with endotoxin. No other penicillin	74	59	80
C. Penicillin by repeated subcutaneous injection. Endotoxin intraperitoneally ...	373	122	33
D. Penicillin inactivated by Penicillinase. Otherwise like C	44	32	73

Among the 373 mice thus treated, some were injected with endotoxin which had previously been mixed with penicillin and some were injected with untreated endotoxin but as no significant difference was observed they were combined in Group C which had an overall mortality of 33 per cent. as compared with a mortality of 89 per cent. for the controls. Penicillin was usually given about 90 and 45 minutes before endotoxin and again about 2, 5, 9 and 24 hours thereafter.

Such substances as saline and casein-digest repeatedly administered failed to influence the lethal action of endotoxin.

Penicillin which had been inactivated by penicillinase[5] was unable to reduce the mortality below 73 per cent.

The protection afforded by penicillin was even more apparent when graded doses of endotoxin were used. The results of such experiments have not been included in Table 1. An illustrative example is given in Table 2.

TABLE 2

RESULTS OF AN EXPERIMENT WITH GRADED QUANTITIES OF ENDOTOXIN

Endotoxin	Control mice untreated	Mice treated with penicillin
	No. died / No. injected	No. died / No. injected
.75 cc	...	13/16
.5	8/8	8/16
.25	8/8	3/16
.1	5/8	.....

Experiments on rabbits weighing 2–3½ kilos were carried out by injection of endotoxin intravenously and penicillin subcutaneously in doses of 10,000–20,000 units every few hours. Among 16 rabbits thus treated, 14 survived and 2 died at 36 and 45 hours, respectively, whereas all of 10 controls died within 24 hours.

These experiments indicate that penicillin repeat-

[1] From the Department of Medicine and the A. B. Kuppenheimer Foundation of the University of Chicago. Aided by a grant from the John and Mary R. Markle Foundation.

[2] Erwin Neter and Dessie Will, *Jour. Bact.*, 48: 261, 1944.

[3] Alden K. Boor, *Proc. Soc. Exp. Biol. and Med.*, 50: 22–25, 1942.

[4] The penicillin was provided by the Office of Scientific Research and Development from supplies assigned by the Committee on Medical Research for experimental investigations recommended by the Committee on Chemotherapeutic and Other Agents of the National Research Council.

[5] Kindly furnshed by Dr. A. J. Liebmann, of the Schenley Research Institute, Lawrenceburg, Indiana.

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]

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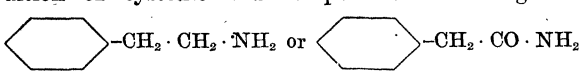
PHYSIOLOGICAL COMPARISON OF TWO STRAINS OF PENICILLIUM

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.

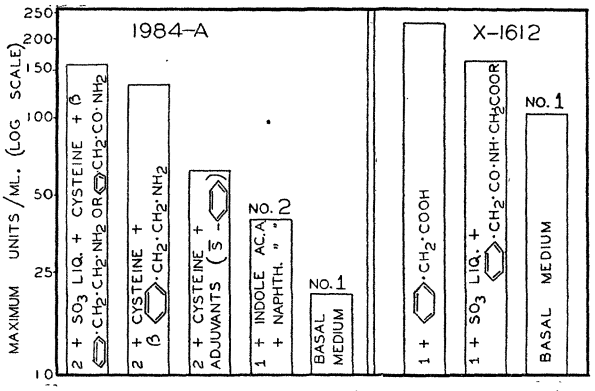


FIG. 1. Maximum yields of penicillin with two strains of penicillium in synthetic media and without various adjuvants.

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.