charged colloids, *e.g.*, gum acacia, to the protamine-treated true cholinesterase restores the original activity-substrate concentration relationship.

The change in the activity curve of the true cholinesterase, brought about by the addition of protamines, in no way affects the fundamental property of the enzyme, its specificity towards choline esters. This shows that the activity-substrate concentration relationship is but a secondary characteristic of the enzyme, determined by its physical environment, whereas the specificity of the enzyme, unchangeable irrespective of environmental conditions, is an inherent property of the true cholinesterase.

Thus, a classification of cholinesterases according to their locale or to their activity-substrate concentration relationship, though seemingly expedient, is at variance with the facts and will inevitably lead to confusion. Specificity alone, therefore, remains the true criterion for a differentiation of cholinesterases.

Alles and Hawes contend that "the findings of Glick on the behavior of the enzyme of the cat superior cervical ganglion, make the acceptance of 'pseudo-cholinesterase' as a suitable name for the serum enzyme seem inadvisable."[10] If the enzyme activity of the superior cervical ganglion were, in fact, due to pseudo-cholinesterase alone, such findings would indeed support the contention of Alles and Hawes. Our experiments, however, have shown that a mixture of both cholinesterases occurs in this ganglion, the true cholinesterase being present in considerable amounts in the ganglion of the cat and predominating in that of the dog.[11]

Further experiments performed by us on pseudo-cholinesterase have tended to minimize the importance of this enzyme. We have found that blood[3] and tissues[12] of ox and sheep do not contain any pseudo-cholinesterase. In rats, moreover, we have been able to reduce the activity of the pseudo-cholinesterase of serum and tissues considerably without effecting any noticeable physiological changes. An 80 per cent. reduction of the pseudo-cholinesterase level in the rat can be brought about by the oral administration of 5 g/Kg of tri-ortho-cresyl phosphate. This chemical, which is toxic to rabbits, was found by Hottinger and Bloch[13] to reduce their cholinesterase level; it does not, however, produce any symptoms in rats, whose true cholinesterase, according to our experiments,[12] is insensitive to tri-ortho-cresyl phosphate—a fact which may help to explain the absence of toxic effects in these animals.

Despite the above findings, the name pseudo-cholinesterase was not chosen to detract from the significance of the non-specific enzyme. The term cholinesterase was retained in order to provide continuity with the earlier mass of literature on this subject, while the prefix "pseudo-" was selected to emphasize the intrinsic property of non-specificity and to avoid the hitherto indiscriminate application of the term cholinesterase, suggestive of specificity towards choline esters.

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[10] G. A. Alles and R. C. Hawes, SCIENCE, 100: 75, 1944.
[11] H. Rudney. To be published shortly.
[12] B. Mendel, J. M. Gunter and E. Mortimer. To be published shortly.
[13] A. Hottinger and H. Bloch, *Helv. Chim. Acta*, 26: 142, 1943.

THE ANTIBACTERIAL ACTION OF PENICILLIN AGAINST GRAM NEGATIVE ORGANISMS[1]

SINCE the discovery of penicillin and the subsequent demonstration of its antibacterial activity *in vivo*, considerable interest has centered on the group of bacterial agents susceptible to its action. Fleming reported in 1929[2] that penicillin possessed a marked bacteriostatic effect against many of the Gram positive organisms, including staphylococci, streptococci and the diphtheria bacillus. This observation was confirmed by Chain *et al.*[3] in 1940 and the list of susceptible organisms extended by Hobby *et al.*[4] and by other workers. With the exception of the meningococcus and gonococcus, however, no activity could be demonstrated against Gram negative organisms.

In 1941 Kocholaty[5] demonstrated that *Penicillium notatum*, from which penicillin is formed, produced a second substance, notatin (also known as penatin, penicillin B or *E. coli* factor) which possessed an antibacterial action against Gram negative as well as Gram positive organisms. It was subsequently shown, however, that notatin is an enzyme effective only *in vitro* in the presence of glucose.

In 1944 Helmholz and Sung[6] demonstrated a weak bactericidal effect of penicillin in urine on *Streptococcus fecalis* and on *Proteus ammoniae* but not on *E. coli* or Aerobacter aerogenes.

In the present communication preliminary data are presented to demonstrate that penicillin produced by *Penicillium notatum* or *Penicillium chrysogenum* possesses an antibacterial action *in vitro* against other Gram negative organisms and is effective in the absence of glucose.

[1] From the Biological Laboratory of Charles Pfizer and Co., Brooklyn, N. Y.
[2] A. Fleming, *Brit. Jour. Exp. Path.*, 10: 226, 1929.
[3] E. Chain *et al.*, *Lancet*, 2: 226, 1940.
[4] G. L. Hobby, K. Meyer and E. Chaffee, *Proc. Soc. Exp. Biol. and Med.*, 50: 227, 1942.
[5] W. Kocholaty, *Jour. Bact.*, 46: 313, 1943; *Arch. Biochem.*, 2: 73, 1943.
[6] H. F. Helmholz and Chieh Sung, *Proc.* of the Staff Meetings of The Mayo Clinic 19(14): 370, 1944.

Experimental: Preliminary experiments were carried out by the serial dilution method[4] using a stock strain of *E. typhosus* as the test organism. Plain beef infusion broth was used throughout.

One hundred and seventeen crude liquors, prepared by the cultivation of *P. notatum* or *P. chrysogenum* in a medium suitable for penicillin production, and eighty-six partially purified penicillin solutions were tested. The potency of these liquids ranged from 35 to 49,000 Oxford Units per cc. All the crude liquors showed some degree of bacteriostatic activity when tested in low dilutions against *E. typhosus.* The partially purified fractions, containing higher concentrations of penicillin, inhibited growth of *E. typhosus* in dilutions from 1:400 to 1:32,000.

In subsequent experiments the crystalline sodium salt of penicillin (G) was tested by the double serial dilution method for bacteriostatic action against both *Staphylococcus aureus* and *E. typhosus.* It was found that this preparation of penicillin was 64 times more potent against *Staphylococcus aureus* than against *E. typhosus.* On this basis crystalline penicillin sodium, having a potency of 1,650 Oxford Units per mg against *Staphylococcus aureus,* was chosen as standard and a value of 26 Typhoid Units per mg assigned to it. All subsequent tests for activity against *E. typhosus* were carried out by the Oxford cup method[7] using a substandard, established at 7 Typhoid Units per mg by titration against the crystalline penicillin sodium. Plain beef infusion agar containing neither glucose nor cerelose was used throughout. Table 1 shows the results on a number of fractions tested.

TABLE 1

BACTERIOSTATIC ACTION OF PENICILLIN AGAINST E. TYPHOSUS

Preparation of penicillin*	Potency against *Staphylococcus aureus*	Potency against *E. typhosus*
	Oxford Units/cc	Typhoid Units/cc
Crude liquors	> 35	0.5–2.0
Partially purified fractions	5,970–13,980	74–92
Liquid concentrates	32,000–49,000	50–800
	Oxford Units/mg	Typhoid Units/mg
Dried penicillin "Commercial"	620–993	7–20
Crystalline penicillin sodium	1,650	26

* All penicillin tested was prepared by Chas. Pfizer and Company, Brooklyn, N. Y.

A variety of Gram negative organisms,[8] in addition to *E. typhosus,* were tested for sensitivity. Table 2 shows the relative susceptibility of the organisms tested. Only two strains of Gram negative organisms, a strain of *E. coli* which produces large amounts of penicillinase and a freshly isolated strain of *Ps. pyocyaneus* were completely resistant.

[7] W. H. Schmidt and A. J. Moyer, *Jour. Bact.,* 47: 199, 1944.

[8] These cultures were received through the kindness of Dr. Hattie Alexander, Babies Hospital, New York, N. Y.

TABLE 2

RELATIVE SUSCEPTIBILITY OF GRAM NEGATIVE ORGANISMS TO ACTION OF PENICILLIN

Strain	Typhoid units per cc causing inhibition
E. typhosus (3 strains)	0.016
Sh. dysenteriae Flexner (V)	0.031
S. paratyphi B*	0.125
Sh. dysenteriae Flexner (W)*	0.125
*S. panama**	0.125
Sh. dysenteriae Flexner (Y)*	0.250
Sh. dysenteriae Flexner (X)*	0.250
Sh. dysenteriae Flexner (Z)*	0.500
Proteus vulgaris	0.500
Br. abortus	0.700
Klebsiella pneumoniae	> 1.400†
Serratia marcescens	> 1.400†
Ps. aeruginosa	> 1.400
E. coli	> 1.400

† Complete inhibition of growth was observed with 62 T.U./cc.

* Cultures were typed by the Salmonella Center, Beth Israel Hospital, New York, N. Y.

The antibacterial action of penicillin against *E. typhosus* is destroyed by clarase, which contains penicillinase, and is partially or completely destroyed at 100° C. for 1½ hours.

Summary: It is apparent that penicillin exerts an antibacterial action against Gram negative as well as Gram positive organisms. This property of penicillin becomes more apparent in high potency preparations. It is possible that a form of penicillin showing greater activity against Gram negative organisms may exist. Studies on the nature of such a substance and on the *in vivo* action of penicillin against Gram negative organisms are in progress.

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RELATION OF DOSAGE TO SURVIVAL TIME OF ARSENITE-INJECTED ROACHES

IN a study, carried on during the last several years, of the mode of action of sodium metarsenite on the American cockroach, *Periplaneta americana* (L.), various concentrations of the poison in volumes of saline proportional to body weight of the insect were injected into the roaches and the survival times determined. When the survival times were plotted against concentrations, hyperbolic curves were obtained. A portion of one of these is shown in Fig. 1. These curves are characterized by a region of inflection (*i,* Fig. 1) and a critical zone, both of which are reproducible in repeated experiments. The critical zone is a region, usually associated with long survival times, in which insects injected with equal doses of the poison have survival times that fall into a bimodal frequency distribution or actually into two separate groups. In the region of inflection, injection of slightly different concentrations of the poison may