sage, but produced hyaluronidase as well after becoming more virulent. While this observation is recorded for its theoretical interest, there is no assurance that the strain recovered after mouse passage was the same strain that was injected, hence the validity of this observation must await confirmation from many sources.

The need for cultivating organisms in the presence of hyaluronic acid in order to demonstrate hyaluronidase is amply borne out by the observation that 49 of 54 strains producing hyaluronidase did so only when grown in media containing hyaluronic acid.

Summary. 1. The isolation of 94 strains of *Cl. welchii* from human feces, soil, and animal feces was facilitated by the use of sodium azide in the culture media. 2. These strains were tested for mouse virulence, and for ability to produce the enzymes lecithinase and hyaluronidase. Fixty-six per cent of the strains producing both enzymes were virulent, as were 47% of those producing only lecithinase. Twenty-four per cent of those strains producing only hyaluronidase and 28% of those producing neither enzyme were virulent. 3. Of 41 mouse virulent strains isolated, 83% produced lecithinase, and 54% produced hyaluronidase. Of all strains isolated, 70% produced lecithinase whereas only 52% produced hyaluronidase.

4. It is concluded that regardless of the role of hyaluronidase in a gangrenous lesion, its *in vitro* production by a given strain of *Cl. welchii* bears no necessary relationship to the virulence of that strain for mice.

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Inactivation of the Antibiotic Activity of Penicillin by Cysteine Hydrochloride. I. Chemical Aspects of Inactivation.

BACON F. CHOW AND CLARA M. MCKEE.

From the Laboratory of Physical Chemistry, and the Division of Microbiology, the Squibb Institute for Medical Research, New Brunswick, N. J.

It is well known that penicillin can be easily inactivated by numerous chemical agents, such as acids, alkalis, etc. Recently Atkinson and Stanley[1] reported the occurrence of "penicidin-suppressors" in a variety of materials, among which they listed thioglycolic acid. They were aware of the fact that the abolition of the antibiotic activity may be the result of either a specific chemical reaction or of an interference with an essential enzyme reaction in a manner similar to the inhibiting effect of p-aminobenzoic acid on sulfonamide bacteriostasis. In another communication,[2] they extended the study to various sulfhydryl compounds including cysteine as well as other reducing agents. Their results suggested to them "that the mechanism of this suppression is a chemical reaction between penicidin and the SH group."

Woodruff and Foster[3] studied the effect of sulfhydryl group (cysteine) on penicillinase, an enzyme which destroys penicillin. In one of their experiments, they used as a control a mixture of penicillin and cysteine with heat-inactivated penicillinase, and found, on incubation, a decrease in the penicillin content. Woodruff and Foster, however, did not extend their investigation of the phenomenon.

In October 1943, during the course of a study on the effect of different chemicals on the stability of penicillin, we observed that penicillin, in contact with neutralized cysteine[4] hydrochloride, rapidly loses its antibiotic activity. In the light of the experiments we are

[1] Atkinson, N., and Stanley, N. F., *Australian J. Exp. Biol. and Med. Science*, 1943, **21**, 249.

[2] Atkinson, N., and Stanley, N. F., *Australian J. Exp. Biol. and Med. Science*, 1943, **21**, 255.

[3] Woodruff, H. B., and Foster, J., *J. Bact.*, (in press).

[4] Cavallito, C. J., and Bailey, J. H., *Science*, 1944, **100**, 390.

TABLE I.
Destruction of the Antibiotic Activity of Crystalline Penicillin by Decreasing Amounts of Cysteine Hydrochloride.

Cysteine hydrochloride Mg/ml	μ Penicillin per ml	Percent Destruction 48 hr
2.5	20	99
1.25	"	99
0.625	"	99
0.313	"	99
0.157	"	99
0.0785	"	97
0.0393	"	50-70
0.000	"	0

The reaction was allowed to proceed at 37° for 48 hours at pH 6.5 in an atmosphere of nitrogen.

to report in this communication, such a loss in activity is probably due to the destructive effect of cysteine.

Experimental. A procedure as described below was used in the early experiments. One ml of a solution of crystalline penicillin* (0.333 mg per ml) was added to each of 2 test tubes containing 8.0 ml of a M/10 phosphate buffer 7.6. To one tube was then added 1.0 ml of a freshly prepared neutralized cysteine hydrochloride solution (10 mg per ml) and to the other was added 1.0 ml of a 0.90% sodium chloride solution. After incubation at 37° for 2 hours, the antibiotic activity was determined by making 2-fold serial dilutions using 0.5 ml amounts, adding constant amounts (0.5 ml of a 10^{-6} dilution) of a penicillin sensitive organism (*Staphylococcus aureus,* Heatley), and incubating at 37° for 16 hours. The potency was determined by comparison with a crystalline penicillin standard tested at the same time. It was found that less than 0.2% of the original activity remained in the penicillin-cysteine mixture whereas there was no appreciable loss of activity in the control. In other words, 10 mg of cysteine had destroyed approximately 550 Oxford units of penicillin. In experiments like the one described above, air was not excluded and a part of the cysteine was oxidized to cystine which has been isolated and identified. In order to minimize the loss of cysteine hydrochloride due to oxidation by air, the reaction in later experiments was allowed to proceed in an atmosphere of nitrogen. The results of such an experiment performed at pH 6.5 and 37° for 48 hours are given in Table I.

It can be seen from Table I that under the experimental conditions about 0.04 to 0.08 mg of cysteine hydrochloride were sufficient to inactivate 0.02 mg of crystalline penicillin. In another experiment in which the reaction extended over a period of only 5 hours (other conditions remaining the same) inactivation was reduced about 15-fold. Expressed in terms of Oxford units destroyed, approximately 30 units were destroyed by 0.08 mg of cysteine in 48 hours while in 5 hours 1.2 mg of cysteine were required to destroy the same amount of penicillin.

Our preliminary experiments indicated that the rate of inactivation was dependent on hydrogen ion concentration. Within the pH range tested (5.3 to 7.6), the rate of destruction increased with increasing alkalinity. Hence we believe that the inactivation of penicillin may be due to a chemical reaction. Since the inactivation may involve any one or more of the reactive groups (the amino-, carboxyl-, and sulfhydryl-groups) in the cysteine molecule, it is of interest to ascertain, if possible, how the reaction takes place. To this end we have tried but failed to inactivate penicillin by means of other amino acids, such as glycine, cystine, methionine, or serine (an analogue of cysteine, having an oxygen atom in place of the sulfur atom). A comparison between the chemical structure of these com-

*The crystalline sodium salt of penicillin, obtained first by MacPhillamy and Wintersteiner in July 1943 (cf. R. D. Coghill, *Ind. & Eng. Chem.*, News Edition, **22**, 593 (1944)) was kindly furnished by Dr. O. Wintersteiner of the Division of Organic Chemistry of this Institute.

TABLE II.
Inactivation of Penicillin by Thioglycolic Acid.

Thioglycolic Acid Mg	Penicillin Oxford Units	pH	Destruction %
200	40	6.4	95
50	40	6.6	30-50
12.5	40	6.7	0
3.1	40	6.8	0
0.0	40	6.5	0
200	40	7.6	95
50	40	7.8	88
12.5	40	8.0	50
0.0	40	7.8	0

The reaction was allowed to proceed at 37° for 48 hours.

pounds and cysteine suggests that a free sulfhydryl group is essential for the destruction of the antibiotic activity. If this hypothesis is correct, one might expect thioglycolic acid to be able to inactivate penicillin. The results of a typical inactivation experiment with thioglycolic acid at different pH's are given in Table II.

Thus thioglycolic acid is a far less potent inactivating agent than cysteine. As a matter of fact, we found that simple mercaptans will not inactivate penicillin. These results, therefore, indicate that the inactivation of penicillin by cysteine involves more than the sulfhydryl group.

Summary. The antibiotic activity of penicillin can be easily abolished by cysteine in a slightly alkaline solution. The rate of inactivation is dependent on the amount of cysteine and pH. In an atmosphere of nitrogen, one mg of penicillin can be inactivated by several mg of cysteine at pH 7.8, but to a lesser extent with thioglycolic acid. Inactivation of penicillin does not take place if other amino acids, *e. g.* cystine, methionine, serine, or glycine are used. Hence we believe that the inactivation of penicillin by cysteine is a chemical reaction and that it may involve both sulfhydryl and amino groups of cysteine.

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Seasonal Fluctuations in Susceptibility of Guinea Pigs to Experimental Cavian Poliomyelitis.*

CLAUS W. JUNGEBLUT.

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York City.

Following the adaptation of SK poliomyelitis virus from monkey to mouse through intermediate cotton rat passage, further transmission of the murine strain to guinea pigs has been described.[1,2] A fixed strain of cavian virus was finally obtained which has now been carried over more than 70 serial passages. The disease thus produced in guinea pigs is characterized by the occurrence of flaccid paralysis, and the lesions in the central nervous system of paralyzed animals are "remarkably like those of poliomyelitis in monkey and man."[3] Serological identity of the murine and cavian strain was established by cross neutralization tests in mice and in guinea pigs. As far as any relationship between simian and cavian polio-

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1 Jungeblut, C. W. and Sanders, M., *J. Am. Med. Assn.*, 1941, **116**, 2136.

2 Jungeblut, C. W., Feiner, R. R. and Sanders, M., *J. Exp. Med.*, 1942, **76**, 31.

3 Wolf, A., *J. Exp. Med.*, 1942, **76**, 53.