Further experiments with *Streptococcus hemolyticus* and *Staphylococcus aureus* will be reported in the near future.

The penicillin employed has now been concentrated by chemical means, thus permitting the oral administration of substantially smaller doses than those reported here.

We desire to express to Dr. G. H. A. Clowes our indebtedness for advice and suggestions in the conduct of these experiments.

Conclusions. Penicillin is an effective chemotherapeutic agent against both parent and sulfonamide-fast pneumococci in mouse infection experiments.

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Formation of Angiotonin-like Pressor Substance from Action of Crystalline Pepsin on Renin-Activator.

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The interesting communication of Croxatto and Croxatto[1] in which they describe the formation of a pressor substance similar in properties to angiotonin or hypertensine prompted us to repeat their work. We wished to ascertain whether the same results could be obtained by using crystalline pepsin instead of the commercial product. Further, the effect of pH on this reaction as well as on that between renin and renin-activator was studied.

Methods. Preparation of the Renin-activator. For the preparation of renin-activator ox serum[2, 3] was used. One volume of 3 M potassium phosphate at pH 6.5 was added to an equal volume of ox serum and the resulting precipitate discarded. To each liter of the clear filtrate 500 cc of 3 M phosphate was added to raise the phosphate concentration to 2 molar. This precipitate which contains the active material was collected on Buchner funnels.

The precipitate was dissolved in distilled water and dialyzed against running tap water. The precipitate which forms in the sac was discarded and the residual solution reprecipitated between 1.5 and 2

1 Croxatto, H., and Croxatto, R., *Science*, 1942, **95**, 101.

2 Kohlstaedt, K. G., Helmer, O. M., and Page, I. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **39**, 214.

3 Kohlstaedt, K. G., Page, I. H., and Helmer, O. M., *Am. Heart J.*, 1940, **19**, 92.

molar potassium phosphate. The precipitate was suspended in as small a volume of distilled water as possible and dialyzed until free of potassium phosphate.

Any precipitate which formed on dialysis was removed by centrifuging, and the final volume adjusted so that 1 cc was equivalent to 10 cc of original ox serum. Sodium chloride was added to a concentration of 0.9%.

The renin-activator and enzyme solutions were adjusted to the desired pH with hydrochloric acid or sodium hydroxide, using a glass electrode as indicator. After mixing, the solutions were incubated for 10 minutes at 40° C, boiled for 10 minutes, cooled and centrifuged. The clear supernatant fluid was used for assay in pithed cats. The latter were prepared by pithing under pentobarbital anesthesia. The crystalline pepsin was dissolved in 85% glycerol and 1 cc ≡ 3.1 mg P.N. per cc. It was diluted 1:1 with saline. The renin was of such pressor activity that 0.2 cc produced a rise of ar-

TABLE I.
Formation of Pressor Substance from Interaction of Pepsin and Renin-Activator as Indicated by the Reaction of Pithed Cats.

Amt of activator, cc	pH		Exp. No.	Vol. inj. cc	Rise in pressure, mm Hg.
5	6.5	5 cc 1:50 dilution renin	2460	0.5	52
5	6.5	5 " saline		0.5	0
5	6.5	U.S.P. pepsin ≡ 10 mg		0.5	0
5	2.0	U.S.P. " ≡ 10 "		0.5	6
5	2.0	U.S.P. " ≡ 10 "		1.0	14
5	2.0	U.S.P. " ≡ 5 "		0.5	0
5	2.0	5 cc 1:50 dilution renin	2462	1.0	0
5	2.0	U.S.P. pepsin ≡ 100 mg		0.5	44
5	2.0	U.S.P. pepsin ≡ 50 mg	2466	0.5	12
5	2.0	U.S.P. " ≡ 50 "		1.0	19
5	6.5	U.S.P. " ≡ 50 "		0.5	0
5	6.5	U.S.P. " ≡ 50 "		1.0	6
5	7.0	5 cc 1:50 dilution renin		0.5	20
5	7.0	5 " 1:50 " "		1.0	38
5	4.0	5 " 1:50 " "		0.5	0
5	4.0	5 " 1:50 " "		1.0	0
5	2.0	5 " 1:50 " "		0.5	0
5	2.0	5 " 1:50 " "		1.0	0
3	2.0	3 cc crystalline pepsin	2469	1.0	10
3	2.0	U.S.P. pepsin ≡ 30 mg		1.0	12
3	6.5	3 cc 1:50 dilution renin		1.0	16
5	2.0	5 cc crystalline pepsin	2470	1.0	24
5	2.0	5 " U.S.P. pepsin ≡ 50 mg		1.0	30
5	6.5	5 " 1:50 dilution renin		1.0	34
		Saline 1 cc and crystalline pepsin content		1.0	0

terial pressure of 50 mm Hg in a cat under pentobarbital anesthesia.

Results. Our results entirely confirm those of Croxatto and Croxatto in demonstrating that incubation of renin-activator with commercial pepsin at pH 2.0 leads to the formation of a pressor substance with properties similar to those of angiotonin (Table I). The crystalline as well as the U.S.P. pepsin caused the formation of a heat stable substance able to produce a sharp rise in the arterial pressure of pithed cats.

The reaction between pepsin and renin-activator proceeds at a satisfactory rate at pH 2.0 but at pH 6.5 it is practically stopped. In sharp contrast to the effect of pH on the pepsin reaction is its action on that between renin and renin-activator. At pH 2.0 to 4.0 apparently no angiotonin is formed while at pH 6.5 to 7.0 it is formed abundantly.

It is of interest to note the relatively much greater potency of renin in the formation of angiotonin as compared with crystalline pepsin. The renin which we employed was free of the enzyme contained in kidney extracts which destroys angiotonin.

Dr. John Northrup was kind enough to supply us with some crystalline pepsin for our study. We are very grateful to him.

Summary. We have been able to confirm the observations of Croxatto and Croxatto that commercial pepsin at pH 2.0 reacts with renin-activator to form a substance similar to and probably identical with angiotonin. Furthermore, we found crystalline pepsin active. The reaction proceeds satisfactorily at pH 2.0 but is halted at pH 6.5 in sharp contrast to that between renin and renin-activator. At pH 6.5 to 7.0 the latter reaction yields angiotonin abundantly but none is formed at pH 2.0 to 4.0.