TABLE I. Effect of Papain and Ficin Feeding on Fatty Acid Content of Liver of 16 Insulin-Treated Depancreatized Dogs.

Pre-operative wt, kg	Final wt, kg	Supplement: Type	Supplement: Amt daily, g*	Supplement: Period fed, wk	Liver: Wt, g	Liver: Total fatty acids, % wet wt
9.5	7.2	None	—	20	670	21.5
6	5.5	"	—	24	580	13.3
7.9	6.5	"	—	19	310	37.3
9.2	8.2	"	—	12	720	17.2
13.1	9.2	Papain	.5	18	433	2.4
13.1	7.2	"	.5	18	443	4.5
12.8	9	"	1	21	433	3.1
12.8	11	"	1	21	382	2.5
10	9.4	"	1	21	432	3.1
9.7	6.3	"	1	21	356	3.5
13.8	10.7	"	4	21	630	4.7
6.4	6.6	"	4	20	463	2.2
14	8.7	"	4	20	495	2.8
8.3	6.6	Ficin	.5	21	400	1.6
9.1	7.8	"	.5	21	390	2.6
9.1	11.4	"	.5	18	420	2.5

* One-half of the amount recorded was fed with each meal.

form, and 2) those providing a means of liberating lipotropic substances from their bound form. There can be little doubt that the two plant enzymes, papain and ficin, act by replacing the loss of digestive capacity of the depancreatized dog. These results indicate that restoration of adequate digestive function, even with substances not specific to the animal organism, can prevent fat deposition in the liver.

Summary. It is shown that the development of fatty liver in insulin-maintained, depancreatized dogs can be prevented by the daily administration of the plant proteolytic enzymes, papain and ficin.

1. Chaikoff, I. L., Entenman, C., and Montgomery, M. L., *J. Biol. Chem.*, 1945, v160, 489.

2. ———, *J. Biol. Chem.*, 1947, v168, 177.

3. Chaikoff, I. L., and Entenman, C., *Advances in Enzymol.*, 1948, v8, 171.

4. Montgomery, M. L., Entenman, C., Chaikoff, I. L., and Feinberg, H., *J. Biol. Chem.*, 1950, v185, 307.

5. Montgomery, M. L., Entenman, C., and Chaikoff, I. L., *Am. J. Physiol.*, 1944, v141, 216.

6. Chaikoff, I. L., and Kaplan, A., *J. Biol. Chem.*, 1934, v106, 267.

7. Hwang, K., and Ivy, A. C., *Ann. N. Y. Acad. Sc.*, 1951, v54, 161.

8. Bergmann, M., *Advances in Enzymol.*, 1942, v2, 49.

9. Walti, A., *J. Am. Chem. Soc.*, 1938, v60, 493.

Received May 6, 1952. P.S.E.B.M., 1952, v80.

Effect of β-propiolactone on Metabolism of *Pseudomonas aeruginosa* and Growth of Certain Fungi. (19556)

FREDERICK BERNHEIM AND GLEN R. GALE.

From Duke University School of Medicine, Department of Physiology and Pharmacology, Durham, N. C.

β-propiolactone has been shown to be both bactericidal and virucidal and to have a relatively low toxicity(1-5). One of the organisms it is effective against, *Pseudomonas aeruginosa,* has been used in the following to study its possible mode of action. In aqueous solution the lactone hydrolyzes to β-hydroxpropionic acid, a fairly strong acid. This

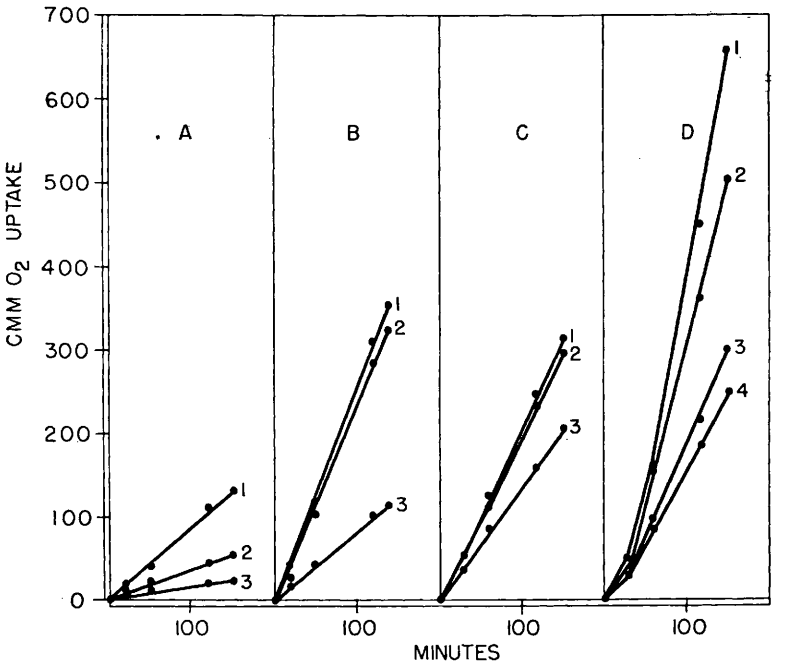

FIG. 1. The effect of β-propiolactone on oxygen uptake of *Pseudomonas aeruginosa* at pH 7.8 and 37°. Concentrations given are in mg/ml. Except in A the autorespiration has been subtracted. A1, .25 mg β-hydroxypropionic acid; A2, .25 mg lactone; A3, autorespiration. B1, 1 mg succinate; B2, + .25 mg β-hydroxypropionic acid; B3, + .25 mg lactone. C1, 1 mg succinate; C2, + .1 mg lactone; C3, + .15 mg lactone. D1, 1 mg succinate + .25 mg $(NH_4)_2SO_4$; D2, + .025 mg lactone; D3, + .1 mg lactone; D4, + .15 mg lactone.

property might make it a useful skin disinfectant, because the effect of the lactone would be augmented by the local increase in hydrogen on concentration. Accordingly some experiments on the effect of the lactone on the growth of certain fungi were undertaken.

Methods. To study the effect of the lactone on certain aspects of bacterial metabolism a strain of *Ps. aeruginosa* was used which was originally supplied by Dr. Koppers. This organism oxidizes acids of the Krebs' cycle with the concomitant assimilation of ammonia(6), it oxidizes amino acids(7), and forms adaptive enzymes for the oxidation of benzoic and p-hydroxybenzoic acids(8). It was grown as previously described(6), the cells washed by centrifugation, and placed in Warburg vessels with 0.05 M K-Na-phosphate buffer pH 7.8. The fluid volume was 2.0 ml. The lactone was obtained from the B. F. Goodrich Chemical Co.

Results. It was first necessary to determine whether the lactone or the product of its hydrolysis was the effective agent. Accordingly, the lactone in a concentration of 1.0 mg/ml in buffer was allowed to stand at room temperature for 24 hours before it was added to the bacterial suspension and its effect compared with a freshly prepared solution. The results are shown in Fig. 1, A & B. The lactone in a concentration of 0.25 mg/ml almost completely inhibited the oxidation of succinate whereas the hydrolyzed product was without effect. Moreover, the latter was oxidized slowly by the bacteria. The lactone increased the oxygen uptake of the bacteria to a very small extent. This indicates that it was fixed by the cells and thus not available for hydrolysis since the free lactone is rapidly hydrolyzed in alkaline solutions. The buffer prevented any pH change resulting from the hydrolysis of the small amounts of lactone used.

When succinate is oxidized in the presence of added ammonia the rate but not the amount of oxygen uptake is increased(6). Certain

antibiotics in low concentrations have no effect on the oxidation of succinate alone but inhibit the increased rate in the presence of added ammonia thereby inhibiting its assimilation(6,7). Fig. 1, C & D, shows that the lactone had a similar effect. As little as 0.025 mg/ml inhibited the increased oxidation rate in the presence of ammonia but had no effect on the rate of oxidation of succinate alone. As the concentration of lactone was increased, inhibition of the succinate oxidation occurred.

The previous addition of succinate protected the enzymes against the lactone. In this experiment, with 0.2 mg of lactone/ml at the end of 2 hours the succinate oxidation was inhibited 49%, and in the presence of ammonia 61%. The lactone was added 10 minutes before the succinate. When the succinate was added 10 minutes before the lactone there was no inhibition of its oxidation, and only a 29% inhibition in the presence of added ammonia. This effect occurred with other substrates and is thus non-specific. The lactone has little effect on the succinoxidase of animal origin.

Of all the amino acids tried, the oxidation of phenylalanine is most sensitive to the action of several antibiotics(7). The possible reason for this is that it utilizes high energy compounds during one stage of its oxidation. The lactone showed a similar selectivity. 0.05 mg/ml inhibited the oxidation of phenylalanine 50%, tyrosine 23%, and had no effect on the oxidation of alanine and serine. It did, however, inhibit the oxidation of propionic acid 52%. Finally, the oxidation of p-hydroxybenzoic acid which requires the formation of an adaptive enzyme and is inhibited by a number of antibiotics(8) was inhibited 36% by 0.05 mg/ml of the lactone. In this organism, therefore, it appears that the lactone has an action similar to that of streptomycin, aureomycin, and certain other antibiotics.

Trichophyton mentagrophytes and *Epidermophyton floccosum* obtained from the Department of Bacteriology, Duke Hospital, were grown on Sabouraud's medium at pH 5.6 and the plates were incubated at room temperature for 10 days. At this pH the hydrolysis of the lactone is delayed and the acidity of the medium remained constant. 1:1000 concentration inhibited growth of these 2 organisms completely as well as that of a 7-day-old yeast culture of *B. dermatitidis* incubated at 37°C. Furthermore, when scrapings were made from these plates at the end of 4 days and streaked on fresh plates without the drug, no growth occurred. A 1:5000 concentration inhibited growth approximately 50%.

Discussion. Because β-propiolactone is fungistatic as well as bacteriostatic it may be useful as a surface disinfectant. It would be necessary to store and apply it in a non-aqueous medium. Once in contact with the skin some of the lactone would be taken up by the organisms and the remainder would hydrolyze slowly, thereby keeping the area acid. The combined effect of the lactone and acidity should inhibit growth. Because of its low toxicity, it could be applied frequently without danger of systemic effects from absorption through the skin.

Summary. 1. β-propiolactone inhibits the oxidative metabolism of a strain of *Pseudomonas aeruginosa*. The mechanism of the inhibition appears similar to that of streptomycin, aureomycin, and certain other antibiotics. 2. Its hydrolytic product, β-hydroxypropionic acid, is without effect. 3. In low concentrations it inhibits the growth of *Trichophyton mentagrophytes, Epidermophyton floccosum,* and *Blastomyces dermatitidis.* 4. Its possible use as a surface disinfectant is discussed.

1. Hartman, F. W., Piepes, S. L., and Wallbank, A. M., *Fed. Proc.*, 1951, v10, 358.

2. Kelley, A. R., and Hartman, F. W., *ibid.*, 361.

3. Mangum, G. H., Kelley, A. R., Sanders, B. E., Piepes, S. L., Wallbank, A. M., and Hartman, F. W., *ibid.*, 220.

4. Kelley, A. R., and Hartman, F. W., *Fed. Proc.*, 1952, v11, 419.

5. Kelley, A. R., *ibid.*, 419.

6. Bernheim F., and DeTurk, W. E., *J. Pharmacol.*, 1951, v103, 107.

7. ———, *ibid.*, in press.

8. ———, *J. Bact.*, in press.

Received April 25, 1952. P.S.E.B.M., 1952, v80.