cal, within the range of 1000–25,000 cal characteristic of most enzymes as determined by Sizer (11).

Discussion. On the basis of the evidence presented, it is likely that the delta hemolysin is an enzyme. In view of the fact that aqueous organic phosphorus is released from phospholipid substrates by the hemolysin, it may have a mode of action similar to that of phospholipase C. Unlike the beta hemolysin, which is a phospholipase C and releases water-soluble phosphorylcholine from sphingomyelin, the delta hemolysin does not attack this substrate. Its activity is not affected by Mg^{2+} ions and is uninhibited by EDTA in contrast with the beta hemolysin. Although its substrate in the erythrocyte has not yet been clearly identified, the delta hemolysin releases water-soluble organic phosphorus from phosphatidylinositol and to a lesser degree from phosphatidylserine. We have detected the presence of small amounts of phosphatidylinositol in the erythrocytes used in this study when their extracts were chromatographed using Marinetti's technique (12). The same species of erythrocytes have also been shown to contain phosphatidylinositol and in addition, phosphatidylserine, according to a recent report by Nelson (13).

Summary. Purified delta hemolysin from the Newman and E-delta strains of *S. aureus* liberates aqueous organic phosphorus from phospholipid extracts of various species of mammalian erythrocytes. Of several phospholipids investigated as substrates, phosphatidylinositol is most susceptible to degradation by the delta hemolysin. Phosphatidylserine was to a lesser extent attacked by the enzyme. In contrast with the beta hemolysin of *S. aureus,* delta hemolysin does not hydrolyze sphingomyelin and its activity is unaffected by EDTA or Mg^{2+} ions.

1. Doery, H. M., Magnusson, B. J., Gulasekharam, J., and Pearson, J. E., J. Gen. Microbiol. **40**, 283 (1965).

2. Wiseman, G. M. and Caird, J. D., Can. J. Microbiol. **13**, 369 (1967).

3. Dolman, C. E. and Wilson, R. J., Can. J Public Health **31**, 68 (1940).

4. Tiselius, A., Hjerten, S., and Levin, O., Arch. Biochem. Biophys. **65**, 132 (1956).

5. Jackson, A. W. and Little, R. M., Can. J. Microbiol. **4**, 435 (1958).

6. Gladstone, G. P. and Yoshida, A., Brit. J. Exptl. Pathol. **48**, 11 (1967).

7. Gladstone, G. P. and van Heyningen, W. E., Brit. J. Exptl. Pathol. **38**, 123 (1957).

8. Rose, H. G. and Oklander, M., J. Lipid Res., **6**, 428 (1965).

9. Wiseman, G. M., J. Pathol. Bacteriol **89**, 187 (1965).

10. Fiske, C. H. and Subbarow, Y., J. Biol. Chem. **66**, 375 (1925).

11. Sizer, I. W., Advan. Enzymol. **3**, 35 (1943).

12. Marinetti, G. V., J. Lipid Res. **3**, 1 (1962).

13. Nelson, G. J., Biochim. Biophys. Acta **144**, 221 (1967).

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The Effect of SC 15396 on Gastrin Stimulated Pancreatic Secretion (33030)

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Bedi *et al.* (1) described the inhibitory effects of 2-phenyl-2-(2-pyridyl) thioacetamide (SC 15396, G. D. Searle Co.) on gastric secretory responses to both pure gastrin II and the synthetic peptide, pentagastrin. They were satisfied that at low doses its antagonism was specific for gastrin and the pentapeptide, and they termed the substance antigastrin. Cook and Bianchi (2) also showed that SC 15396 was an inhibitor of gastrin induced gastric secretion in rats and dogs. Others (3) used larger doses and suggested that the effect is not specific because inhibition was demonstrated against both insulin and histamine induced gastric acid secretion.

Gastrin (4) stimulates pancreatic secre-

tion, being a strong stimulant for protein output and a weak one for volume flow and bicarbonate secretion. It was the purpose of the present study to observe the effects of SC 15396 on gastrin induced pancreatic secretion.

Methods. The gastrin used in this study was prepared from hog antral mucosa by the method of Gregory and Tracy (5) carried only through the isopropanol stage. The same batch of gastrin was used throughout. Doses of gastrin are expressed as wet weight of antral mucosa extracted.

SC 15396 is not easily soluble. One hundred mg of the powder was dissolved in 10 ml of 100% propylene glycol during stirring with a magnetic stirring bar for 4 hours, and 40 ml of 0.15 *M* NaCl was added; thus the solution contained 2 mg/ml.

Three mongrel dogs weighing between 14 and 20 kg were used. A chronic pancreatic fistula was made (4) and at the same time a Thomas cannula was inserted into the most dependent portion of the ventral wall of the stomach, just proximal to the antrum, to form a gastric fistula. Three weeks were allowed for recovery of the animals before experiments were started.

The animals were fasted for 18 hours before each experiment. Throughout each experiment the pancreatic and gastric fistulas were open and secretion was collected every 15 min. A polyethylene catheter was inserted into a leg vein and an infusion of sterile 0.15 *M* NaCl was maintained throughout by a Harvard peristaltic pump at a rate of 60 ml/hour. After at least two 15-min periods to confirm basal levels, gastrin extract was added to the saline infusion to give a dose level of 0.4 gm (equivalent wt. wet mucosa) per kg per hour.

The tests were divided into two sets, each comprising three tests in each of the three dogs. In both sets a constant infusion of gastrin dissolved in 0.15 *M* saline was given for 4.5 hours. In the control set 10 ml of sterile 0.15 *M* saline were injected intravenously 2 hours after the start of the infusion of gastrin. In the second set at 2 hours 2 mg/kg of SC 15396 was injected intravenously. Tests with saline or SC 15396 were performed alternately; not more than three experiments were performed in any 1 week.

The concentration of acid in gastric juice was measured by titration of 0.2-ml samples to pH 7.0 with 0.2 *N* NaOH using a glass electrode and an automatic titrator (Autoburet, Radiometer, Copenhagen, Denmark). The output of acid is expressed in milliequivalents secreted per 15 min.

The concentration of bicarbonate in pancreatic juice was measured by adding 1.0 ml of 0.1 *N* HCl to 0.5-ml samples, heating the mixture to the first evidence of boiling, and, when cooled, backtitrating the residual acid to pH 7.0 with 0.2 *N* NaOH using the automatic titrator. The output of bicarbonate is expressed as microequivalents secreted per 15 min.

Total protein concentration was used as an index of pancreatic enzyme secretion. Keller *et al.* (6) have shown that there is good correlation between the total protein concentration and the proteolytic enzyme in pancreatic juice. The protein concentration was measured using an AutoAnalyzer (Technicon) with a Beckman DB spectrophotometer measuring absorbance at 280 mμ. This was compared with a standard solution of bovine serum albumin. The protein output is expressed as milligrams secreted per 15 min.

The mean values for the second hour of gastrin infusion, just before the intravenous injection of SC 15396, were taken as 100% and all subsequent values are expressed as percentage of these control values. All values following ± signs are standard errors of the mean.

In separate tests in each dog the effectiveness of the gastric fistula to totally drain gastric juice from the stomach was tested (7). These tests consisted of intravenous infusion of 0.16 mg of histamine dihydrochloride/kg per hour for 5 hours. For the first 2 hours of this infusion the secretions from both the gastric and pancreatic fistulas were collected, and for the last 3 hours the gastric fistula was closed and only pancreatic secretion was collected. In the hour before the gastric fistula was closed the mean gastric acid output was 8.39 ± 0.95 meq/15 min,

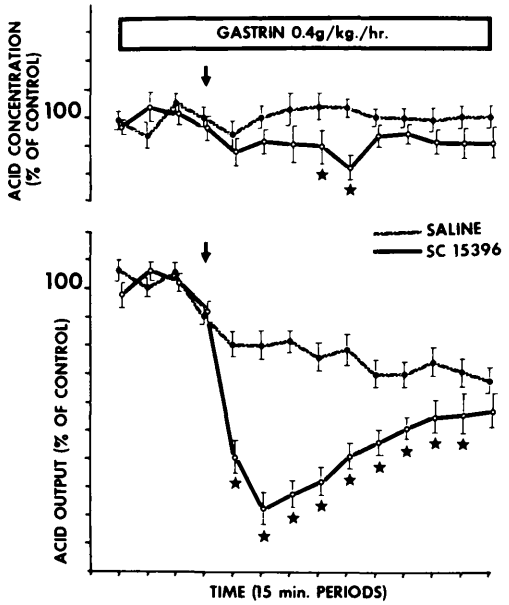

FIG. 1. Top: Gastric acid concentration. Control (100%) acid concentration: 144 ± 2.4 m*M*, saline; 142 ± 2.7 m*M*, SC 15396. Bottom: Gastric acid output. Control (100%) acid output: 6.84 ± 0.51 meq/15 min, saline; 6.56 ± 0.45 meq/15 min, SC 15396. In this and subsequent figures, each point represents the mean of nine experiments in three dogs, the vertical bars the SE of the mean, the stars points of statistical difference ($p < 0.05$), and the arrow the time of SC 15396 or saline administration.

and the pancreatic bicarbonate output was 101.6 ± 6.9 μeq/15 min. The pancreatic bicarbonate output in the second hour following closure of the gastric fistula was 1906.0 ± 25.6 μeq/15 min. It was concluded that, in these dogs, the gastric fistula adequately drained the gastric juice from the stomach.

The standard *t* test for unpaired values was used to determine statistical differences (8).

Results. During the control period (second hour of gastrin infusion) none of the parameters of gastric or pancreatic secretion were significantly different in the saline tests as compared with the tests with SC 15396.

Gastric acid response. A single intravenous injection of 2 mg/kg of SC 15396 caused marked reduction of gastric acid output; inhibition was greatest (23% of control) 30 min after the injection and remained for 135 min (Fig. 1) at a level significantly lower than with infusion of gastrin alone. Acid concentration was lower to a significant extent only in the 60- and 75-min periods after SC 15396 (Fig. 1).

Pancreatic response. The volume flow rate did not show significant differences between drug and saline injections at any time before or after the injections (Fig. 2).

The control bicarbonate concentration, taken as 100% was 74.8 ± 3.5 m*M* in the tests with SC 15396 and 68.1 ± 4.2 m*M* in the other group. The bicarbonate concentration in both groups gradually fell throughout the experiment, to 85% of control in the group that received the SC 15396 injection and to 92% of control in the group that received a saline injection.

Following the injection of SC 15396 the bicarbonate output decreased (Fig. 2), but a significant difference from the saline injection was found at only the 75-minute period.

The control protein concentration was 39.2 ± 3.2 mg/ml in the tests with SC 15396,

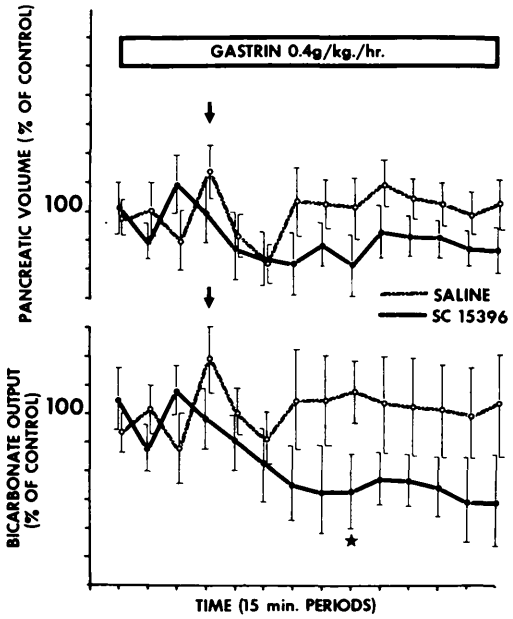

FIG. 2. Top: Pancreatic volume response. Control (100%) volume: 3.75 ± 0.24 ml/15 min, saline; 4.43 ± 0.27 ml/15 min, SC 15396. Bottom: Pancreatic bicarbonate output response. Control (100%) bicarbonate output: 255 ± 24.8 μeq/15 min, saline; 331.5 ± 29.0 μeq/15 min, SC 15396.

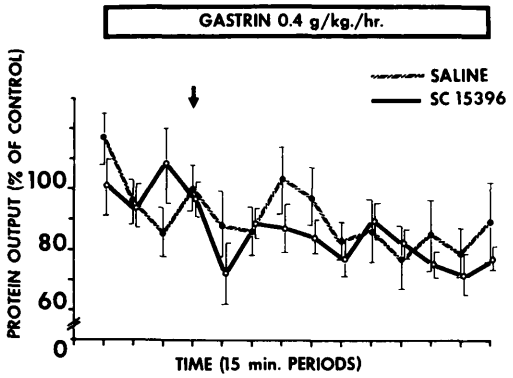

FIG. 3. Pancreatic protein output response. Control (100%) protein output: 154.5 ± 8.4 mg/15 min, saline; 167.8 ± 11.8 mg/15 min; SC 15396.

and 45.7 ± 4.3 mg/ml in the tests with a saline injection. There was no significant change in the protein concentration of either group throughout the tests.

In both groups the protein output gradually declined throughout the tests with no significant differences between the saline or SC 15396 injections (Fig. 3).

Discussion. The inhibitory effect of SC 15396 on gastric acid output stimulated by gastrin as reported by Bedi *et al.* (1) in both innervated and denervated fundic pouches has been confirmed in gastric fistula dogs in the present study. The percentage inhibition of gastric acid output was about the same as they reported in vagally innervated pouches and lasted for a similar period. The acid concentration was also decreased following SC 15396 administration, but the maximal reduction was only 18.5%, thus the major factor in decreased acid output was decreased volume flow.

Pancreatic volume, bicarbonate output, and to a lesser extent bicarbonate concentration, were uniformly lower after SC 15396 than after saline. However, most of the differences were not satistically significant and the percentage reduction of pancreatic secretion was very much less than that of gastric secretion.

Gastrin extracts (4) have been shown to be more powerful stimulants of pancreatic enzyme secretion than of volume flow and bicarbonate secretion. In these experiments there was no difference in either protein concentration or protein output from the pancreas following a dose of SC 15396 or saline.

A possible explanation for the small decrease in pancreatic volume flow and bicarbonate output is that some acid reached the duodenum instead of being totally diverted by the gastric fistula, and that following administration of SC 15396 and the subsequent lowering of gastric acid output, the delivery of acid to the duodenum was decreased. However, as was described under "Methods," the efficiency of each dog's gastric fistula to drain gastric juice was tested and found to be adequate.

Summary. The effect of SC 15396 on gastrin stimulated pancreatic and gastric secretion in conscious dogs with gastric and pancreatic fistulas was tested. A dose of SC 15396 that caused marked inhibition of gastric secretion produced only a small and statistically insignificant depression of pancreatic secretion.

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1. Bedi, B. S., Gillespie, G., and Gillespie, I. E., Lancet **1**, 1240 (1967).

2. Cook, D. L. and Bianchi, R. G., Life Sci. Part 1 **6**, 1381 (1967).

3. Connell, A. M., Sircus, W., Hill, R. A., Maclead, I. B., and Thomson, C. G., Lancet **2**, 720 (1967).

4. Preshaw, R. M. and Grossman, M. I., Gastroenterology **48**, 36 (1965).

5. Gregory, R. A. and Tracy, H. J., Gut **5**, 103 (1964).

6. Keller, P. J., Cohen, E., and Neurath, H., J. Biol. Chem. **233**, 344 (1958).

7. Preshaw, R. M., Cooke, A. R., and Grossman, M. I., Gastroenterology **50**, 171 (1966).

8. Snedecor, G. W. "Statistical Methods," 5th ed. Iowa State Univ. Press, Ames, Iowa, 1956.

Received Jan. 22, 1968. P.S.E.B.M., 1968, Vol. 128.