PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 150, 773–779 (1975)

Cyclic-AMP and Pancreatic Bicarbonate Secretion in Response to Secretin in Dogs (39123)

SIGURD DOMSCHKE, STANISLAW J. KONTUREK, WOLFRAM DOMSCHKE, ARTUR DEMBIŃSKI, PIOTR THOR, RYSZARD KRÓL, AND LUDWIK DEMLING

(Introduced by E. D. Jacobson)

Department of Medicine, University of Erlangen–Nürnberg, Erlangen, Germany, and Institute of Physiology, Medical Academy, Cracow, Poland

In the last decade, many gastrointestinal hormones have been shown to influence their target tissue by increasing or decreasing the steady-state level of cyclic-AMP in the cell. However, mediation of the effects of secretin via the cyclic-AMP (adenosine-3′:5′-monophosphate) pathway remains a controversial issue. Secretin causes accumulation of cyclic-AMP in the cat pancreas *in vitro* and *in vivo* as reported by Case and coworkers (1). Moreover, in cats, dibutyryl cyclic-AMP and the phosphodiesterase inhibitor theophylline are also able to stimulate pancreatic secretion (2). In dogs, however, Guelrud *et al.* (3) found theophylline did not stimulate basal and maximal secretin-stimulated pancreatic secretion. In contrast, Tompkins and Kuchenbecker (4) recently demonstrated an enhancing effect of theophylline on canine pancreatic secretion during submaximal secretin stimulation.

These conflicting results promoted the present study in order to evaluate the role of cyclic-AMP in the stimulation of bicarbonate secretion in the canine pancreas both in acute experiments and in conscious animals. Experiments were designed primarily to focus on measurements of bicarbonate secretory rates and cyclic-AMP levels in both pancreatic juice and pancreatic tissue after stimulation by exogenous and endogenous secretin administered either alone or in combination with phosphodiesterase inhibitors. In this way, cyclic-AMP changes and pancreatic secretory responses to physiological stimulation could be examined at the same time.

Methods. The studies were performed on three groups (A, B, and C) of mongrel dogs, weighing 15–18 kg. The animals of group A (four dogs) and B (four dogs) were anesthetized with intravenous thiobarbital sodium and supplied with oxygen by means of a Starling pump and an endotracheal tube. The abdomen was opened by midline incision and the pylorus was ligated. Polyethylene tubes were inserted into the stomach and upper part of the duodenum for the drainage of gastric juice and intraduodenal infusion, respectively. The lesser pancreatic duct was ligated. The main pancreatic duct was drained by a polyethylene tube inserted ahead of the point where the duct opens into the duodenum, and the juice was collected in iced samples. The common bile duct was also cannulated to divert the bile to the exterior (Fig. 1).

The animals of group C (three dogs) were prepared with gastric fistulas (GF) by inserting a Thomas cannula (5) into the stomach and with pancreatic fistula (PF) made by a modification of the method of Herrera *et al.* (6). The studies reported here started about 5 months after surgery. Food was withheld from the cages for at least 18 hr before each test. At the beginning of each experiment, the stomach was washed and then the gastric juice was drained throughout the rest of the study to prevent acid from the stomach from entering the duodenum and releasing secretin. During each experiment an infusion of 0.15 *M* NaCl was given by peristaltic pump through a polyethylene tube inserted into a leg vein.

Two series of experiments were performed on anesthetized dogs. In animals of Group A pancreatic secretion was stimulated by secretin (GIH Research Unit, Karolinska Institutet, Stockholm, Sweden) dissolved in saline and infused intravenously in doses

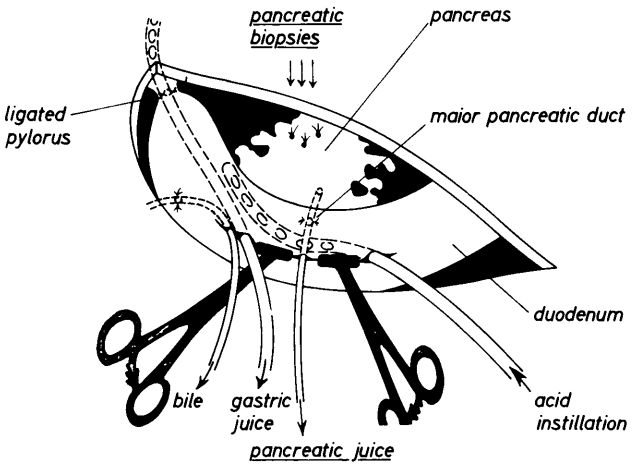

FIG. 1. Schematic drawing of the preparation in anesthetized dogs.

doubled every 60 min from 0.5 to 8.0 U/kg/hr. In animals of Group B, pancreatic secretion was induced by hydrochloric acid instilled intraduodenally through the polyethylene tube placed in the upper part of the duodenum. The stimulant for release of endogenous secretin was infused intraduodenally at a constant concentration of 100 mEq/liter at rates doubling every 60 min from 2 to 16 mEq/hr. In all experiments on anesthetized animals, samples of pancreatic tissue were taken from the body and tail of the pancreas at each dose level of intravenous secretin or intraduodenal acid load. Biopsies were immediately frozen in liquid nitrogen and then kept frozen at −20° until assayed for DNA and cyclic-AMP content. DNA was determined by using a diphenylamine reagent (7). Cyclic-AMP was estimated by means of an isotope dilution procedure (8). Pancreatic secretion was collected continuously and divided into 15-min samples. The volume of each sample was recorded to the nearest 0.1 ml. Bicarbonate concentration was determined by adding an excess of 0.1 *N* H_2SO_4 to an aliquot and back-titrating to pH 7.0 with 0.1 NaOH while N_2 was bubbled continuously through the mixture. Protein was measured according to Lowry *et al.* (9).

In animals of Group C, identical secretory studies were performed using either secretin or intraduodenal infusion of hydrochloric acid. In addition, a constant dose of caffeine (20 mg/kg/hr), aminophylline (10 mg/kg/hr), or papaverine hydrochloride (2 mg/kg/hr) was infused intravenously in combination with graded doses of secretin. In separate studies it was shown that intravenous administration of graded doses of caffeine (10–80 mg/kg/hr), aminophylline (5–40 mg/kg/hr), or papaverine hydrochloride (1–8 mg/kg/hr) did not produce any significant changes from the basal levels in pancreatic volume or bicarbonate outputs.

Secretion from PF in conscious dogs was collected continuously and divided into 15-min samples. Pancreatic secretion was examined first under basal conditions during two 15-min periods. No tests were started unless there were at least two consecutive 15-min periods with basal secretion less than 1.5 ml. The experimental protocol resembled that of Groups A and B. Volume, bicarbonate, protein, and cyclic-AMP (in some tests) were estimated as in acute experiments.

Results were evaluated statistically by means of Wilcoxon's signed rank test; as a measure of the degree of correlation between variables, the rank correlation coefficient (r_S) according to Spearman was computed (10).

Results. As shown in Fig. 2, subsequent to increasing doses of iv secretin there was a dose-dependent rise in the cyclic-AMP content of the pancreatic tissue (increases from the basal level: $p < 0.01$). DNA concentrations fell slightly, but significantly ($P < 0.05$) during secretin infusion. Moreover, as

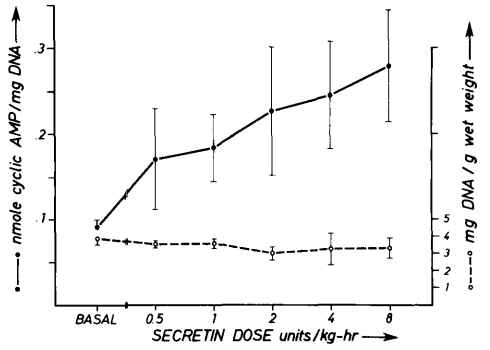

FIG. 2. Dose-dependent effect of a continuous iv infusion of secretin on cyclic-AMP and DNA concentrations in canine pancreas. Value represent average of determinations in four anesthetized dogs. Vertical bars indicate standard deviation (SD).

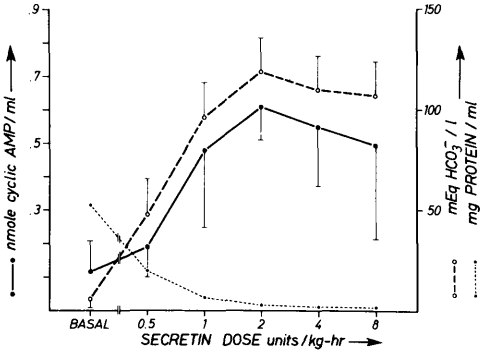

FIG. 3. Pancreatic dose–response curves to secretin Cyclic-AMP is depicted as a solid curve, bicarbonate as a dashed curve, and protein as a dotted curve. Means ± SD of four tests in four anesthetized dogs.

may be seen in Fig. 3, both bicarbonate and cyclic-AMP concentrations in the pancreatic juice were elevated dose-dependently. Peak concentrations were attained with a secretin dose of 2 U/kg/hr, whereas the bicarbonate and cyclic-AMP outputs approached maximal values during 4 U/kg/hr owing to a further increase in pancreatic juice flow. The effect of increasing secretin doses on bicarbonate and cyclic-AMP outputs was remarkably similar. The bicarbonate and cyclic-AMP outputs were significantly correlated ($P < 0.001$), Spearman's rank correlation coefficient being 0.94. The tissue cyclic-AMP concentrations were significantly correlated with both the cyclic-AMP (r_S = 0.572, $P < 0.01$) and bicarbonate outputs ($r_S = 0.707$, $P < 0.001$).

In conscious dogs provided with chronic GF and PF (Group C), pancreatic volume flow and bicarbonate outputs in response to secretin showed a dose-related increase (Fig. 4). As may be seen in Fig. 4, the outputs of bicarbonate and cyclic-AMP were correlated ($r_S = 0.909$, $p < 0.001$). Both cyclic-AMP and bicarbonate concentrations in the pancreatic juice were significantly correlated with the pancreatic volume flow ($r_S = 0.615$, $P < 0.005$; $r_S = 0.814$, $P < 0.001$, respectively). Cyclic-AMP and bicarbonate concentrations in pancreatic juice were correlatively elevated in response to stepwise increasing doses of secretin (Fig. 4; $r_S = 0.559$, $P < 0.01$). In contrast, the protein output did not increase significantly

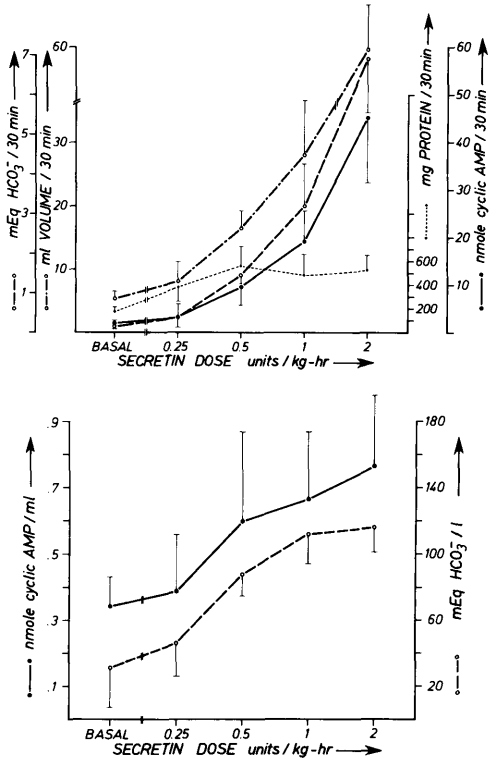

FIG. 4. Dose–response curves to secretin in conscious dogs. Exogenous secretin vs pancreatic juice flow, cyclic-AMP, bicarbonate, and protein outputs, respectively (above). Pancreatic juice concentrations of bicarbonate and cyclic-AMP (below). Means ± SD of six experiments in three dogs.

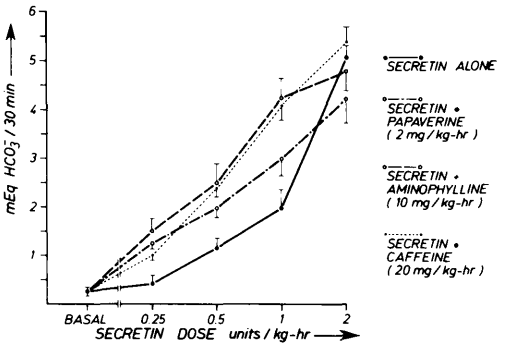

FIG. 5. Effect of phosphodiesterase inhibitors on secretin-induced bicarbonate outputs in conscious dogs. Means ± SD of six experiments in three animals. Basal secretion was obtained when neither secretin nor phosphodiesterase inhibitors were given.

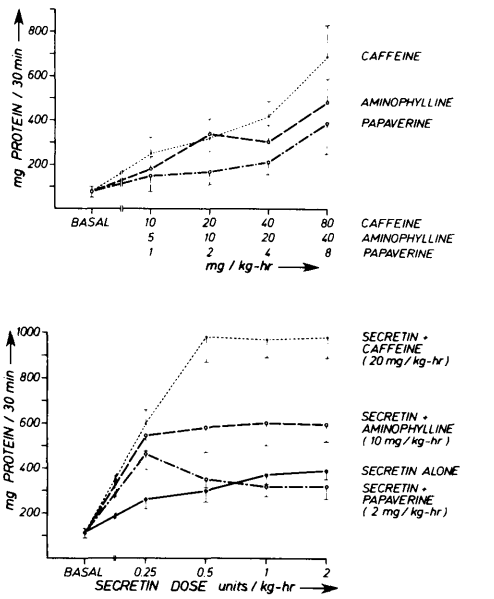

FIG. 6. Effect of phosphodiesterase inhibitors alone (above) and in combination with secretin (below) on pancreatic protein secretion in conscious dogs. Mean ± SD of six experiments in three dogs.

because of decreasing concentration with increasing doses of secretin.

In the studies with secretin infusion during the administration of a constant dose of caffeine, aminophylline, or papaverine, the highest pancreatic bicarbonate outputs occurred at the same dose as in tests with secretin (2 U/kg/hr). There was no statistically significant difference in the highest volume or bicarbonate outputs between tests with secretin alone and secretin combined with any of the phosphodiesterase inhibitors used (Fig. 5). At the lower dose levels, the pancreatic volume and bicarbonate outputs were significantly higher in tests with secretin combined with phosphodiesterase inhibitors than with secretin alone ($P < 0.01$). Pancreatic protein outputs in tests with secretin combined with caffeine or aminophylline were significantly higher than with secretin alone ($P < 0.01$). The addition of papaverine to secretin infusion did not augment pancreatic protein output (Fig. 6).

The results of pancreatic bicarbonate responses obtained from duodenal perfusion with hydrochloric acid in anesthetized dogs (Group B) are shown in Table I. Duodenal instillation of acid led to a dose-dependent increase in both pancreatic bicarbonate concentration and outputs accompanied by an increase in cyclic-AMP concentration and outputs. In conscious dogs (Group C), duodenal acidification with graded acid loads also produced a stepwise increase in pancreatic volume flow and bicarbonate outputs (Fig. 7). These secretory changes were accompanied by corresponding rises in the cyclic-AMP concentrations and outputs in the pancreatic juice. The pancreatic bicarbonate and cyclic-AMP outputs were correlated ($r_S = 0.915$, $P < 0.001$).

Discussion. The concentrations of cyclic-

TABLE 1. PANCREATIC BICARBONATE AND CYCLIC-AMP RESPONSES TO DUODENAL ACIDIFICATION[a]

	Duodenal acidification (mEq/hr)				
	Basal	4	8	16	32
mEq HCO_3^-/liter	14 ± 4	16 ± 8	58 ± 18	89 ± 15	113 ± 9
mEq HCO_3^-/hr	0.011 ± 0.009	0.023 ± 0.016	0.37 ± 0.27	1.05 ± 0.38	2.39 ± 0.60
nmole cAMP/ml	0.189 ± 0.091	0.208 ± 0.137	0.246 ± 0.118	0.396 ± 0.172	0.335 ± 0.069
nmole cAMP/hr	0.152 ± 0.130	0.299 ± 0.191	1.16 ± 1.52	4.96 ± 3.17	6.86 ± 0.48

[a] In anesthetized dogs. Means ± SD of four tests in four dogs.

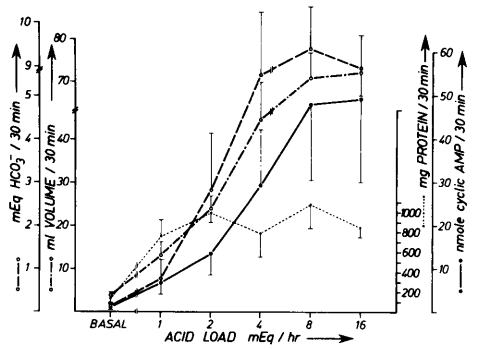

FIG. 7. Duodenal acidification in three conscious dogs. Pancreatic juice flow, cyclic-AMP, bicarbonate, and protein outputs vs intraduodenal acid load. Mean ± SD of six tests in three conscious dogs.

AMP in the pancreatic juice have not yet been studied. It has now been found that they are in the same order of magnitude as reported for bile (11). The relatively high levels (10^{-7} to 10^{-6} *M* range) are comparable to those in urine (12) and those in milk and semen, which are both exocrine secretions (13). In contrast, relatively low levels of the cyclic nucleotide (10^{-8} *M* range) have been found in the plasma (13), cerebrospinal fluid (12), gastric secretion (14), and amniotic fluid (13). Changes in the extracellular levels of cyclic nucleotide have been shown to reflect alterations in the intracellular levels of the substance in response to a variety of endocrinologic manipulations (13). This study reveals a statistically significant correlation between pancreatic tissue cyclic-AMP levels and cyclic-AMP output in pancreatic juice. Obviously, some of the cyclic nucleotide escapes from its origin into the extracellular fluid. This rate of extrusion of cyclic-AMP into the extracellular space seems to be an important factor in the regulation of the intracellular levels of nucleotide (15).

Although cyclic-AMP concentrations rose several fold after secretin administration, juice concentrations never reached tissue levels. However, since tissue cyclic-AMP levels and juice outputs of this cyclic nucleotide were correlated, cyclic-AMP outputs may be chosen as a readily accessible indicator reflecting changes in the pancreatic tissue cyclic-AMP.

In anesthetized dogs, exogenous secretin was capable of elevating the intracellular levels of cyclic-AMP in canine pancreas. The effect occurred at dose levels of secretin that also initiated a physiological response, i.e., water and bicarbonate secretion. It can be assumed that pancreatic tissue samples taken at the end of each secretin infusion period were representative of the whole period, since Case *et al.* (1) found that during secretin infusion the initial rise of cyclic-AMP in cat pancreas was maintained as long as secretin was infused. The slight fall in pancreatic DNA concentration following secretin is probably due to an increase in blood flow (16) or edema.

Secretin caused a dose-dependent increase in pancreatic secretion of cyclic-AMP and bicarbonate. Both parameters were significantly correlated. The pancreatic bicarbonate output was also correlated with the tissue cyclic-AMP levels, thus indicating a participation of cyclic-AMP in pancreatic secretion.

Pancreatic protein outputs were shown not to be systematically dependent on secretin doses. Only an initial and transitory increase in protein output in response to secretin was found, probably representing the wash-out of preformed enzymes from the duct system (1). However, since in all periods of secretin administration the outputs of protein were slightly but significantly above the basal level, a weak stimulation of enzyme secretion by secretin cannot be ruled out. These results are in agreement with those of Stening *et al.* (17), who have demonstrated that pure secretin is a weak stimulant of protein secretion in conscious pancreatic fistula dogs.

Further support for the hypothesis that cyclic-AMP is involved in water and electrolyte secretion has been sought by using agents that alter the activity of phosphodiesterase. In this report the effects of aminophylline, caffeine, and papaverine on the cyclic-AMP and bicarbonate secretion in the pancreatic juice in conscious dogs have been tested. The results have shown that all the phosphodiesterase inhibitors used augmented pancreatic bicarbonate secretion induced by secretin at lower dose levels,

leaving the response to the highest dose of this hormone virtually unchanged. The shift in the bicarbonate dose–response curve to the left by methylxantines and papaverine indirectly suggests that these phosphodiesterase inhibitors act synergistically with secretin to stimulate bicarbonate secretion. This remains in accord with one of the postulates of Sutherland (18) that agents that inhibit phosphodiesterase should potentiate the submaximal effect of the hormone, the physiological action of which is mediated by cyclic-AMP. We are unable to explain the failure of these agents to initiate bicarbonate secretion from the pancreas. Perhaps the limited cell permeability of these agents or the inherent low activity of phosphodiesterase in the bicarbonate-producing pancreatic cells might contribute to these results. It is of interest that methylxantines used in this study also augmented pancreatic protein response to secretin. This effect may be a secondary phenomenon attributable to the action of these agents on the release of antral hormone, which has been sought to be mediated by cyclic-AMP (19). Gastrin is known to be a strong stimulant of pancreatic protein secretion in the dog (17).

The concurrent increase in bicarbonate and cyclic-AMP outputs following intraduodenal instillation of acid strongly suggests that cyclic nucleotide plays a role in pancreatic bicarbonate secretion also under physiological conditions, i.e., in response to endogenous secretin. Adenylate cyclase may be the site of action of secretin, since this hormone has been shown to stimulate adenylate cyclase in rat pancreas homogenate (20, 21), fat ghost cells (22), and liver homogenate (23).

Summary. In anesthetized dogs given secretin intravenously in doses doubling every 60 min and ranging from 0.5 to 8 units per kg body weight per hr, cyclic-AMP levels in pancreatic tissue rose continuously, whereas DNA concentrations were slightly decreased. Bicarbonate concentrations and bicarbonate outputs, cyclic-AMP tissue concentrations and bicarbonate outputs, as well as cyclic-AMP tissue concentrations and juice outputs, were significantly correlated. In conscious pancreatic fistula dogs, there was also a significant correlation between cyclic-AMP and bicarbonate concentrations and outputs in the pancreatic juice after stimulation by exogenous secretin. Accordingly, enhanced release of endogenous secretin achieved by intraduodenal acidification led to a dose-dependent increase in bicarbonate and cyclic-AMP outputs in both conscious and anesthetized dogs. Phosphodiesterase inhibitors (aminophylline, caffeine, and papaverine) given alone to the conscious dogs did not initiate pancreatic bicarbonate secretion, but they potentiated bicarbonate responses to exogenous secretin. These data suggest that cyclic-AMP plays a part in secretin-stimulated pancreatic secretion.

1. Case, R. M., Johnson, M., Scratcherd, T., and Sherratt, H. S. A., *J. Physiol. London* **223,** 669 (1972).
2. Case, R. M., and Scratcherd, T., *J. Physiol. London* **223,** 649 (1972).
3. Guelrud, M., Rudick, J., and Janowitz, H. D., *Gastroenterology* **60,** 671 (1971).
4. Tompkins, R. K., and Kuchenbecker, S. L., *J. Surg. Res.* **14,** 172 (1973).
5. Thomas, J. E., *Proc. Soc. Exp. Biol. Med.* **46,** 260 (1941).
6. Herrera, F., Kemp, D. R., Tsukamoto, M., Woodward, E. R., and Dragstedt, L. R., *J. Appl. Physiol.* **25,** 205 (1968).
7. Croft, R. M., and Lubran, M., *Biochem. J.* **95,** 612 (1965).
8. Van den Berghe, G., De Wulf, H., and Hers, H. G., *Eur. J. Biochem.* **16,** 358 (1970).
9. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.* **193,** 265 (1951).
10. Snedecor, J. W., and Cochran, W. G., "Statistical Methods." Iowa State Univ. Press, Ames (1967).
11. Levine, R. A., and Hall, R. C., *Gastroenterology* **61,** 873 (1972).
12. Butcher, R. W., and Sutherland, E. W., *J. Biol. Chem.* **237,** 1244 (1962).
13. Broadus, A. E., Hardman, J. G., Kaminsky, N. I., Ball, J. H., Sutherland, E. W., and Liddle, G W., *Ann. NY Acad. Sci.* **185,** 50 (1971).
14. Bieck, P. R., Oates, J. A., Robinson, G. A., and Adkins, R. B., *Amer. J. Physiol.* **224,** 158 (1973).
15. Sutherland, E. W., *JAMA* **214,** 1281 (1970).
16. Aune, S., and Semb, L. S., *Acta Physiol. Scand.* **76,** 406 (1969).
17. Stening, G. F., Johnson, L. R., and Grossman, M. I., *Amer. J. Physiol.* **217,** 251 (1969).
18. Sutherland, E. W., Robinson, G. A., and Butcher, R. W., *Circulation* **37,** 279 (1967).

19. Gabryś, B. F., Alavi, S. M., Nyhus, L. M., and Bombeck, C. T., *Gastroenterology* **64**, 730 (1973).
20. Marois, C., Morrisset, J., and Dunningan., J., *Rev. Can. Biol.* **31**, 253, (1972).
21. Rutten, W. J., de Pont, H. M., and Bonting, S. L., *in* "Advances Cyclic Nucleotide Research," Vol. 1, *Physiology and pharmacology of cyclic AMP*, (P. Greengard, G. A. Robinson, and R. Paoletti, eds.), p. 586. Raven Press, New York (1972).
22. Rodbel, M., Birnbaumer, L., and Pohl, S. L., *J. Biol. Chem.* **245**, 718 (1970).
23. Thompson, W. J., Williams, R. H., and Little, S. A., *Biochem. Biophys. Acta* **302**, 329 (1973).

Received July 11, 1975. P.S.E.B.M. 1975, Vol. 150.