function of the decreasing pO_2. O_2 consumption was then related to the dry weight of the preparation.

The results of our experiments (Figure) show that at no pO_2 between 40% and 90% of the saturation point (285÷642 mm Hg) it is possible to evidence a critical pO_2 level in the incubating medium above that at which O_2 consumption of the sacs remains at a steady state: in fact, O_2 consumption always varied along with pO_2.

It is therefore clear that O_2 requirements of the preparation are never completely satisfied; the large amount of lactic acid produced by the everted sacs of rat small intestine during incubation in a medium containing glucose should consequently be considered, at least partially, as a consequence of anaerobic glycolysis resulting from inadequate O_2 availability.

Riassunto. Misurando il consumo di O_2 in funzione della pO_2 nel mezzo di incubazione è stato dimostrato che i sacchetti di intestino tenue rovesciato di ratto si trovano in condizione di parziale anaerobiosi nel corso dell'incubazione in liquido fisiologico contenente glucoso. Ciò viene posto in relazione con la notevole produzione di acido lattico da parte dell'intestino isolato mantenuto in tali condizioni.

P. Pietra and V. Cappelli

Institute of General Physiology and Institute of Human Physiology, University of Pavia, I-27100 Pavia (Italy), 14 November 1969.

Significance of Cyclic AMP in the Regulation of Exocrine Pancreas Secretion

Since its discovery[1], adenosine-3′,5′-monophosphate (cAMP) has been implicated as an important regulatory mechanism in the control of a wide variety of divergent processes[2–4]. Early investigators have provided evidence that cAMP is closely linked to the endocrine system: ACTH, vasopressin, luteinizing hormone, TSH, MSH, and glucagon activities appear to be related to cAMP formation[5–10]. More recently, questions regarding the influence of cAMP on the exocrine systems have been raised. Alonso and Harris[11] have demonstrated that cAMP stimulates acid secretion by frog gastric mucosa; Kulka and Sternlicht[12] have shown that cAMP stimulates the secretion of amylase by mouse pancreas, and Case et al.[13] have postulated that cAMP mediates the action of secretin on the exocrine cat pancreas. We have examined the effects of cAMP on the secretion of the exocrine rabbit pancreas.

Materials and methods. New Zealand white male rabbits weighing 2.6–3.6 kg were anesthetized with 0.7 ml/kg Dial-Urethane (CIBA Pharmaceutical Co.) given i.v. The pancreas was removed and mounted according to the method of Rothman and Brooks[14,15]. Krebs-Hanseleit bicarbonate solution gassed with 95% O_2 and 5% CO_2 and 95 mg/100 ml added glucose was used as the bathing medium; pH was maintained in the range 7.2–7.4. Pancreatic secretion was collected at $^1/_2$ h intervals for a period of 5 h; collection periods 1 and 2 were allowed for equilibration and washout of the secretion already present in the main duct. N^6-2-*O*-dibutyryl cyclic adenosine-3′,5′-monophosphate (Schwarz BioResearch Inc.), $1\times10^{-5}M$, and theophylline (Cal BioChem), $1\times10^{-3}M$, and $1\times10^{-2}M$, were added to the bath singularly and in combination after collection period 4; effect on enzyme concentration, enzyme output, and volume were determined. After activation with enterokinase (Cal BioChem), esterase activity of trypsin and chymotrypsin was determined from the initial reaction velocities of their respective hydrolysis of TAMe[16] (*p*-toluenesulfonyl-L-arginine methyl ester) and ATEe[17] (acetyl-L-tyrosine ethyl ester) with a radiometer titrator. Levels of significance were determined employing the Student *t*-test for paired data comparison.

Results. In our control animals, and in previous controls done in this laboratory[18], there has been a constant downward progression of enzyme concentration and output with volume remaining fairly constant over a 5 h period (Figure 1). When dibutyryl cyclic AMP (DcAMP), $1\times10^{-5}M$, was added to the bath, enzyme concentration and output markedly increased as compared to controls while volume remained stable (Figure 2). Theophylline, $1\times10^{-3}M$, caused a volume increase and a rise in trypsin-chymotrypsin concentration and output, although only the trypsin output was significantly increased. Addition of theophylline, $1\times10^{-2}M$, produced a volume rise and a statistically significant increase in trypsin and chymotrypsin concentration and output. DcAMP, $1\times10^{-5}M$, and theophylline, $1\times10^{-3}M$, combined resulted in volume, trypsin and chymotrypsin concentration similar to those seen with DcAMP alone and a slight increase in enzyme output over that observed in DcAMP-only experiments. Summation of these results and statistical comparison is shown in the Table.

[1] E. W. Sutherland and T. W. Rall, J. biol. Chem. *232*, 1077 (1958).
[2] E. W. Sutherland, I. Oye and R. W. Butcher, Recent Prog. Horm. Res. *21*, 623 (1965).
[3] E. W. Sutherland and G. A. Robison, Pharmac. Rev. *18*, 145 (1965).
[4] G. A. Robison, R. W. Butcher and E. W. Sutherland, Ann. N.Y. Acad. Sci. *139*, 703 (1966–67).
[5] R. C. Hayes jr., J. biol. Chem. *233*, 1220 (1958).
[6] J. Orloff and J. S. Handler, J. clin. Invest. *41*, 702 (1962).
[7] J. M. Marsh and K. Sauard, J. biol. Chem. *239*, 1 (1964).
[8] S. Tarui, K. Nonaka, Y. Ikara and K. Shima, Biochem. biophys. Res. Commun. *13*, 329 (1963).
[9] M. W. Bitensky and S. R. Burstein, Nature *208*, 1282 (1965).
[10] J. R. Turtle and D. M. Kipnis, Biochem. biophys. Res. Commun. *28*, 797 (1967).
[11] J. B. Harris and D. Alonso, Fedn. Proc. *24*, 1368 (1965).
[12] R. G. Kulka and E. Sternlicht, Proc. natn. Acad. Sci. *61*, 1123 (1968).
[13] R. M. Case, T. J. Laundy and T. Scratcherd, Proc. phys. Soc. *47* (June 1969).
[14] S. S. Rothman, Nature *204*, 84 (1964).
[15] S. S. Rothman and F. P. Brooks, Am. J. Physiol. *208*, 1171 (1965).
[16] G. W. Schwert, H. Neurath, S. Kaufman and J. E. Snoke, J. biol. Chem. *172*, 221 (1948).
[17] J. E. Snoke and H. Neurath, J. biol. Chem. *182*, 577 (1950).
[18] L. Solberg and F. P. Brooks, in print (1970).

Discussion. Response to DcAMP addition was a clear increase in enzyme concentration and output over that observed in control animals. Comparison of control and experimental animal ratios for collection periods 6, 7, 8/3, 4 showed statistically significant increased trypsin and chymotrypsin concentration and output without a significant increase in volume (Table). This response is similar to that produced by pancreoyzmin activity, although DcAMP did not produce a several-fold increase in enzyme output such as has been noted previously with pancreozymin stimulation[20,21]. Adenyl cyclase is thought to reside on the cell membrane[22], and its stimulation by pancreozymin would be immediate without any delay for intracellular penetration of pancreozymin. In contrast, slow intracellular penetration of DcAMP may not allow the rapid build-up of intracellular nucleotide to levels necessary to cause enzyme release such as those produced quickly by pancreozymin. Enzyme release in our experiments was a slower, more sustained release than the rapid response which would be expected for extracellularly-acting pancreozymin. Larger concentrations of DcAMP than those we used might be expected to raise intracellular levels of nucleotide more rapidly and produce a more traditional pancreozymin response.

Theophylline often mimics the effects of cAMP. This was generally true in our experiments with the additional finding that theophylline also caused an increase in volume (Table). When all experiments in which theophylline was involved were compared with controls, volume increase was just short of statistical significance ($0.05 < p < 0.1$). Theophylline may have a dual action on the pancreas. Response to addition of DcAMP, $1 \times 10^{-5} M$,

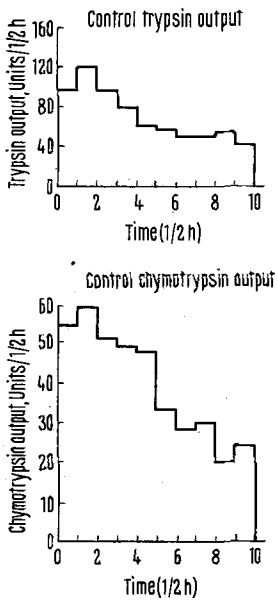

Fig. 1. Downward progression of enzyme output with time in a typical control animal.

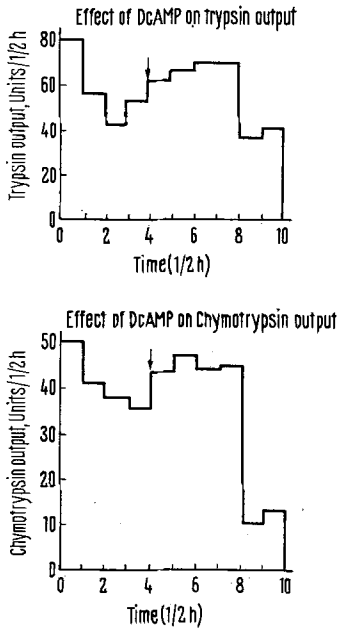

Fig. 2. DcAMP addition prevents downward progression of enzyme output with time in typical experimental animals.

Mean percentages for average values of 6,7,8/3,4

Bath additive	Trypsin concentration	Trypsin output	Chymotrypsin concentration	Chymotrypsin output	Volume
Controls (6)	52.3 ± 5.7	56.0 ± 7.7	57.2 ± 5.9	62.8 ± 9.8	105.0 ± 12.1
DcAMP, $1 \times 10^{-5} M$ (5)	98.6 ± 12.4 $p < 0.005$	107.2 ± 13.4 $p < 0.005$	96.4 ± 10.9 $p < 0.01$	104.9 ± 12.4 $p < 0.02$	108.0 ± 4.7 $p < 0.90$
Theophylline, $1 \times 10^{-3} M$ (3)	72.8 ± 23.6 $p < 0.4$	97.1 ± 19.5 $p < 0.05$	66.3 ± 17.3 $p < 0.6$	98.4 ± 12.1 $p < 0.2$	142.3 ± 16.5 $p < 0.1$
Theophylline, $1 \times 10^{-2} M$ (4)	99.7 ± 17.8 $p < 0.02$	127.8 ± 27.4 $p < 0.02$	101.0 ± 18.1 $p < 0.05$	129.5 ± 28.4 $p < 0.05$	124.0 ± 8.7 $p < 0.3$
DcAMP, $1 \times 10^{-5} M$ + Theophylline, $1 \times 10^{-3} M$ (6)	85.5 ± 9.3 $p < 0.01$	114.0 ± 16.4 $p < 0.01$	79.2 ± 9.7 $p < 0.1$	110.8 ± 15.5 $p < 0.025$	130.6 ± 9.6 $p < 0.2$

Mean percentages ± S.E.M. for enzyme concentration, enzyme output, and volume average values for collection periods 6, 7 and 8 when compared with those for collection periods 3 and 4. Levels of significance determined for comparison of mean percentages of averaged enzyme and volume for collection periods 6,7,8/3,4 in control and experimental animals. Number of experiments listed in parentheses.

plus theophylline, $1 \times 10^{-3} M$, was somewhat puzzling. There was a small increase in enzyme output, a moderate increase in volume, and a small decrease in enzyme concentration over that observed when only DcAMP was added. Synergistic stimulation of enzyme concentration and output by theophylline in combination with DcAMP may be masked by a separate antagonistic secretin-like effect of theophylline.

Cyclic AMP is known to serve a regulatory role related to a number of enzyme activities and hormonal systems. DcAMP addition produced increased enzyme output with little secretory volume change. This may reflect an absent role for cAMP in the regulation of secretin activity in the in vitro rabbit pancreas preparation or a concentration of DcAMP insufficient to stimulate secretin activity. Theophylline enhances enzyme output in the in vitro rabbit pancreas preparation, most probably on the basis of phosphodiesterase inhibition. Secondly, it appears theophylline has a second secretin-like effect on pancreatic secretion which may be unrelated to cAMP[23,24].

Zusammenfassung. Nachweis, dass die Zufuhr von dibutyryl-zyklischem AMP (DcAMP) in vitro die Enzymproduktion erhöht und zwar mit nur geringer Gewichtsveränderung des Kaninchenpankreas. Die Enzymproduktion wird ebenso durch Theophyllin vermehrt und es wird vermutet, das DcAMP der Vermittler der pankreozymen Wirkung im Kaninchen ist.

R. G. KNODELL, P. P. TOSKES, H. A. REBER and F. P. BROOKS[25]

University of Pennsylvania School of Medicine, Hospital, Philadelphia (Pennsylvania 19104, USA), 8 November 1969.

[19] S. S. ROTHMAN, Nature *213*, 460 (1967).
[20] S. S. ROTHMAN, Am. J. Physiol. *211*, 777 (1966).
[21] R. M. CASE, A. A. HARPER and T. SCRATCHERD, J. Physiol., Lond. *201*, 335 (1969).
[22] P. R. DAVOREN and E. W. SUTHERLAND, J. biol. Chem. *238*, 3016 (1963).
[23] The authors gratefully acknowledge the technical assistance of Mrs. SARA M. LEE.
[24] Supported in part by USPHS Grants Nos. T01-AM-05462-05 and 5T01-GM-01540.
[25] Send requests for reprints to F. P. BROOKS, Hospital, University of Pennsylvania.

Failure of the D-Analog of Angiotensin II-Amide [D-asp (NH_2)-D-arg-D-val-D-tyr-D-val-D-his-D-prol-D-phe (OH)] to Influence Water Diuresis in the Rat[1]

An analog of val[5]-angiotensin II-amide composed of the D-amino acids instead of the L-amino acids has been synthesized by VOGLER et al.[2] and proved biologically inactive on the guinea-pig ileum (which contracts under the influence of val[5]-angiotensin II-amide) and on the cat's blood pressure.

Val-[5]-angiotensin II-amide possesses a natriuretic and diuretic effect in the rat (PETERS[3], BONJOUR et al.[4,5]) which, in animals in water diuresis, results in a large increase in the osmolar clearance without a notable change or with a slight increase in the clearance of free water. The present experiments were undertaken in order to check whether the D-analog shared this somewhat peculiar effect of L-val[5]-angiotensin II-amide.

Water diuresis was induced in a group of 10 rats by infusing a hypotonic solution of glucose and fructose containing minimal amounts of NaCl (BONJOUR et al.[5]). After equilibration and a few control periods, the D-analog of angiotensin II-amide (kindly supplied by Dr. R. O. STUDER, Hoffmann-La Roche Ltd., Basel) was added to the infusion fluid at a rate of 0.25 µg/animal/min. No change occurred in urine flow. The same experiment was repeated in a second group of animals, increasing the dose of the D-analog of angiotensin II to 2.5 µg/animal/min. This higher dose also proved completely ineffective (Table).

Zusammenfassung. Während L-val[5]-Angiotensin II bei Ratten einen ausgesprochenen diuretischen Effekt zeigt, fehlt diese Wirkung bei dem D-Analogon.

J. PH. BONJOUR[6] and G. PETERS

Institut de Pharmacologie de l'Université de Lausanne, CH-1005 Lausanne (Switzerland), 19 December 1969.

	Urine flow	C_{OSM} (ml/kg/min)	C_{H_2O} (ml/kg/min)
Control periods	0.59 ± 0.05	0.28 ± 0.03	0.30 ± 0.02
D-angiotensin (2.5 µg/min)			
1–15 min	0.62 ± 0.05	0.32 ± 0.02	0.30 ± 0.03
16–30 min	0.57 ± 0.05	0.31 ± 0.03	0.26 ± 0.03
Control periods	0.60 ± 0.04	0.33 ± 0.02	0.27 ± 0.02

Urine flow, osmolar clearance (C_{OSM}) and clearance of free water (C_{H_2O}) in a group of 10 rats in water diuresis, as influenced by D-angiotensin. Values are means ± standard error (S.E.).

[1] Supported by Fonds National Suisse de la Recherche Scientifique, Grant No. 4190.
[2] K. VOGLER, R. O. STUDER, W. LERGIER and P. LANZ, Helv. chim. Acta *48*, 1407 (1965).
[3] G. PETERS, Proc. Soc. exp. Biol. Med. *112*, 771 (1963).
[4] J. PH. BONJOUR, G. PETERS, F. CHOMETY and D. REGOLI, Europ. J. Pharmac. *2*, 88 (1967).
[5] J. PH. BONJOUR, D. REGOLI, F. ROCH-RAMEL and G. PETERS, Am. J. Physiol. *215*, 1133 (1968).
[6] Present address: Physiopathologisches Institut der Universität Bern (Switzerland).