between curve b and curve a is statistically significant: the t calculated was 3.659 > 3.250 for p = 0.01 probability. These results point to a potentiation provoked by catecholamines on ADP-induced platelet clumping in vivo, evidenced by a slowing down of the disaggregation. This effect of catecholamines can be further shown by the calculation of the area delimited by curve a (area A = ADP) and curve b (area B = catecholamines + ADP). In fact if we consider that the rectangular area delimited by time (30 min), in abscissa, and 100% in platelet count, in ordinate, represents the total amount of responsive platelets in the animal, it appears that the area over each curve represents the percentual of platelets which have been aggregated, and the area below each curve represents the percentual of free circulating platelets. In this way it was calculated that area B (catecholamines + ADP) is 24.34% of the total area, whereas area A (ADP) is 16.30% of the total area. Comparing the 2 areas in absolute values, it appears that B is about 50% larger than A, thus indicating a potentiation.

Discussion. Present results show that the infusion of a mixture of catecholamines in the rat, causes a low drop in the basal platelet count and also potentiates aggregation induced by ADP. Literature[1-3] indicates that aggregation by epinephrine and l-norepinephrine in vitro is present in humans but not in rats, and this discrepancy with our results points to the differences often occurring between in vitro and in vivo experiments. On the other hand, our results confirm the in vitro finding that catecholamines potentiate aggregation by ADP[4,5,7]. These findings give further evidence that there are species differences in the response of platelets to aggregating agents. Mills[3] ascribed the different behaviour of human platelets, compared to those of rats or of other animals, to the greater amount of ADP contained by human thrombocytes in the 'nucleotide storage pool'. This fact may also explain the mechanism of action underlying potentiation: authors suggest that it may involve α-receptors[5-7] and that may be mediated by endogenous ADP released by platelets in the extracellular medium[12]. Really our results show that the in vivo potentiation induced by catecholamines on ADP clumping, does not consist in increasing the maximal extent of aggregation, but in slowing the recovery. This phenomenon indicates a persistence of platelet aggregates in blood, which may be circulating, or may be filtered by some districts, for example lungs or kidneys, as previously suggested[9,13].

12 D. G. McMillan, Nature, Lond. *211*, 140 (1966).
13 M. G. Doni, Experientia *30*, 550 (1974).

Effect of pancreatic polypeptide on DNA-synthesis in the pancreas[1]

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Summary. Bovine pancreatic polypeptide increases DNA-synthesis in the rat pancreas; no effect is observed in stomach (oxyntic area), duodenum or liver. BPP neither augments or inhibits the trophic action of cholecystokinin.

Regulation of growth in the digestive tract is one of the important physiological actions of gastrointestinal hormones. Gastrin has been shown to promote the growth of acid-secreting gastric mucosa[2], while cholecystokinin (CCK) increases DNA-synthesis in pancreatic acinar tissue[3,4]. In contrast, secretin[5] and more recently motilin[6] have been shown to inhibit pentagastrin-stimulated growth in the stomach.

A trophic role for pancreatic polypeptide (PP), a new hormone found primarily in the pancreas, has not hitherto been investigated. Because PP appears to conteract CCK-mediated pancreatic enzyme secretion[7], a similar inhibitory effect on pancreatic growth might be anticipated. The purpose of this study was therefore 2fold: first to establish if PP had any trophic effects in the gastrointestinal tract and second to observe whether it could influence the trophic action of CCK.

Materials and methods. 48 male Sprague-Dawley rats (100–120 g) were fasted for 24 h in individual metabolic cages. Water was provided ad libitum. They were randomly divided into 4 groups (n = 12 for each group) and injected once i.p. as follows: Group I (control): NaCl, group II: 18 nmoles/kg cholecystokinin-octapeptide (CCK-OP), group III: 12 nmoles/kg bovine pancreatic polypeptide (BPP) and group IV, a combination of II and III. This dose of BPP has previously been shown to approximately halve CCK-OP-induced pancreatic trypsin output in the dog[7]. The volume of injection was equal in the 4 groups. After 15 h, 0.5 mCi/kg ^{3}H-thymidine (5 Ci/mmole) was injected and the animals sacrificed at 16 h. The liver, pancreas, duodenum and oxyntic area of the stomach were quickly removed, frozen in liquid nitrogen and stored at −20 °C.

Tissue samples were homogenized in 5% trichloracetic acid (1 ml/100 mg of tissue) at 4 °C. The homogenate was centrifuged and the pellet washed twice with 3 ml of 5% TCA. The DNA-containing pellet was then suspended in 3 ml of 5% TCA and heated for 10 min at 90 °C. This suspension was centrifuged and the supernatant saved. The pellet was then resuspended in 1 ml of 5% TCA and again heated and centrifuged. The supernatants were combined and incorporation of ^{3}H-thymidine into DNA determined by counting 1 ml of the supernatant in 10 ml of scintillation fluid (Instagel) in an Intertechnique scintillation counter. The DNA-content of the samples was determined using calf thymus DNA as a standard[8].

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2 L. R. Johnson and A. M. Chandler, Am. J. Physiol. *224*, 937 (1973).
3 D. L. Mainz, O. Black and P. D. Webster, J. clin. Invest. *52*, 2300 (1974).
4 L. R. Johnson and P. D. Guthrie, Gastroenterology *70*, 59 (1976).
5 L. R. Johnson and P. D. Guthrie, Gastroenterology *67*, 601 (1974).
6 P. Mitznegg, W. Domschke, S. Domschke, D. Belohlavek, W. Sprügel, U. Strunz, E. Wünsch, E. Jaeger and L. Demling, Scand. J. Gastroent. *11*, 657 (1976).
7 T. M. Lin and R. E. Chance, Gastroenterology *67*, 737 (1974).
8

Disintegrations per minute of ^{3}H-thymidine per mg of DNA

Tissue	Group Control	CCK-OP	BPP	BPP + CCK-OP
Pancreas	9.4 ± 1.41	18.7 ± 4.25[a]	17.9 ± 2.61[c]	19.7 ± 2.91[d]
Liver	15.4 ± 2.83	12.9 ± 1.79	15.7 ± 2.98	14.1 ± 2.19
Duodenum	107.4 ± 16.62	154.0 ± 24.95	146.3 ± 20.94	152.2 ± 16.18
Stomach	17.7 ± 3.01	12.8 ± 1.79	15.4 ± 1.97	15.0 ± 1.83

[a] Results expressed as mean ± SEM; n = 12 for each group. p-values were determined using Student's t-test: [b] < 0.05, [c] < 0.01, [d] < 0.005 when compared to control values.

Results. The experimental observations appear in the table. In the pancreas BPP significantly ($p < 0.01$) increased DNA-synthesis from control values of 9.4 ± 1.4 dpm/μg, DNA to 17.9 ± 2.6 (mean ± SEM). Similarly, a significant ($p < 0.05$) 2fold increase in DNA-synthesis was observed with CCK-OP. BPP when given in combination with CCK-OP neither augmented nor inhibited the effect observed with CCK-OP alone. The 2fold increase in DNA-synthesis was comparable to that observed with the individual hormones, and was significantly above control values ($p < 0.005$).

Although in the duodenum CCK, BPP and the combination, resulted in levels 50% higher than those observed in NaCl-injected rats, the difference did not reach statistical significance. BPP, CCK-OP, or the combination did not increase the rate of DNA-synthesis in the stomach (oxyntic gland area) or the liver.

Discussion. Whereas protein and RNA-synthesis are measures of cell hypertrophy, DNA-synthesis is held to be a specific index of cell division and hence cell growth[9]. In the present study, bovine pancreatic polypeptide was found to increase the rate of DNA-synthesis in the rat pancreas. This hormone, together with gastrin and CCK therefore becomes the third peptide demonstrated to have a trophic effect on this organ. In contrast, BPP had only a weak effect on the duodenum and failed to stimulate DNA-synthesis in the stomach or the liver. Pharmacological studies in the dog have demonstrated that low dose infusions of BPP relax the gall bladder and inhibit CCK-mediated pancreatic enzyme production[7]. Because CCK stimulates DNA-synthesis in the pancreas a similar inhibitory effect by BPP might also have been predicted. However, in this study, the 2fold increases in pancreatic DNA-synthesis observed with CCK was neither inhibited or augmented by the addition of BPP.

More recently, it has been demonstrated that PP is released by CCK-like peptides[11]. In this study, the increase in pancreatic DNA-synthesis by PP was identical to that observed for CCK. As CCK causes release of endogenous PP, it is conceivable that its trophic action is mediated entirely through PP release. This hypothesis is supported by the fact that the reported trophic response pattern of CCK in the pancreas, duodenum and stomach[3] confirmed in this study exactly parallels that observed for PP.

9 K. Burton, Biochem. J. *62*, 315 (1956).
10 L. R. Johnson, Gastroenterology *70*, 278 (1976).
T. E. Adrian, S. R. Bloom, M. G. Bryant, J. M. Polak, Ph. Heitz and A. J. Barnes, Gut *17*, 940 (1976).
11 T. E. Adrian, S. R. Bloom, H. S. Besterman, A. J. Barnes, T. J. C. Cooke, R. C. G. Russel and R. G. Faber, Lancet *1*, 161 (1977).

The effects of topical succinylcholine on single unit and electrocorticographic activity

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Summary. Topical succinylcholine induces massive epileptiform discharges in the electrocorticogram, coinciding with the bursting or tonic activity of single cortical cells.

The neuromuscular blocking agent succinylcholine (SCh) consisting of 2 acetylcholine molecules is known to stimulate the muscle spindles eliciting massive afferent discharge in relatively small dosages[1]. This drug also depolarizes the end plate free part of the muscle membrane[2] and reacts with the cholinoceptive site at the first node of the motor nerve terminal[3]. In addition to these SCh effects, it has recently been found that it may act as a potent convulsant when applied topically to the cerebral cortex[4]. It was assumed that topical SCh may strongly depolarizes the cortical cells. An attempt was made to verify this hypothesis in the present work.

Materials and methods. The experiments were carried out in 6 adult cats spinalized at C_1 and maintained by artificial respiration. Under initial ether anesthesia, the trachea and the left femoral vein with the femoral artery were cannulated. The latter was used to monitor the blood pressure, which was always kept above 70 mm Hg. The left cerebral cortex was exposed by craniotomy, the dura was removed, and this area was filled with warmed

1 R. Granit, S. Skoglund and S. Thesleff, Acta physiol. scand. *28*, 134 (1953).
2 R. Ras, A. Den Hertog and W. Lammers, Pflügers Arch. *333*, 187 (1972).
3 L. C. Blaber and J. W. Goode, Int. J. Neuropharmac. *7*, 429 (1968).
4 Ü. Tan, Electroenceph. clin. Neurophysiol. *42*, 252 (1977).