cells of rat by ATP[14]. On this point, as well as the mechanisms by which adenosine phosphates influence anaphylaxis as observed, further investigation is being conducted in our laboratory[15].

[13] B. DIAMANT and P. G. KRÜGER, Acta physiol. scand. *71*, 291 (1967).
[14] B. DIAMANT and C. PETERSON, Acta physiol. scand. *80*, 299 (1970).
[15] Acknowledgment. Research supported in part by the Research-Aid Fund (1971) from the Prefecture of Fukushima. The author wishes to thank Prof. TAKEO YOSHIDA and Dr. JACK G. MAKARI for making the present study possible.

Zusammenfassung. Im Schultz-Dale'schen Versuch wird festgestellt, dass Adenosin-Triphosphat und Adenosin-Diphosphat die anaphylaktische Reaktion verstärken. D-Glukose-6-Phosphat wirkt abschwächend, während Kreatin-Phosphat keinen Einfluss auf die Reaktion hat.

T. OKAZAKI

Second Department of Medicine, Prefectural Medical College of Fukushima, Fukushima 960 (Japan), 10 June 1971.

Effect of Adrenaline on the in vitro Enzyme Secretion in the Guinea-Pig Pancreas

It is known in several animal species that injection of adrenaline brings about a marked inhibition of the exocrine secretion of the pancreas[1–4]. The mechanism of this effect is still debated. Hence, the inhibition has been interpreted either as the result of a direct action of the drug on the secretory cells[4] or as the consequence of vasoconstriction in the splancnic area[3].

Two types of exocrine cells are known to be present in the pancreas: the acinoductular cells, which secrete water and electrolytes, and the acinar cells which are responsible for the secretion of digestive (pro)enzymes. Recently HUBEL[5] has provided convincing evidence, obtained by in vitro experiments, indicating that in the rabbit pancreas secretion of water and electrolytes is inhibited by adrenaline by a direct mechanism acting at the cell level. In a system of pancreatic tissue slices we have investigated the in vitro effect of adrenaline on the other type of pancreas exocrine cells, the acinar cells.

In tissue slice systems the rate of in vitro enzyme secretion is usually determined by assaying the activity of one or more secretory enzymes released in the incubation medium. Such a method is rather insensitive because it does not permit discrimination between the enzyme molecules which are actually secreted over the time of the experiment and those which at the beginning of the experiment were already secreted and stored within the duct system. Recently JAMIESON and PALADE[6] have developed a more sensitive radiochemical procedure based on the determination of radioactive proteins released in the medium during a pulse-chase experiment. In our work we have used both the traditional and the new radiochemical procedures and in neither case we were able to demonstrate any effect of adrenaline on in vitro pancreatic enzyme secretion.

Methods. Male albino guinea-pigs (gift of Sigurtà Drug Co., Milan, Italy) weighing ~600 g were starved for 18–20 h.

[1] A. A. HARPER and C. C. N. VASS, J. Physiol., Lond. *99*, 415 (1941).
[2] H. GREENGARD, R. A. ROBACK and A. C. IVY, J. Pharmac. exp. Ther. *74*, 309 (1942).
[3] E. THOMAS, in *External Secretion of the Pancreas* (C. C. Thomas, Springfiled 1950), p. 114.
[4] T. E. BARLOW, J. R. GREENWELL, A. A. HARPER and T. SCRATCHERD in *Blood Flow Through Organs and Tissue* (Eds. W. H. BAIN and A. M. HARPER, Livingstone, Edinburgh 1968), p. 469.
[5] K. A. HUBEL, Am. J. Physiol. *219*, 1590 (1970).
[6] J. D. JAMIESON and G. E. PALADE, J. Cell Biol. *48*, 503 (1971).

Effect of adrenaline on the rate of secretion of pancreas tissue slices incubated in vitro

		A		B	
	Treatment	TCA-insoluble dpm in medium (%)	% of controls	α-amylase (mg maltose/5 min at 30°/mg protein)	% of controls
I	None	4.6 (3.6–5.9)	100	8.8 (7.3–11.0)	100
	Adrenaline 3 μM	5.8 (4.2–7.5)	125	7.2 (6.7–7.7)	72
	Adrenaline 30 μM	4.6 (2.7–6.5)	100	8.5 (7.0–10.2)	98
	Adrenaline 300 μM	4.4 (2.4–6.4)	94	9.0 (6.4–11.6)	102
II	Caerulein	13.1 (9.2–18.2)	285	14.3 (9.8–17.1)	162
	Caer. + Adr. 3 μM	10.8 (10.7–11.0)	237	13.1 (9.0–17.3)	149
	Caer. + Adr. 30 μM	9.4 (8.4–10.5)	210	14.1 (10.0–18.2)	160
	Caer. + Adr. 300 μM	10.8 (9.9–11.7)	237	11.4 (8.5–14.4)	130
III	Carbamoylcholine	11.1 (8.2–16.5)	241	10.8 (8.1–13.6)	123
	Carb. + Adr. 3 μM	11.1 (8.7–14.5)	241	13.7 (9.5–16.5)	155
	Carb. + Adr. 30 μM	13.3 (9.6–18.9)	289	10.6 (8.6–13.7)	121
	Carb. + Adr. 300 μM	12.9 (9.6–18.9)	280	10.6 (8.6–13.7)	121

Slices were first labeled for 5 min with ^{14}C-L-leucine, then chased for 80 min in a non-radioactive medium and finally reincubated for 20 min in the latter containing various concentrations of adrenaline, alone (I) or in the presence of either caerulein (II) or carbamoylcholine (III). In A) the rate of secretion is estimated by the amount of radioactive protein recovered in the medium, expressed as percentage of radioactive proteins present in slices + medium; in B) by the activity of the secretory enzyme α-amylase recovered in the medium, expressed as mg of maltose formed in 5 min of incubation at 30 °C per mg of slice protein. Values given are averages of 2 to 6 experiments. Ranges are in parentheses.

Pancreas tissue slices, prepared as in [7] were pulse labelled at 37°C in 5 ml of Krebs-Ringer bicarbonate solution containing glucose and a complete set of amino acids with ^{14}C-L-leucine (0.08 mM; 5 μC/μmole) as a tracer. After 5 min of labelling the slices were washed with warm, non-radioactive incubation medium containing an excess of ^{12}C-L-leucine (2 mM, chase medium) and reincubated in the latter for 80 min. This time of chase incubation is sufficent for labelled secretory proteins to be transported from their site of synthesis in the endoplasmic reticulum to zymogen granules at the cell apex[6]. The slices were then washed again and transferred to new incubation flasks with fresh medium containing the drugs whose effect on secretion had to be investigated. Concentrations used were as follows: adrenaline 3, 30 and 300 μM; carbamoylcholine, 100 μM; caerulein, 0.003 μM; pancreozymin, 12 Crick-Harper-Raper Units/ml. Incubation was continued for 20 additional min; media were then decanted and centrifuged at high speed to remove any particles and cell debris; slices were washed and homogenized in 2 ml of distilled water.

Protein radioactivity was determined in tissue homogenates and media as previously described[7]. Protein was assayed according to LOWRY et al.[8] and α-amylase activity according to BERNFELD[9].

Materials. All chemicals were reagent-grade. Carbamoylcholine and caerulein were a gift of Farmitalia Labs for Basic Res., Milan, Italy; pancreozymin (1800 Crick-Harper-Raper Units/mg) was a gift of Prof. E. JORPES, the Karolinska Institute, Stockholm, Sweden. Adrenaline was purchased from BDH, Poole, England, and ^{14}C-L-leucine (S.A. 250 mC: mM) from NEN, Langen, Germany.

Results and discussion. The results are summarized in the Table. It is evident that the radiochemical method is much more sensitive than the assay of α-amylase, a secretory enzyme, in detecting changes in the rate of enzyme secretion. Thus, in agreement with previous data[6,7] we observed that incubation of pancreas slices in the presence of either carbamoylcholine or caerulein results in a large increase of percentage of pulse labelled proteins recovered in the medium (+141 and +185%, respectively) while with the α-amylase assay only a much smaller increase could be detected. Analogous, but less reproducible results were found using pancreozymin as a segretagogue (not shown in the Table). However, when the effect of various concentrations of adrenaline on enzyme secretion was investigated, by neither method we could detect a significant change of the low, basal secretion rate of unstimulated cells (Table, I) as well as of the high secretion rates elicited by the segretagogues, i.e., carbamoylcholine and caerulein (Table, II and III).

In different secretory cells, the concentrations of adrenaline that we used have been found capable of influencing in vitro the secretion rate either by exerting an inhibition (for instance in pancreatic acino-ductular[5] and in islet β-cells[10]) or a stimulation (for instance in salivary acinar cells[11,12]). Furthermore, the doses of adrenaline effective in vivo in inhibiting the pancreatic exocrine secretion are such that the concentration of the drug at the level of the effector organs should be at the most of the order of the lowest used in our in vitro studies. Therefore, the lack of any detectable effect of adrenaline on pancreatic enzyme secretion in vitro strongly suggests that the observed changes in vivo are effected at a level other than the acinar cells.

Two possible sites of action can be envisaged. Injection of adrenaline is known to produce in several animal species a vasoconstriction in the pancreas[1-4]. This could reduce the access of secretory cells to segretagogues as well as oxygen and metabolites. It is well known that the supply of amino acids is rate-limiting for the synthesis of pancreatic secretory enzymes[13,14]; and that their synthesis, intracellular transport and release are all energy-requiring processes[6,15]. Hence vasoconstriction might be responsible of the in vivo inhibition of secretion. The possibility that in the pancreas the exocrine secretion is influenced by an adrenergic mechanism regulating the blood supply to the organ is also suggested by the finding that adrenergic fibers are distributed virtually exclusively to blood vessels and islets, as demonstrated by histological and histochemical studies[16,17].

On the other hand the in vivo effects could also depend, at least in part, on the inhibition of the acino-ductular cells[5]. This would cause the (pro)enzymes released to the duct system to be drained very slowly to the gut as a consequence of the impaired secretion of water and electrolytes.

Riassunto. In un sistema di fette di pancreas di cavia incubate in vitro l'adrenalina non modifica l'attività secretoria delle cellule acinari né in condizioni basali né in risposta a farmaci segretagoghi (ceruleina e carbamoilcolina). Sembra quindi probabile che l'inibizione della secrezione pancreatica esocrina prodotta in vivo dalla iniezione di adrenalina sia dovuta ad un meccanismo indiretto, quale la vasocostrizione pancreatica e(o) l'inibizione della secrezione di acqua ed elettroliti a livello delle cellule acino-duttulari.

J. MELDOLESI and G. MACCHI

Department of Pharmacology, University of Milan, Via Vanvitelli 32, I-20129 Milano (Italy), and CNR Center of Cytopharmacology, Milan (Italy), 5 July 1971.

[7] J. MELDOLESI, Br. J. Pharmac. *40*, 721 (1970).
[8] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[9] P. BERNFELD in *Methods in Enzymology* (Eds. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1955), p. 149.
[10] H. G. COORE and P. J. RANDALL, Biochem. J. *93*, 66 (1964).
[11] H. BABAD, R. BEN-ZVI, H. BDOLAH and M. SCHRAMM, Eur. J. Biochem. *1*, 96 (1967).
[12] R. J. GRAND and P. R. GROSS, J. biol. Chem. *244*, 5608 (1969).
[13] L. E. HOKIN, Biochem. J. *50*, 216 (1951).
[14] J. D. JAMIESON and G. E. PALADE, J. Cell Biol. *50*, 135 (1971).
[15] J. D. JAMIESON and G. E. PALADE, J. Cell Biol. *39*, 589 (1968).
[16] C. A. RICHINS, J. comp. Neurol. *83*, 223 (1945).
[17] B. FALK and B. HELLEMAN, Experientia *19*, 139 (1963).

Testosterone Induced Alterations in Histamine Metabolism in Mice

The urinary histamine content in mice can be assayed on a segment of isolated guinea-pig ileum. On assaying the excretion of free histamine, it was revealed that profound changes in the histamine metabolism occurred during pregnancy in mice[1].

Testosterone has been reported to reduce the excretion of free histamine when given to castrated female rats[2-4]. In the present study, the effect of testosterone on the urinary excretion of free histamine and metabolites was investigated in normal female mice.