THE RIBONUCLEIC ACID CONTENT OF PANCREAS AND PAROTID GLANDS DURING ENZYME SYNTHESIS AND SECRETION *IN VITRO*

by

L. E. HOKIN* AND MABEL R. HOKIN**

*Research Institute, the Montreal General Hospital, Montreal (Canada)*** *and the Department of Biochemistry, University of Sheffield (England)*

INTRODUCTION

There have been reports of both increases (GUBERNIEV AND KOVYREV[1]) and decreases (VERNE[2]; RIES[3]) in the ribonucleic acid (RNA) concentration of the pancreas after *in vivo* stimulation of secretion. The very high concentration of RNA in the pancreas (CASPERSSON[4]; BRACHET[5]; DAVIDSON AND WAYMOUTH[6]) forms part of the basis of the theory that RNA is concerned in protein synthesis[4,5]. It therefore seemed of interest to study the RNA content of pancreas slices when they are stimulated to synthesise or secrete enzymes *in vitro* (HOKIN[7,8]).

In the present investigation we have found that the RNA content of pancreas slices decreases about 30% during 2–3 hours incubation, but that this decrease seems to take place mainly in damaged cells. The RNA concentrations of pancreas slices which were stimulated *in vitro* either to synthesise or secrete enzymes were the same as in unstimulated slices. RABINOWITCH *et al.*[9], DALY AND MIRSKY[10] and DE DEKEN-GRENSON[11] have recently reported that there is no change in the RNA content of mouse pancreas after the stimulation of secretion *in vivo* with pilocarpine.

EXPERIMENTAL

Preparation and incubation of tissues

Pancreases from pigeons given food *ad lib.* were used. When enzyme synthesis was studied, 0.15 mg of carbamylcholine was injected intramuscularly 1 hour before killing. The slices were prepared and incubated as previously described[7,8]. Medium III (KREBS[12]), containing 200 mg% glucose was used. It was gassed with oxygen. Enzyme secretion was stimulated by carbamylcholine. Enzyme synthesis was stimulated by a complete mixture of amino acids[8]. Slices of rabbit parotid gland were prepared and incubated in a similar manner.

Amylase and RNA assays

After incubation the tissue was ground in sand, and water was added to a final volume of 3.0 ml. Aliquots of this material were taken for amylase assays. The remaining material was then treated with trichloracetic acid as described previously[13]. All operations were carried out at about 0–4° C.

* Merck Postdoctoral Fellow in Natural Sciences of the National Research Council.
** Fellow of the National Cancer Institute of Canada.
*** Present address.

References p. 240.

RNA was estimated by the method of SCHNEIDER[14]. The washed acid-insoluble residue was extracted in 5 ml of 5 % trichloracetic acid at 100° C for 15 minutes. After heating, the contents were centrifuged, the supernatant and one washing were made up to 10 ml and ribose was estimated in a 0.5 ml sample by the method of MEJBAUM[15]. The RNA content of the tissue is expressed here as RNA-ribose/mg dry weight. It should be pointed out that only the ribose from purine nucleotides is liberated to give the color reaction.

RESULTS

Change in the RNA content of pigeon pancreas slices and rabbit parotid slices during incubation

In Tables I–III are shown typical experiments in which the RNA content of pigeon pancreas slices was followed during incubation in oxygenated saline at 40° C. It can be seen that the RNA content fell by approximately 25–50 % during 2–3 hours' incubation. In some experiments the RNA concentration tended to reach a constant level after 1–2 hours' incubation, while in others it fell at a fairly constant rate over the experimental period.

In view of the fact that pancreas slices are capable of respiring and synthesising amylase at fairly constant rates for 2–3 hours[7,13] it seemed rather surprising that such a large fall in the RNA content should occur without affecting either respiration or enzyme synthesis. It thus seemed possible that the major portion of the loss in RNA during incubation occurred in damaged cells rather than in viable cells. This view is supported by the experiments shown in Table I. In this experiment the loss of RNA from slices cut from the surface of the gland (*i.e.* having one cut surface and one surface covered with peritoneum) was compared with the loss of RNA from slices prepared in the usual way (*i.e.* having two cut surfaces). If the observed fall in RNA mainly occurred in damaged cells this fall should be only half as great in slices with only one cut surface. This was found to be the case. It is therefore likely that the major portion of the breakdown of RNA occurred in damaged cells.

TABLE I

CHANGE IN RNA CONCENTRATION IN PIGEON PANCREAS SLICES DURING INCUBATION

Slices incubated in Medium III (KREBS[12]) containing 200 mg % glucose and complete amino acid mixture[8]; temperature 40° C; gas phase O_2.

Incubation time (min)	*RNA in tissue (μg RNA-ribose/mg dry weight)*	
	One cut surface	*Two cut surfaces*
0	23.2	24.0
		23.6
60		19.2
120		16.0
180	19.6	15.2
	18.8	

In connection with studies on amylase secretion by rabbit parotid slices, we had occasion to follow the RNA concentration in this tissue, incubated under the same conditions as the pigeon pancreas slices. The results of these studies are shown in Table II. The RNA-ribose content of rabbit parotid was found to be about one-tenth to one-fifth

References p. 240.

of that found in pigeon pancreas. The agreement between the RNA contents of duplicate parotid slices was not as good as in pigeon pancreas, due to the large admixture of connective tissue in the parotid. In contrast to pigeon pancreas slices there was no fall in the RNA concentration of parotid slices during incubation.

TABLE II

EFFECT OF CARBAMYLCHOLINE ON AMYLASE SECRETION AND RNA CONCENTRATION IN PIGEON PANCREAS AND RABBIT PAROTID SLICES

Slices incubated in Medium III (KREBS[12]) containing 200 mg% glucose; temperature 40° C; gas phase, O_2; (—) = no added carbamylcholine; (+) = added carbamylcholine (1 mg% final concentration); pigeon pancreas slices pre-incubated 15 minutes to remove amylase from damaged cells.

Tissue	*Time (min)*	*Amylase in medium (Units/mg dry wt.)*		*RNA in tissue (μg RNA-ribose/mg dry wt.)*	
		—	+	—	+
Pigeon pancreas	0	0	0	15.4	15.4
	30	23.6	31.2	13.9	14.5
	90	28.8	50.0	12.4	13.8
	150	30.8	55.6	9.3	8.7
Rabbit parotid	0	0	0	3.5	3.5
	30	15.6	26.0	3.1	3.3
	60	23.0	42.7	3.5	3.1
	120	31.5	43.7	3.6	3.3

The loss in RNA in pancreas slices during incubation was probably due to the large quantities of ribonuclease in this tissue. This view is further supported by the observation that less than 5% of the RNA which disappeared from the tissue could be recovered as RNA in the medium.

Amylase secretion and the RNA concentration in pigeon pancreas and rabbit parotid slices

To test the possibility that the RNA concentration of pancreas changes during the secretory cycle, pancreas slices were incubated in the presence and absence of carbamylcholine, which is capable of stimulating enzyme secretion *in vitro*[7]. The results of such experiments are recorded in Table II. Although the RNA content of pancreas slices fell by about 40% during 150 minutes incubation, the RNA content of stimulated and unstimulated slices agreed within 10%. Thus, stimulation of enzyme secretion in pancreas *in vitro* is not accompanied by any significant changes in the RNA content of the tissue.

When carbamylcholine was added to respiring rabbit parotid slices the secretion of amylase was stimulated (Table II). In contrast to pigeon pancreas, respiration was also stimulated by about 50%; DEUTSCH AND RAPER[16] have reported a similar increase in respiration when rabbit parotid slices were incubated with acetylcholine. As with pigeon pancreas, the RNA content of rabbit parotid slices was not altered when enzyme secretion was stimulated by carbamylcholine.

The possibility that RNA might be secreted by the pancreas, even though there is no net change in the cellular RNA content, was examined *in vivo*. Pancreas slices proved unsatisfactory because of the large quantities of ribonuclease present in the incubation medium. The pancreatic duct of an anaesthetised rabbit was cannulated,

References p. 240.

and the secretion was collected. The secretions were immediately chilled, and samples were taken for amylase and RNA assays. The injection of 1 mg of pilocarpine intravenously resulted in about a fifteen-fold increase in the secretion of amylase. No RNA was found in the pancreatic juice of either the unstimulated or stimulated gland. The failure to observe any RNA in pancreatic juice is in agreement with the recent findings of DALY AND MIRSKY[10].

Effect of amino acids on amylase synthesis and RNA content of pigeon pancreas slices

As reported earlier the addition of an appropriate mixture of amino acids to respiring pancreas slices stimulates the rate of amylase synthesis about 100–200%[7,8]. This mixture of amino acids also stimulates the synthesis of lipase and ribonuclease[17]. In the experiments reported here only amylase synthesis was measured. The rate af amylase synthesis was more than doubled by the additions of amino acids; however, the concentration of RNA in slices in which enzyme synthesis was stimulated agreed with control slices within experimental error (Table III).

TABLE III

AMYLASE SYNTHESIS AND RNA CONCENTRATION IN PIGEON PANCREAS SLICES

Fed pigeon injected with 0.15 mg carbamylcholine 1 h before killing; slices incubated in Medium III (KREBS[12]) containing 200 mg% glucose; temperature 40° C; gas phase O_2.

Incubation time (min)	*Amylase synthesised (units/mg dry wt.)*		*RNA in tissue (μg RNA-ribose/mg dry wt.)*	
	Without amino acids	*With amino acids*	*Without amino acids*	*With amino acids*
0	0	0	22.0	22.0
60	6.8	14.8	18.8	21.2
120	11.2	29.2	15.6	16.8

DISCUSSION

The RNA concentration of cells undergoing rapid division is higher than in corresponding non-dividing cells; this has been observed in embryonic cells (CASPERSSON[18]; CASPERSSON AND THORELL[19]; BRACHET[20]), tumour cells (SENTESSON AND CASPERSSON[21]) bacteria (MALMGREN AND HEDEN[22]; CALDWELL, MACHOR AND HINSHELWOOD[23]) yeast (THORELL AND WISING[24]; THORELL[25]) and tissue cultures (DAVIDSON AND LESLIE[26]). This parallelism between the rate of cell division and the RNA concentration in the cytoplasm has been regarded as evidence for a functional relationship between RNA and protein synthesis (CASPERSSON[4]; BRACHET[5]). It should be pointed out, however, that the RNA content has not been found to parallel the rate of protein synthesis at all stages of growth (DAVIDSON AND LESLIE[26]).

Protein synthesis can be followed in pancreas slices *in vitro* uncomplicated by cellular growth. Under these conditions the stimulation of protein synthesis by the addition of amino acids is not accompanied by changes in the RNA content. The RNA concentration also appears to remain constant in the various phases of the secretory cycle.

ACKNOWLEDGEMENTS

We wish to thank Professors J. H. QUASTEL, F.R.S. and H. A. KREBS, F.R.S. for their interest and encouragement.

References p. 240.

SUMMARY

The RNA content of respiring pancreas slices incubated *in vitro* falls about 30–50% during 2–3 hours incubation at 40° C. This fall is probably due to the action of ribonuclease on the RNA of damaged cells. There is practically no fall in the RNA content of rabbit parotid slices during incubation. Stimulation of enzyme secretion in either pigeon pancreas slices or in rabbit parotid slices is not accompanied by any significant changes in the RNA concentration. Stimulation of enzyme synthesis in pigeon slices by a complete amino acid mixture is also not parallelled by changes in the RNA content.

RÉSUMÉ

La teneur en ARN de coupes de pancréas qui respirent diminue de 30 à 50% au cours d'une incubation de 2 à 3 heures à 40° C *in vitro*. Cette diminution résulte probablement de l'action de la ribonucléase sur l'ARN des cellules endommagées. On ne l'observe pas dans des coupes de parotides de lapin incubées. La stimulation de la sécrétion enzymatique des coupes de pancréas de pigeon ou des coupes de parotides de lapin ne s'accompagne d'aucune modification significative de la concentration en ARN. La stimulation de la synthèse des enzymes dans les coupes de pancréas de pigeon par un mélange complet d'acides aminés n'est pas suivie non plus d'une modification de la teneur en ARN.

ZUSAMMENFASSUNG

Der RNA-Gehalt von *in vitro* bebrüteten respirierenden Pankreasschnitten nimmt bei einer Bebrütung bei 40° während 2–3 Stunden um 30–50% ab. Diese Abnahme ist wahrscheinlich der Einwirkung von Ribonuclease auf die RNA beschädigter Zellen zuzuschreiben. Bei Ohrspeicheldrüsenschnitten von Kaninchen tritt während der Bebrütung praktisch keine Abnahme des RNA-Gehaltes auf. Die Stimulierung der Enzymsekretion sowohl in Pankreasschnitten von Tauben wie in Ohrspeicheldrüsenschnitten von Kaninchen geht nicht einher mit irgendwelchen bedeutsamen Veränderungen der RNA-Konzentration. Die durch eine vollständige Aminosäure-mischung verursachte Stimulierung der Enzymsynthese in Pankreasschnitten von Tauben geht ebenfalls nicht parallel mit Veränderungen des RNA-Gehaltes.

REFERENCES

1 M. A. GUBERHIEV AND I. G. KOVYREV, *Doklady Akad. Nauk. S.S.S.R.*, 69 (1949) 889.
2 J. VERNE, *Bull. Hist. appl.*, 4 (1927) 110.
3 E. RIES, *Z. Zellforsch. mikr. Anat.*, 22 (1935) 523.
4 T. CASPERSSON, *Sym. Soc. expl. Biol.*, 1 (1947) 127.
5 J. BRACHET, *Sym. Soc. exp. Biol.*, 1 (1947) 207.
6 J. N. DAVIDSON AND C. WAYMOUTH, *Biochem. J.*, 38 (1944) 39.
7 L. E. HOKIN, *Biochem. J.*, 48 (1951) 320.
8 L. E. HOKIN, *Biochem. J.*, 50 (1951) 216.
9 M. RABINOVITCH, V. VALERI, H. A. ROTHSCHILD, S. CAMARA, A. SESSO AND L. C. U. JUNQUEIRA, *J. Biol. Chem.*, 198 (1952) 815.
10 M. M. DALY AND A. E. MIRSKY, *J. gen. Physiol.*, 36 (1952) 243.
11 M. DE DEKEN-GRENSON, *Biochim. Biophys. Acta*, 10 (1953) 488.
12 H. A. KREBS, *Biochim. Biophys. Acta*, 4 (1950) 249.
13 M. R. HOKIN AND L. E. HOKIN, *J. Biol. Chem.*, 203 (1953) 967.
14 W. C. SCHNEIDER, *J. Biol. Chem.*, 161 (1945) 293.
15 W. MEJBAUM, *Z. physiol. Chem.*, 258 (1939) 112.
16 W. DEUTSCH AND H. S. RAPER, *J. Physiol.*, 87 (1936) 275.
17 R. SCHUCHER AND L. E. HOKIN, *Abstr. Proc. XIX Intern. Physiol. Cong. Montreal* (1953) 744.
18 T. CASPERSSON, *Naturwissenschaften*, 29 (1941) 33.
19 T. CASPERSSON AND B. THEORELL, *Chromosoma*, 2 (1941) 132.
20 J. BRACHET, *Arch. Biol., Paris*, 53 (1942) 207.
21 L. SENTESSON AND T. CASPERSSON, *Acta radiol., Stockholm*, Suppl. 46 (1942).
22 B. MALMGREN AND C. HEDEN, *Acta path. microbiol. scand.*, 42 (1947) 417.
23 P. C. CALDWELL, E. L. MACKOR AND C. HINSHELWOOD, *J. Chem. Soc.*, (1950) 3151.
24 B. THORELL AND P. WISING, *Nord. Med. Ark.*, 24 (1944) 1842.
25 B. THORELL, *Exp. Cell. Res.*, Suppl. 1 (1949) 414.
26 J. N. DAVIDSON AND I. LESLIE, *Exp. Cell Res.*, 3 (1951) 367.

Received July 27th, 1953