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THE INCORPORATION OF ^{32}P INTO THE NUCLEOTIDES OF RIBONUCLEIC ACID IN PIGEON PANCREAS SLICES

by

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It was reported in a preliminary note to this Journal[1] that when enzyme secretion was stimulated in pigeon pancreas slices by cholinergic drugs there was 50–100% increase in the rate of incorporation of ^{32}P into the nucleotides of ribonucleic acid (RNA). In this earlier study the nucleotides were isolated by paper chromatography of the SCHMIDT-THANNHAUSER ribonucleotide fraction[2] in 70% *iso*propanol-water-ammonia[3]. After chromatography the nucleotide spots were cut out, pooled and eluted. It was subsequently found that when the ribonucleotides in the SCHMIDT-THANNHAUSER fraction were separated by ionophoresis on paper, only the uridylic acid showed an increased specific activity (about 100%) in the stimulated pancreas. While this work was in progress DAVIDSON AND SMELLIE[4] reported that when the SCHMIDT-THANNHAUSER ribonucleotide fraction from liver was separated by ionophoresis on paper the uridylic acid was contaminated with an unknown phosphorus compound ("Substance D") immediately preceding it. We therefore cut out the area of paper immediately preceding uridylic acid, eluted with ammonia and assayed the eluate for radioactivity. The total radioactivity in the eluted material derived from stimulated pancreas slices was in some cases 800% higher than that from unstimulated slices. We have found that the incorporation of ^{32}P into those phospholipids which are extractable with ethanol and 3:1 hot ethanol-ether is 500–1500% greater in pancreas slices stimulated with cholinergic drugs than in unstimulated slices[5]. It seems possible therefore that the stimulated contaminating material in the chromatographed or ionophoresed SCHMIDT-THANNHAUSER RNA fraction is derived from phospholipids not extracted with neutral organic solvents—possibly inositol phospholipids[6].

It became clear that traces of phosphate substances whose phosphate turnover is so markedly stimulated with cholinergic drugs and which remain in the SCHMIDT-THANNHAUSER fraction could account for the 50–100% increases in the specific activities of the chromatographically isolated nucleotides of RNA from stimulated pancreas slices. It was thus imperative to rid the nucleotides of all contamination, and this was achieved by a procedure based on that of DAVIDSON AND SMELLIE[4], which could be adapted to quantities of pancreas tissue as low as 100 mg fresh weight. In this procedure the sodium nucleate is isolated by extraction with NaCl and precipitation with ethanol prior to its hydrolysis with alkali to free nucleotides. The free nucleotides are then separated by ionophoresis on paper. The following results were obtained using this improved technique.

When amylase synthesis was stimulated over 100% by the addition of an appropriate mixture of amino acids[7] there was about a 20–40% increase in the specific activities of the nucleotides of RNA (Table I). However, there was also a 20–40% increase in respiration and the specific activities

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TABLE I

EFFECT OF AMINO ACID MIXTURES ON AMYLASE SYNTHESIS AND THE INCORPORATION OF ^{32}P INTO THE NUCLEOTIDES OF RNA

(80 minutes incubation at 40° C in bicarbonate saline containing glucose and pyruvate. Specific activities corrected to 100,000 counts/min/μg P for inorganic P in medium)

Amino acid mixture	*Amylase synthesis (units/mg dry wt.)*	*Specific activities (counts/min/μg P.)*				
		Acid-soluble phosphate esters	*Ribonucleotides*			
			Cytidylic	*Adenylic*	*Guanylic*	*Uridylic*
None	14.0	2,960	20.2	26.7	18.4	31.1
Complete	25.2	4,150	28.3	39.3	22.4	36.6
Without tryptophan	13.6	3,820	27.8	34.8	22.7	38.4

of the acid soluble phosphate ester fraction, suggesting that the increase in ^{32}P uptake in RNA was not specifically associated with protein synthesis. This was confirmed by an experiment, also shown in Table I, in which the omission of tryptophan from a complete amino acid mixture abolished the stimulatory effect of the amino acid mixture on amylase synthesis, but did not decrease the respiratory rate, the specific activity of the acid soluble phosphate ester fraction or the specific activities of the nucleotides of RNA.

When enzyme secretion was stimulated with cholinergic drugs in pancreas slices incubated in oxygenated saline containing glucose, sodium pyruvate and in some cases a mixture of 22 amino acids, there was no increase in the rate of incorporation of ^{32}P into the nucleotides of RNA (Table II). These observations indicate that the higher specific activity of the RNA from stimulated pancreas slices, previously reported by one of us[1] was in all likelihood due to the stimulation of the incorporation of ^{32}P into substances other than RNA, which are present in the SCHMIDT-THANNHAUSER fraction and which are not completely separated from the ribonucleotides either by chromatography or by ionophoresis.

TABLE II

EFFECT OF CARBAMYLCHOLINE ON AMYLASE SECRETION AND THE INCORPORATION OF ^{32}P INTO THE NUCLEOTIDES OF RNA

(Conditions as in Table I. (—) – No added carbamylcholine. (+) – Added carbamylcholine)

Time (min)	*Amylase in medium (units/mg dry wt.)*		*Specific activities of ribonucleotides (Counts/min/μg P)*							
			Cytidylic		*Adenylic*		*Guanylic*		*Uridylic*	
	—*	+	—	+	—	+	—	+	—	+
40	25	34	8.4	7.7	10.2	10.1	7.7	6.7	14.0	11.8
80	33	44	23.6	22.2	30.2	28.1	23.1	22.7	29.9	27.8
120	32	49	22.8	23.7	38.1	37.4	25.4	27.5	31.3	30.8

* Most of the enzyme found in the medium in the absence of carbamylcholine is not due to active secretion.

The experiments reported here fail to provide evidence of any link between enzyme synthesis or secretion and RNA metabolism; however, it should be emphasised that RNA may participate in these processes by mechanisms which are not metabolic in nature.

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