THE INCORPORATION OF ^{32}P INTO THE NUCLEOTIDES OF RIBONUCLEIC ACID IN PANCREAS SLICES DURING ENZYME SYNTHESIS AND SECRETION

by

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CASPERSSON[1] and BRACHET[2] and their collaborators have found that protein secreting cells (*e.g.*, the acinar cells of the pancreas and the chief cells of the stomach) and rapidly dividing cells (*e.g.*, growing bacteria, embryonic cells and tumour cells) contain high concentrations of ribonucleic acids (RNA). They have regarded these observations as evidence for a functional relationship between RNA and protein synthesis.

GUBERNIEV AND IL'INA[3] reported that when glandular secretion was stimulated in dogs by the intravenous injection of pilocarpine there were increases in the rates of incorporation of ^{32}P into the "nucleoproteins" (isolated by the method of SCHMIDT AND THANNHAUSER[4]) of pancreas (1200%), parotid (400%) and liver (500%). They assumed that the stimulation of enzyme secretion was accompanied by an increased rate of enzyme synthesis, and they thus regarded their findings as evidence for a parallel synthesis of nucleic acids and proteins in digestive glands—a suggestion which had been made earlier by LANDSTRÖM-HYDEN, AQUILONIUS AND CASPERSSON[5] from their cytological studies of the pancreas in different functional states.

Pancreas slices *in vitro* afford a means of studying enzyme synthesis and enzyme secretion separately under controlled conditions[6,7]. The present report deals with studies in this system of the relationship between the incorporation of ^{32}P into the nucleotides of RNA and enzyme synthesis and enzyme secretion.

EXPERIMENTAL

Preparation and incubation of tissues

Pigeons were either fasted for 48 hours or given food *ad lib.*, as indicated. When enzyme ynthesis was studied, 0.15 mg of carbamylcholine was injected intramuscularly 1 hour before killing. The slices were prepared and incubated as previously described[6,7,8]. Approximately 50 μC of $NaH_2{}^{32}PO_4$ was added to each vessel. Bicarbonate saline (KREBS AND HENSELEIT[9]) was used throughout. It was gassed with either 7% CO_2 in O_2 (aerobic experiments) or 7% CO_2 in N_2 (anaerobic experiments). Sodium pyruvate (0.02 M final concentration) and glucose (200 mg% final concen-

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tration) were added to all vessels. Enzyme secretion was stimulated by cholinergic drugs, as indicated. Enzyme synthesis was stimulated by a complete mixture of amino acids[7].

Isolation of the nucleotides of R.N.A.

After incubation the slices were ground with sand in 3 ml water and aliquots of the tissue suspension and medium were taken for amylase assays[7]. 2 ml of 20% trichloroacetic acid were then added to the tissue suspension. The acid soluble substances and the phospholipids were extracted from the residue as described previously[8]. In the earlier experiments the residue remaining after extraction of acid-soluble substances and lipids was hydrolysed in *M* KOH for 18 hours at 37°, according to the method of SCHMIDT AND THANNHAUSER[4]. The digest was neutralised in the cold with *M* $HClO_4$, and the supernatant was either chromatographed with 70% *iso*propanol-water-ammonia[10] or ionophoresed on paper. As described below, neither the chromatography nor the electrophoresis of this fraction completely removed all contaminating radioactive phosphorus from the nucleotides.

While this work was in progress DAVIDSON AND SMELLIE[11] described a method in which the ribonucleotides could be obtained free of all contaminating ^{32}P containing substances. We found this method applicable to quantities of pancreas tissue weighing as little as 100 mg. Unless otherwise specified all data presented here are from experiments using the method of DAVIDSON AND SMELLIE, in a slightly modified form, as follows.

The residue remaining after extraction of acid-soluble and lipid substances was extracted twice in 1 ml of 10% NaCl at 100° C. Two volumes of 95% ethanol were then added to the pooled extracts, and the mixture was kept at 0° C for about 30 minutes. The resulting white flocculent sodium nucleate precipitate was washed twice with 95% ethanol and dried *in vacuo*. The RNA was then hydrolysed in 0.2 ml of 0.3 *N* KOH at 37° for 18 hours. After hydrolysis the digest was acidified to about pH 2 with 0.15 *M* $HClO_4$ at 0° C. The desoxyribonucleic acid (DNA) and potassium perchlorate precipitate was removed by centrifugation, and the supernatant, containing the ribonucleotides, was adjusted to about pH 4 with 0.3 *N* KOH. The nucleotides were then ready for separation by ionophoresis on paper.

The method of ionophoresis combined features published by DAVIDSON AND SMELLIE[11], CONSDEN AND STANIER[12] and MARKHAM AND SMITH[10]. The nucleotides were applied as bands along the entire width of Whatmann No. 3 MM paper strips (10 cm × 57 cm), 16 cm from one end, under a stream of hot air from a commercial hair drier. The ribonucleotides obtained from 100–200 mg of tissue were applied to each strip. The ionophoresis apparatus consisted of two perspex troughs (58 cm long × 7½ cm wide × 10 cm high), which lay against the ends of two glass trays (33 cm long × 27 cm wide × 10 cm high), lying side by side. Carbon rod electrodes ran the full length of the troughs. The troughs contained 0.02 *M* citrate buffer, pH 3.5[11]. The trays were filled with monochlorobenzene[12]. The paper strips were wetted with buffer, gently blotted, and placed in glass frames similar to that described by CONSDEN AND STANIER[12]. The frames containing the strips were then submerged in the monochlorobenzene and the ends of the paper strips were placed in the buffer. About 1200 V were then applied from a high tension electronic power supply (maximum voltage 1400 V). Adequate separation was achieved in three hours.

After electrophoresis the strips were immediately removed and dried in a stream of warm air. The nucleotides were located by photography in the ultraviolet according to the method of MARKHAM AND SMITH[13]. The nucleotides appeared as four bands in the following order from the starting line: cytidylic, adenylic, guanylic and uridylic. The paper strips were superimposed over their photographs, and the edges of the nucleotide bands were marked lightly in pencil. The bands were then cut out.

The bands were cut into thin strips about 0.5 cm wide. The strips were superimposed over each other to form a rectangular column, which was pierced at one end and suspended directly under a burette. Molar ammonia was then dropped on to the paper column. The eluted material was collected in a graduated tube until a little over 1.5 ml had been collected. 0.5 ml of the eluate was diluted with molar ammonia to 4.0 ml for determination of the ribonucleotide concentration. For counting, a 1.0 ml sample was pipetted on to an aluminum disc (area 3.8 sq.cm) with slightly elevated edges and dried under an infra-red lamp.

Ultraviolet absorption spectra were determined for each nucleotide in molar ammonia; the wavelength of maximum absorption found for each nucleotide was as follows: cytidylic, 270 mμ; adenylic, 260 mμ; guanylic, 256 mμ; and uridylic, 259 mμ. The optical density of each sample was read at the maximum wavelength and at 290 mμ, a procedure previously employed by MAGASANIK *et al.*[14].

In several standard samples the difference in optical density at the two wavelengths (Δ) was divided by the phosphorus concentration (in μg/ml). By this method the nucleotide concentration in routine experiments could be expressed in terms of μg P per ml. The following values were obtained for Δ/μg P/ml: cytidylic, 0.231; adenylic, 0.464; guanylic, 0.347; and uridylic, 0.230.

All plates containing nucleotide samples were counted to $\pm$ 3% accuracy. No correction for self-absorption was required. A standard plate of the sample of inorganic ^{32}P added to the vessels was always counted. All specific activities were expressed as counts/min/μg P, and corrected to an

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initial specific activity of 100,000 counts/min/μg P for the inorganic P in the medium, except in Table I. The bicarbonate saline used contained 37.0 μg P/ml.

To test their radiopurity, the ribonucleotides obtained by the above method were chromatographed in 8 *N iso*propanol—2 *N* HCl in water (WYATT[15]). The specific activities remained constant.

The specific activities of the acid-soluble phosphate esters were also determined in some experiments, as described previously[8]. It was found that one treatment with magnesia reagent was sufficient to give constant specific activities.

In vivo mouse experiments

Albino mice which had been inbred in this Institute for the past two years were used. They were fed *ad lib.* with Purina Fox Chow.

Female mice, weighing about 25 g, were injected with 100 μC of $NaH_2{}^{32}PO_4$. The experimental group also received 1 mg of pilocarpine hydrochloride along with the $NaH_2{}^{32}PO_4$. Marked salivation, defecation and prostration resulted from this dose of pilocarpine. One hour later the animals were killed by decapitation, their pancreases were removed and immediately cooled to 0° C. The amylase contents and specific activities of the nucleotides of RNA and the specific activities of the acid soluble phosphate esters were determined as described above.

RESULTS

The incorporation of ^{32}P into the nucleotides of RNA in pancreas slices

It became apparent at the outset that the rate of incorporation of ^{32}P into the nucleotides of RNA in pancreas slices was quite low. It was therefore necessary to add considerable quantities of ^{32}P (of the order of 50 μC per vessel). Table I shows that the specific activities of the ribonucleotides were proportional to the amount of added ^{32}P within the range used experimentally (up to 80 μC/vessel). It was thus clear that the incorporation of ^{32}P into the nucleotides was not affected by the radiation emitted by the ^{32}P over the experimental period. The concentration of RNA was also not significantly altered when pancreas slices were incubated with as much as 140 μC/vessel for 2 hours.

TABLE I

EFFECT OF INCREASING QUANTITIES OF ^{32}P ON THE SPECIFIC ACTIVITIES OF THE NUCLEOTIDES OF RNA

Pancreas from fed pigeon. 80 minutes incubation in oxygenated saline, containing 200 mg% glucose and 0.02 *M* sodium pyruvate, at 40° C.

μC ^{32}P *vessel*	*Specific activities of ribonucleotides(counts/min/μg P)*			
	Cytidylic	*Adenylic*	*Guanylic*	*Uridylic*
10	25.2	30.6	23.1	38.8
20	62.2	78.4	51.7	87.2
40	112	150	87.6	163
80	210	290	189	314

In Table II are shown the increases with time in the specific activities of the various nucleotides of RNA. It will be noted that there was a lag in the rate of incorporation of ^{32}P into the ribonucleotides. This lag was not due to an initially slow rate of penetration of phosphate into the cells, since the rates of incorporation of ^{32}P into the acid soluble phosphate esters[8] or the phospholipids[8] in pancreas slices are linear from the beginning of incubation. The most probable explanation for this apparent lag is that it is an artifact due to an initial dilution of metabolically active RNA of intact cells by metabolically inert RNA of damaged cells. This RNA of damaged cells is broken

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down during the course of incubation[16] so that its diluting effect decreases with time.

In the experiment shown in Table II the rate of incorporation of ^{32}P into the ribonucleotides did not decrease to any significant extent over the three hour incubation period. On the other hand, in the experiment shown in Table VI the specific activities of the nucleotides of RNA showed little or no increase after 80 minutes. It should also be noted, however, that in this latter experiment the rates of incorporation of ^{32}P into the ribonucleotides during the first 40–80 minutes were about four times more rapid than in the experiment shown in Table II and that the final specific activities at 120 minutes were considerably higher than the specific activities at 160 minutes in the experiment of Table II.

TABLE II

RATES OF INCORPORATION OF ^{32}P INTO THE NUCLEOTIDES OF RNA IN PANCREAS SLICES

(Conditions as in Table I; all counts corrected to a specific activity of 100,000 counts/min/μg for inorganic P in medium)

Duration of incubation (min)	*Specific activities of ribonucleotides(counts/min/μg P)*			
	Cytidylic	*Adenylic*	*Guanylic*	*Uridylic*
40	1.15	1.30	1.01	1.66
80	4.17	5.14	3.94	6.24
120	9.46	12.7	9.38	13.5
160	13.7	18.0	12.5	17.2

The specific activities of the various nucleotides of RNA, after 80 minutes incubation, were not the same. The average ratios of the specific activities (guanylic being taken as 1.0) were: cytidylic, 1.06; adenylic, 1.45; guanylic, 1.00 and uridylic, 1.55. These ratios are in close agreement with those found by BOULANGER AND MONTREUIL[17] in the liver of the rat 60 minutes after injection of ^{32}P. These workers also found that the specific activity of the uridylic acid was highest during the first hour but was surpassed by the adenylic in the second hour. DAVIDSON AND SMELLIE[11] obtained similar ratios for the specific activities of the ribonucleotides in rat liver 2 hours after injection of ^{32}P.

The incorporation of ^{32}P into the nucleotides of RNA under anaerobic conditions

It has been previously shown that endergonic processes such as amylase synthesis and amylase secretion are abolished when pancreas slices are incubated anaerobically[6]. As is shown in Table III, the incorporation of ^{32}P into the nucleotides of RNA was inhibited about 90–95% under anaerobic conditions. The incorporation of ^{32}P into RNA seems therefore to be largely dependent upon energy derived from respiratory processes. It will be observed in Table III that the specific activities of the acid-soluble phosphate esters were inhibited only about 70–80%; this proportionately smaller inhibition of the turnover of the acid-soluble phosphate esters is probably due to the presence of intermediates of glycolysis in the acid-soluble phosphate ester fraction.

The effect of amino acids on amylase synthesis and the rate of incorporation of ^{32}P into the nucleotides of RNA

The synthesis of amylase, lipase and ribonuclease can be stimulated in pancreas slices by the addition of a complete mixture of amino acids to the incubation medium[6,18].

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TABLE III

EFFECT OF ANAEROBIC INCUBATION ON INCORPORATION OF ^{32}P INTO THE NUCLEOTIDES OF RNA IN PANCREAS SLICES

(Pigeons fasted 48 hours; 80 minutes incubation in saline, containing 200 mg% glucose and 0.02 *M* sodium pyruvate, at 40° C. All counts corrected to 100,000 counts/min/μg P for inorganic P in medium)

Exp. No.	*Gas phase*	*Specific activities (counts/min/μg P)*				
		Acid-soluble phosphate esters	*Ribonucleotides*			
			Cytidylic	*Adenylic*	*Guanylic*	*Uridylic*
1	7% CO_2 in O_2	2520	13.7	20.7	13.0	27.9
	7% CO_2 in N_2	720	2.2	2.8	1.4	4.8
2	7% CO_2 in O_2	3120	18.2	29.2	14.6	35.5
	7% CO_2 in N_2	730	1.1	1.5	0.9	2.5

TABLE IV

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

(80 minutes incubation; conditions as in Table I. 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

Experiments were undertaken in an attempt to discover whether the stimulation of enzyme synthesis by an appropriate mixture of amino acids would be accompanied by an increase in the rate of incorporation of ^{32}P into the nucleotides of RNA. In these experiments amylase was measured as an example of general enzyme synthesis. In Table IV are shown the results of a representative experiment, in which amylase synthesis and the specific activities of the ribonucleotides and the acid-soluble phosphate ester fraction were measured after 80 minutes' incubation under the following conditions: (1) no added amino acids, (2) 22 amino acids added, and (3) tryptophan omitted from the complete amino acid mixture. It should be pointed out that the omission of tryptophan from the complete amino acid mixture completely abolishes the stimulatory effects of the amino acid mixture on amylase synthesis[7].

An approximate doubling of the rate of amylase synthesis by the addition of the complete amino acid mixture was accompanied by a 20–40% increase in the specific activities of the nucleotides of RNA. The specific activities of the acid-soluble phosphate esters were increased to the same extent. In other experiments, using Medium III of KREBS, respiration was found to be increased in the presence of the amino acid mixture to the same extent as the specific activities of the acid-soluble phosphate ester fraction. These observations suggest that the stimulation of the incorporation of ^{32}P into the

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nucleotides of RNA by the amino acid mixture was secondary to an increased metabolism of the tissue, rather than being due to any link between RNA metabolism and protein synthesis. This view is further supported by the observation that the omission of tryptophan from the complete amino acid mixture abolished the stimulatory effect of the amino acid mixture on amylase synthesis, but did not decrease the specific activity of the acid-soluble phosphate ester fraction or the specific activities of the nucleotides of RNA (Table IV). Respiration was also unaffected by the omission of tryptophan.

Effect of carbamylcholine on amylase secretion and the rate of incorporation of ^{32}P into the nucleotides of RNA

The secretion of amylase, lipase and ribonuclease can be stimulated in pancreas slices by the addition of a cholinergic drug such as acetylcholine (with eserine) or carbamylcholine[6,18]. Stimulation of enzyme secretion is not accompanied by increased enzyme synthesis[6,18].

It was reported in a preliminary note[19] that the specific activities of the pooled nucleotides of RNA, obtained by chromatography of the SCHMIDT-THANNHAUSER ribonucleotide fraction, were 50–100% higher in pancreas slices stimulated to secrete enzymes by the addition of carbamylcholine. When the nucleotides in this fraction were separated by ionophoresis on paper, only the uridylic acid showed an increased specific activity, ranging from 50–150%. While this work was in progress DAVIDSON AND SMELLIE[11] reported that when the SCHMIDT-THANNHAUSER ribonucleotide fraction from liver was separated by ionophoresis on paper the uridylic acid was contaminated by 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 the stimulated pancreas slices was in some cases as much as 800% higher than that from unstimulated slices.

When the ribonucleotides were isolated by the method of DAVIDSON AND SMELLIE, as slightly modified here, the specific activities of cytidylic, adenylic and guanylic acids were essentially the same as after ionophoresis of the SCHMIDT-THANNHAUSER ribonucleotide fraction. However, the specific activity of the uridylic acid was lower, and it was not increased in the tissue stimulated to secrete with carbamylcholine. An example of the results obtained by ionophoresis after SCHMIDT-THANNHAUSER fractionation and after DAVIDSON-SMELLIE fractionation of the same samples of RNA is shown in Table V.

TABLE V

SPECIFIC ACTIVITIES OF THE RIBONUCLEOTIDES ISOLATED BY ELECTROPHORESIS OF SCHMIDT-THANNHAUSER AND DAVIDSON-SMELLIE FRACTIONS

Pancreas from fed pigeon. Conditions as in Table I. (−) = No added carbamylcholine; (+) = added carbamylcholine (1 mg% final concentration).

Fractionation procedure	*Specific activities of ribonucleotides (counts/min/μg P)*								*Total activity of "Substance D" (counts/min)*	
	Cytidylic		*Adenylic*		*Guanylic*		*Uridylic*			
	−	+	−	+	−	+	−	+		
SCHMIDT-THANNHAUSER	10.0	10.0	16.1	16.3	7.7	8.1	22.1	33.8	23	162
DAVIDSON-SMELLIE	7.7	8.0	18.3	14.3	7.5	7.3	11.1	11.8		

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The above observations indicate that the higher specific activity of the RNA from stimulated pancreas slices, previously reported by one of us, was in all likelihood due to the stimulation of the incorporation of ^{32}P into substances other than RNA, which were present in the SCHMIDT-THANNHAUSER ribonucleotide fraction and which were not completely separated from the ribonucleotides either by the method of chromatography or ionophoresis used. 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[8]. The contaminating material in the chromatographed or ionophoresed SCHMIDT-THANNHAUSER RNA fraction may therefore be derived from phospholipids not extracted with neutral organic solvents. FOLCH[20] has shown that inositol phospholipids in brain are not extracted with neutral organic solvents, but liberate inositol diphosphate on incubation with alkali for 18 hours at 37° C. They would thus be present in the SCHMIDT-THANNHAUSER ribonucleotide fraction. It is noteworthy that pancreas contains the greatest amounts of inositol phosphatides per mole of phospholipid phosphorus of all tissues analysed by TAYLOR AND MCKIBBIN[21].

TABLE VI

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

(Conditions as in Table I. (—) = No carbamylcholine; (+) = carbamylcholine added (1 mg% final concentration).

Exp. No.	*Nutritional state*	*Incubation time*	*Further additions*	*Amylase in medium (units/mg dry wt.)*		*Specific activities of ribonucleotides (counts/min/μg P)*							
						Cytidylic		*Adenylic*		*Guanylic*		*Uridylic*	
				—	+	—	+	—	+	—	+	—	+
1	Fed	40	None	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
2	Fasted 48 hours	80	None	104	148	12.4	14.6	17.7	18.7	10.7	12.2	18.6	21.6
3	Fasted 48 hours	80	Complete amino acid mixture	50	65	13.8	14.4	21.3	20.0	12.9	12.5	21.4	22.0

In Table VI are shown the results of several experiments in which enzyme secretion was stimulated with carbamylcholine under various conditions. There was no increase in the specific activities of the nucleotides of RNA when carbamylcholine was added to pancreas slices from fed or fasted pigeons, in the presence or absence of amino acids. These experiments indicate that stimulation of enzyme secretion *in vitro* is not accompanied by increased ^{32}P turnover in RNA. It should be pointed out that in pancreas slices from pigeons which had been fasted 48 hours or longer carbamylcholine or acetylcholine occasionally inhibited the incorporation of ^{32}P into the nucleotides of RNA as much as 50%. The incorporation of ^{32}P into the acid-soluble phosphate esters was inhibited to the same extent. Respiration was not affected. ABOOD AND GERARD[22] found that parasympathomimetic drugs inhibit oxidative phosphorylation in rat brain and spinal cord *in vitro*.

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The effect of pilocarpine on enzyme secretion and the incorporation of ^{32}P into the nucleotides of RNA in mouse pancreas in vivo

Enzyme secretion in pigeon pancreas is not markedly stimulated *in vitro*: 10–30 units of amylase per mg dry weight are usually discharged in response to cholinergic drugs. Fasted glands may contain as much as 400 units per mg dry weight. GUBERNIEV'S AND IL'INA'S finding of a 1200% increase in the incorporation of ^{32}P into the nucleoprotein of dog pancreas following injection of pilocarpine may have been due to a much greater stimulation of enzyme secretion *in vivo*. We therefore studied the effects of pilocarpine on the incorporation of ^{32}P into the nucleotides of RNA in mouse pancreas *in vivo*. The results of these experiments are shown in Table VII. A considerable variation in the specific activities of the acid-soluble phosphate ester fraction and the ribonucleotides was observed in pancreases from control mice; this was probably due to variations in the quantity of ^{32}P reaching the pancreas in different mice. However, if the specific activities of the ribonucleotides were expressed as per cent of the specific activity of the acid-soluble phosphate ester fraction, they showed good agreement in pancreases from different control mice. In Table VII the specific activities of the ribonucleotides are expressed in this manner, referred to as "relative specific activity". When enzyme secretion was stimulated in the pancreas by the intraperitoneal injection of 1 mg of pilocarpine there was an approximate 45% decrease in the relative specific activities of the nucleotides of RNA 1 hour after injection of $NaH_2{}^{32}PO_4$. The injection of pilocarpine resulted in the extrusion of approximately two-thirds of the amylase from the pancreas. The relative specific activities of the ribonucleotides shown in Table VII are from the linear portion of the specific activity-time curve: in separate experiments the specific activities of the nucleotides of RNA were found to level off about 90 minutes after injection of ^{32}P.

TABLE VII

EFFECT OF PILOCARPINE ON ENZYME SECRETION AND THE INCORPORATION OF ^{32}P INTO THE RIBONUCLEOTIDES OF RNA OF MOUSE PANCREAS *in vivo*

(Approx. 100 μC $NaH_2{}^{32}PO_4$ injected intraperitoneally 1 hour before killing. 1 mg pilocarpine injected with $NaH_2{}^{32}PO_4$ as indicated. Relative specific activities of ribonucleotides are expressed as counts/min/μg P of ribonucleotides ÷ counts/min/μg P of acid-soluble phosphate esters × 100. Values are from 6 individual mice).

Drug injected	*Tissue amylase (units/mg dry wt.)*	*Relative specific activities of ribonucleotides*			
		Cytidylic	*Adenylic*	*Guanylic*	*Uridylic*
None	122	0.95	0.96	0.76	1.15
	148	0.83	0.86	0.74	1.11
	117	1.07	1.12	0.91	1.30
Pilocarpine	45.6	0.58	0.58	0.52	0.82
	50.0	0.55	0.55	0.49	0.81
	40.0	0.48	0.48	0.34	0.60

The decreases in the relative specific activities of the nucleotides of RNA may be due to actual increases in the specific activities of the acid-soluble phosphate esters, although this was not apparent in our experiments; however, the wide scatter in the specific activities of the acid-soluble phosphate ester fraction could have obscured this effect. Our findings certainly indicate that at least in the mouse the stimulation of

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enzyme secretion is not accompanied by increases in the incorporation of ^{32}P into RNA The findings of GUBERNIEV AND IL'INA[3] that the incorporation of ^{32}P into the nucleoprotein of dog pancreas is increased 1200% following injection of pilocarpine may have been due to the presence in their nucleoprotein fraction of phosphate substances, other than RNA, whose turnover is markedly stimulated by cholinergic drugs, as we have found to be the case in the SCHMIDT-THANNHAUSER RNA fraction of pigeon pancreas slices. It seems unlikely that the turnover of DNA, which would also presumably be present in their nucleoprotein, would vary with physiological changes in the cytoplasm, but contaminating phospholipids or derivatives from them could account for their results.

DISCUSSION

The present experiments indicate that the stimulation of enzyme secretion *in vitro* or *in vivo* is not accompanied by an increased rate of RNA synthesis or turnover. Stimulation of enzyme secretion in mouse pancreas *in vivo* by injection of pilocarpine is actually accompanied by an approximate 50% decrease in the specific activity of RNA relative to that of the acid-soluble phosphate ester fraction.

The experiments reported here also indicate that enzyme synthesis is not paralleled by RNA synthesis or by a turnover of phosphate in preformed RNA. In a preliminary note, DE DEKEN-GRENSON[23] recently reported that the incorporation of ^{32}P into RNA was not increased in mouse pancreas when enzyme synthesis was presumably increased by stimulation of enzyme secretion several hours before injection of ^{32}P. HAMMARSTON *et al.*[24], found that the incorporation of glycine-^{15}N into the purines of the polynucleotides in regenerating bone marrow and regenerating liver of hens was paralleled by the uptake of this amino acid into protein. He thought this correlation indicated a "general tendency to simultaneous metabolic activity of polynucleotides and protein". In growing cells this parallelism between nucleic acid synthesis and protein synthesis is not surprising, since all cellular constituents are being rapidly synthesised during growth.

Our experiments do not exclude the possibility that RNA plays a role in protein synthesis. They do suggest that if this role exists it does not involve a turnover of phosphate in preformed RNA or a synthesis of RNA. The observations of SWENSON AND GIESE[25] and SWENSON[26] strongly suggest that nucleic acids are in fact concerned in protein synthesis. These investigators found that adaptive enzyme formation is inhibited by ultraviolet light, and the magnitude of this inhibition at different wavelengths parallels the ultraviolet absorption of nucleic acids. These findings are compatible with a structural rather than a metabolic role for nucleic acids in protein synthesis; *i.e.*, nucleic acids may determine the specificity of the newly formed proteins by directing the arrangement of amino acids in the protein. It has been suggested that nucleic acids may do this by acting as templates for the alignment of amino acids in patterns (HAUROWITZ[27]; CALDWELL AND HINSHELWOOD[28]; CHANTRENNE[29]; DOUNCE[30]). We wish to suggest an alternative view: nucleic acids may direct the arrangement of amino acids in the proteins by acting as frameworks for the alignment of peptidases in specific patterns. The resultant patterns of peptidases in turn catalyse the synthesis of proteins. We favour this hypothesis for several reasons. In the first place, reversible salt-like combinatons between nucleic acids and proteins (or enzymes) have been demonstrated. OHLMEYER[31] has arranged a series of proteins in a spectrum depending on their affinity for DNA.

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A similar combination between nucleic acids and amino acids has not been shown. Secondly, it is known that the ribonucleic acids of cytoplasmic organelles, such as microsomes and mitochondria, exist in close association, or possibly in combination, with enzymes. The organization of enzymes in these cytoplasmic particles may thus be dependent on a matrix of ribonucleic acids. Thirdly, the combination between nucleic acids and enzymes affords greater possibilities for specificity than the alignment of amino acids on nucleic acid templates.

The experiments of JACOBS, TORRIANI AND MONOD[32] offer indirect support for the above concept. These investigators found that the ability of cultures of *E. coli* to adaptively synthesise β-galactosidase was destroyed by ultraviolet light, but the organisms were still capable of synthesising bacteriophage when the latter were introduced into the irradiated bacterial suspension. These observations could be explained by assuming that in the adapted bacterium certain specific nucleic acids organise appropriate patterns of peptidases which catalyse the formation of β-galactosidase. Ultraviolet light could alter the structure of these nucleic acids and consequently the pattern of peptidases. When the phage, which is a new nucleoprotein framework, is introduced, the host enzymes are organised to synthesise phage.

The concept that nucleic acids organise patterns of enzymes which catalyse the synthesis of specific proteins can be generalised to include the synthesis of other polymers such as nucleic acids and type-specific polysaccharides. This mechanism may thus be a means by which genes, viruses and the "pneumococcus transforming factor" replicate themselves. In the case of virus synthesis, COHEN[33], on the basis of the kinetics of virus synthesis, has already suggested that viruses organise host enzymes for their own reproduction. There is additional evidence that viruses may reproduce themselves in this manner. Viruses which have been inactivated by ultraviolet light inhibit the synthesis of viruses of the same strain (LURIA AND DELBRUCK[34]). This inhibition could be explained as being due to a competition between viable virus and inactivated virus for host enzymes. Different strains of influenza virus also interfere with each others' synthesis (ZIEGLER, LAVIN AND HORSFALL[35]; HENLE AND HENLE[36]; FITZGERALD AND LEE[37]). Lysine polypeptides inhibit the multiplication of tobacco mosaic virus, and this inhibition involves a salt-like combination between the acid groups of the virus and lysine polypeptide (BURGER AND STAHMAN[38]). This inhibition could be due to a competition between the lysine polypeptide and the host enzymes for sites on the nucleic acid framework.

In the case of the desoxyribonucleic acids with transforming properties, the desoxyribonucleic acids may organise enzymes in the recipient bacteria in patterns capable of catalysing the synthesis of the type-specific polysaccharides of the donor bacteria as well as more of the original desoxyribonucleic acids.

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SUMMARY

The incorporation of ^{32}P into the nucleotides of RNA has been followed in pigeon pancreas slices under a variety of conditions. This incorporation is inhibited 90–95% anaerobically. Stimulation of enzyme synthesis about 100% by the addition of a complete amino acid mixture is accompanied by 20–40% increases in respiration and the specific activities of RNA and acid-soluble phosphate esters. Omission of tryptophan abolishes the stimulatory effect of the complete amino acid mixture on amylase synthesis but has no effect on respiration or the turnover of RNA or acid-soluble phosphate esters. These observations suggest that the slight stimulation of ^{32}P incorporation into RNA by the complete amino acid mixture is due to the stimulation of general metabolism by the amino acid mixture, rather than to a link between enzyme synthesis and RNA synthesis. Stimulation of enzyme secretion *in vitro* with carbamylcholine or acetylcholine is not accompanied by any increase in the rate of incorporation of ^{32}P into RNA. Stimulation of enzyme secretion in mouse pancreas *in vivo* is accompanied by an approximate 50% decrease in the specific activity of RNA relative to that of the acid-soluble phosphate esters.

A possible mechanism whereby nucleic acids may function in the synthesis of proteins and other specific polymers (*e.g.* nucleic acids and type-specific polysaccharides) is discussed.

RÉSUMÉ

L'incorporation de ^{32}P dans les nucléotides de l'ARN de coupes de pancréas de pigeon a été étudiée dans diverses conditions. Cette incorporation est inhibée à 90 ou 95% en an aérobiose. En augmentant de 100% la synthèse des enzymes par l'addition d'un mélange complet d'aminoacides, on augmente la respiration et les activités spécifiques de l'ARN et des esters phosphoriques acides solubles d'environ 20 à 40%. L'omission du tryptophane supprime l'effet stimulant du mélange d'acides aminés sur la synthèse de l'amylase mais n'a pas d'effet sur la respiration ou le turnover de l'ARN et des esters phosphoriques acides solubles. Ces observations suggèrent que le faible stimulation de l'incorporation de ^{32}P dans l'ARN par le mélange d'acides aminés complet est due à la stimulation du métabolisme général par le mélange d'acides aminés, et qu'en outre la synthèse des enzymes n'est pas liée à la synthèse de l'ARN. La stimulation *in vitro* de la sécrétion enzymatique par la carbamylcholine ou l'acétylcholine n'est suivie d'aucune augmentation de la vitesse d'incorporation de ^{32}P dans l'ARN. La stimulation *in vivo* de la sécrétion des enzymes du pancréas de souris s'accompagne d'une diminution d'environ 50% de l'activité spécifique de l'ARN par rapport à celle des esters phosphoriques acides solubles.

Un mécanisme possible, où des acides nucléiques auraient une fonction dans la synthèse de protéines et d'autres polymères spécifiques (p. ex. acides nucléiques et polysaccharides type-spécifiques) est discuté.

ZUSAMMENFASSUNG

Der Einbau von ^{32}P in die Nucleotide der RNA in Pankreasschnitten von Tauben wurde unter den verschiedensten Bedingungen verfolgt. Bei anaeroben Bedingungen wird der Einbau zu 90–95% gehemmt. Die durch Zugabe einer vollständigen Aminosäuremischung um 100% stimulierte Enzymsynthese steigert die Respiration und die spezifischen Aktivitäten der RNA und der säurelöslichen Phosphorsäureester um ungefähr 20–40%. Weglassen von Tryptophan hebt die stimulierende Wirkung der vollständigen Aminosäuremischung auf die Amylasesynthese auf, zeigt aber keine Wirkung auf die Respiration oder "turnover" der RNA oder die säurelöslichen Phosphorsäureester. Diese Beobachtungen lassen vermuten, dass die geringe, durch die vollständige Aminosäuremischung verursachte Stimulierung des ^{32}P-Einbaus in RNA der Stimulierung des allgemeinen Stoffwechsels durch die Aminosäuremischung zuzuschreiben ist und sie lassen weiter vermuten, dass die Enzymsynthese nicht mit der RNA-Synthese verbunden ist. Die Stimulierung der Enzymsekretion mit Carbamylcholin oder Acetylcholin *in vitro* geht nicht mit irgendeinem Ansteigen der Geschwindigkeit des Einbaus von ^{32}P in RNA einher. Die Stimulierung der Enzymsekretion im Pankreas von Mäusen *in vivo* geht mit einer Abnahme der spezifischen Aktivität der RNA um ungefähr 50% bezüglich zu der der säurelöslichen Phosphorsäureester einher.

Ein möglicher Mechanismus, wobei Nucleinsäuren bei der Synthese von Proteinen und anderen spezifischen Polymeren (z.B. Nucleinsäuren und typenspezifischen Polysacchariden) fungieren, wird besprochen.

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