Effects of Pancreozymin, Urecholine and Actinomycin D on the Metabolism of Ribonucleic Acid by Canine Pancreas

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1. Canine pancreas slices were incubated with [6-¹⁴C]orotic acid and the rate of its incorporation into RNA was measured. RNA was fractionated by shaking homogenates with phenol at 2°, 50°, 65° and 80°. Cytoplasmic RNA was extracted at the lowest temperature and nuclear RNA at the higher temperatures. The samples were centrifuged through sucrose gradients and the E_{260} and ¹⁴C-sedimentation patterns determined. Incorporation of orotic acid was very rapid into cytoplasmic 4s RNA. This probably represents end-group turnover. No incorporation into cytoplasmic ribosomal RNA was observed. 2. The nuclear 50°-RNA exhibited two E_{260} peaks, at 18s and 28s. This portion of the sample contained but moderate amounts of [¹⁴C]RNA. The highly labelled material had sedimentation coefficients in the range 35–50s. The nuclear 65°-RNA showed an E_{260} peak at 16s. The [¹⁴C]RNA peak occurred at 25–35s and this portion demonstrated the highest specific activity of any RNA fraction. 3. The 50°-RNA, 65°-RNA and 80°-RNA were hydrolysed and their base compositions were determined. All three samples possess a ribosomal type of composition $(G + C)/(A + U) = (1.4-1.7)$. For this reason they are considered to contain ribosomal precursor RNA as their major constituent. 4. Actinomycin D (0.5 µg./ml.) in the incubation medium inhibited incorporation of orotic acid into both nuclear fractions but not into 4s RNA. 5. The cholinergic drug Urecholine inhibited incorporation into the heavy, high-specific-activity portions of the nuclear fractions but did not inhibit incorporation into the ribosomal precursor type of nuclear RNA. A similar result was also obtained with the hormone pancreozymin. Moderate inhibition of incorporation of orotic acid into 4s RNA likewise resulted from the presence of the drug and the hormone.

The pancreas of mammalian species has long been known to synthesize proteins at a high rate. These proteins are contained in the zymogen granules of the exocrine portion of the gland and are discharged into the pancreatic juice as the result of hormonal, cholinergic drug, or nervous stimulation (Babkin, 1950). Information about events subsequent to stimulation is due largely to the work of Hokin & Hokin (1954, 1956) and can be summarized as follows. (1) The rate of protein synthesis remains relatively constant as judged from the amylase content of pigeon pancreas slices which had been treated with pancreozymin (Hokin & Hokin, 1956). Similar results were obtained by Morris & Dickman (1960) in mice which had been injected with pilocarpine. (2) The turnover of phospholipid is rapidly accelerated as judged by the incorporation of [³²P]phosphate into this fraction (Hokin & Hokin, 1956). (3) The rate of incorporation of [³²P]phosphate into RNA is

unaffected (pigeon pancreas slices), or decreased by 50% relative to that of acid-soluble nucleotides (mouse pancreas *in vivo*) (Hokin & Hokin, 1954). Recent developments in the fractionation of RNA prompted us to investigate the effects of two stimulants, Urecholine [*N*-(2-carbamoyloxypropyl)-*NNN*-trimethylammonium (chloride)] and pancreozymin, on the incorporation of [6-¹⁴C]orotic acid into nuclear and cytoplasmic RNA. The DNA-dependent RNA-polymerase inhibitor, actinomycin D, was included for comparative purposes. Canine pancreas was selected for the present study because of its relatively large size and its low content of ribonuclease.

There is good evidence for the hypothesis that most RNA is produced in the nucleus of cells (Smellie, 1963). Consequently it was thought desirable to separate this organelle from the cytoplasm before fractionation. Nuclear RNA has been subdivided into two major types: ribosomal-like

(R-RNA), and DNA-like (D-RNA) (Georgiev, Samarina, Lerman, Smirnov & Severtzov, 1963). The R-RNA fraction of a number of tissues was shown to sediment through a sucrose gradient with sedimentation coefficients 18s and 28s. D-RNA showed $S_{20,w}$ about 18s, and the low-molecular-weight material $S_{20,w}$ 4–8s. Rats injected with [^{32}P]phosphate formed D-RNA with about twice the specific activity of R-RNA on fractionation of the liver 30 min. later (Georgiev *et al.* 1963). Because of this rapid uptake of precursor, the D-RNA fraction was postulated to contain considerable amounts of messenger RNA (m-RNA).

METHODS AND MATERIALS

Incubation. Freshly excised canine pancreas was placed immediately in ice-cold 0.25M-sucrose, then trimmed and cut free-hand with a razor blade into slices 0.8–1.0 mm. thick. The average length was 2 cm. The slices were placed in a beaker which contained chilled Krebs III medium (Krebs, 1950), 1 g./10 ml. Each flask was quickly warmed to 25°, then preincubated at 37° in a Dubnoff shaker for 10 min. The hormone or drugs or both were added at the beginning of the preincubation. [$6\text{-}^{14}\text{C}$]Orotic acid or [$8\text{-}^{14}\text{C}$]adenine was added to a final concentration of 1 $\mu\text{C}/\text{ml}$. and the incubation was carried out at 37° for the period indicated.

Homogenization. At the end of the incubation the mixtures were filtered through two layers of cheese-cloth and the tissue was well rinsed with cold 0.14M-NaCl. All subsequent operations were performed at 2–4°. The slices were finely cut with scissors and then homogenized with 10 vol. of 0.14M-NaCl in a Potter-Elvehjem homogenizer fitted with a Teflon pestle.

Isolation of RNA. The homogenates were shaken for 15 min. at 4° with an equal volume of redistilled phenol, saturated with 0.14M-NaCl, pH 6.0 (Georgiev *et al.* 1963). The solutions were centrifuged at 1700g for 25 min. and the aqueous layer was collected. The phenol was discarded and the intermediate layer was treated twice with phenol as described above. The interphase layer consists of nuclei coated with phenol and the combined aqueous layer contains cytoplasmic RNA.

The phenolic nuclei were suspended in 1.5 ml. of 0.14M-NaCl/g. of original tissue. An equal volume of phenol was added and the mixture extracted twice at 50° for 20 min. with stirring. The suspensions were centrifuged at 9800g for 15 min. The RNA of the combined aqueous layer is termed 50°-RNA. The interphase layer was extracted twice more at 65° as described above. The RNA of this aqueous layer is termed 65°-RNA. Two additional extractions of the interphase layer at 80° led to the isolation of material which is termed 80°-RNA. All the RNA samples were precipitated by the addition of 3 vol. of cold 95% ethanol, stored at –20° and centrifuged. Each sample was redissolved in 0.1M-acetate and reprecipitated with ethanol. The samples were stored at –20° until analysis.

Sedimentation through sucrose gradients. Sucrose gradients (5–20%) in 0.1M-acetate, pH 6.0, were prepared in the cold with the device of Britten & Roberts (1960). The samples of RNA (0.5–1.0 mg.) in 100–200 μl . of 0.1M-acetate were carefully layered on the sucrose. They were centrifuged in

the SW 39 rotor of a Beckman-Spinco model L centrifuge at 39000 rev./min. for 210 min. Calculations of $S_{20,w}$ values were based on the equation of Martin & Ames (1961).

Ultraviolet and ^{14}C analyses. The solutions were displaced from the tubes by 90% glycerol, which was introduced at a constant rate into the bottom of the tubes through a needle. The sucrose solutions were passed through a flow cell (0.2 cm. light-path) placed in a monochromator set at 260 m μ and equipped with a Gilford automatic recording attachment. When ^{14}C analyses were desired, the effluent was collected in 23 equal portions in glass vials. To each was added 1 ml. of 1M-Hyamine in methanol, 2 ml. of absolute ethanol and 10 ml. of toluene which contained 0.6% 2,5-diphenyloxazole and 0.01% 1,4-bis-(5-phenyloxazol-2-yl)-benzene. Radioactivity was measured in a Nuclear-Chicago automatic liquid-scintillation spectrometer.

A co-precipitation of [^{14}C]RNA and sucrose occurred in an ethanol-toluene mixture. This phenomenon resulted in a marked decrease in counting efficiencies at sucrose concentrations above 5%. The addition of Hyamine maintained a constant efficiency of counting of [^{14}C]RNA at all concentrations of sucrose used in this work even though most of the sucrose crystallized on the bottom of the vial.

Analytical procedures. DNA was determined by the method of Ceriotti (1952), and protein by the procedure of Nayyar & Glick (1954). RNA was estimated in homogenates which were treated according to Hallinan, Fleck & Munro (1963). The concentration of RNA in the final acid supernatant solution was calculated by $E_{260 \text{ m}\mu}^{1 \text{ cm}} = 20.7 \text{ cm}^3/\text{mg}$.

Materials. All chemicals were of reagent grade. Actinomycin D and Urecholine were obtained from Merck, Sharpe and Dohme, Rahway, N.J., U.S.A. Pancreozymin (Cecekin) was purchased from Vitrum, Akt. Stockholm, Sweden. [$6\text{-}^{14}\text{C}$]Orotic acid (20–30 mC/m-mole) and [$8\text{-}^{14}\text{C}$]adenine (12 mC/m-mole) were bought from Nuclear-Chicago Corp., Des Plaines, Ill., U.S.A.

RESULTS

Intracellular distribution of nucleic acids after incubation of pancreas slices

Many factors might influence the amount of a nucleic acid in a particular fraction under conditions of incubation of tissue slices. Therefore quantitative analyses for DNA and RNA were performed on the nuclear material and for RNA on the cytoplasmic extracts. The data set out in Table 1 demonstrate that recovery of DNA and the various fractions of RNA remains relatively constant for incubation periods up to 2 hr. in duration. There is no indication of any large loss of nucleic acid due to nuclease action or marked shifts in nuclear RNA from one fraction to another.

Analysis of nuclear fractions. The RNA, DNA and protein content of nuclear fractions obtained by extraction of 'phenol nuclei' at three temperatures, 50°, 65° and 80°, is presented in Table 2. At 50°, only RNA is solubilized; at 65° a small amount of DNA also comes into solution. The 80° fraction

contained large amounts of DNA and protein in addition to RNA. Two to three times as much RNA was extracted at 50° as at 65° or 80°.

Base composition. Phenol nuclei were extracted at 50°, 65° and 80° and the RNA fractions were purified by the procedure of Lerman, Mantieva & Georgiev (1964). This accomplishes further removal of protein and DNA by treatment of the RNA with sodium dodecylsulphate, CHCl_3 and precipitation with 2.5M-NaCl. After hydrolysis in 0.3N-KOH at 37° for 18hr., the acidified solutions were placed on columns of Dowex 50 (H^+ form) and the nucleotides eluted with HCl, followed by water (Katz & Comb, 1963). Similar results were obtained with the technique of Cohn (1960). In contrast with the results of Georgiev *et al.* (1963) on rat-liver nuclear RNA the pancreas fractions were quite similar in base composition (Table 3). The high $(\text{G} + \text{C})/(\text{A} + \text{U})$ ratios suggest that the fractions may be considered as precursors of ribosomal RNA. The amounts of any DNA-like RNA appear to be very low in these samples. Because of this similarity in composition, they are referred to below as the 50°-RNA, the 65°-RNA and the 80°-RNA respectively.

Incorporation of [^{14}C]orotic acid into RNA fractions

Cytoplasmic RNA. The sedimentation patterns of RNA extracted at low temperature from slices which had been incubated for 30, 60 and 120min. are shown in Fig. 1(a). The labelled RNA occurs exclusively in the region 4–8s. This high rate of incorporation into transfer RNA has been frequently observed (Tamaoki & Mueller, 1962; Hiatt, 1962). Turnover of the CCA end groups is probably responsible for most of this incorporation (Herbert, 1959; Moulé & Landin, 1965). The complete lack of incorporation of [^{14}C]orotic acid into ribosomal RNA suggests that the rate of turnover of the RNA of these particles is very slow in this tissue.

Nuclear RNA. (a) RNA extracted at 50°. The E_{260} curve of Fig. 1(b) indicates that the bulk of this material sediments at similar rates to the cytoplasmic ribosomal RNA, i.e. 16s and 24s. The rapidly labelled material, however, sediments in the range 35–55s. Owing to the relatively short period during which the slices remained viable, an investigation of the further metabolism of this material was not possible. It may constitute the ribosomal precursor-RNA which has been described

Table 1. *Intracellular distribution of nucleic acids from canine pancreas slices after incubation*

Recovery is calculated from the amount of nucleic acid in a fraction divided by the total DNA or RNA in the homogenate of non-incubated tissue.

Incubation time (min.)	Recovery of nucleic acids (%)			
	DNA	Nuclear		Cytoplasmic RNA
		50°-RNA	65°-RNA	
0	96	2.72	1.04	70
10	98	2.94	1.76	70
30	95	2.90	0.91	84
60	98	3.80	1.80	79
120	92	3.50	1.34	87

Table 2. *Composition of canine pancreas nuclear fractions*

'Phenol nuclei' were prepared from fresh pancreas and RNA was extracted and precipitated as described in the text. The RNA content was calculated from E_{260} . DNA was determined by the procedure of Ceriotti (1952) and protein by the method of Nayyar & Glick (1954).

Temperature of extraction	Weight of component (mg.)		
	RNA	DNA	Protein
50°	12.3	0.0	0.0
65	4.7	0.04	0.0
80	5.0	0.92	2.4

Table 3. *Base composition of nuclear ribonucleic acids*

Samples were obtained from 'phenol nuclei' and purified by the procedure of Lerman *et al.* (1964). They were hydrolysed and analysed by the method of Katz & Comb (1963).

Temperature of extraction	Nucleotide composition (moles/100 moles)				
	G	C	A	U	$\frac{(\text{G} + \text{C})}{(\text{A} + \text{U})}$
50°	35.1	28.0	16.6	20.3	1.71
65	33.4	25.9	18.0	22.7	1.47
80	34.0	22.9	16.5	25.5	1.38

Fig. 1. Sedimentation patterns of ^{14}C -labelled RNA fractions from canine pancreas. Experimental details are described in the text. (a) Cytoplasmic RNA. (b) Nuclear RNA, extracted at 50° . (c) Nuclear RNA, extracted at 65° . Incubation periods: ▼, 30 min., ■, 60 min., ●, 120 min. ----, E_{260} values.

Fig. 2. Effect of actinomycin D on the incorporation of $[6\text{-}^{14}\text{C}]$ orotic acid into RNA. Canine pancreas slices were incubated for 60 min. and the RNA was extracted as described in the text. (a) Cytoplasmic RNA. (b) Nuclear RNA, extracted at 50° . (c) Nuclear RNA, extracted at 65° . Concentrations of actinomycin D in the incubation medium: ●, none; ■, $0.5\text{ }\mu\text{g./ml.}$; ▼, $2.5\text{ }\mu\text{g./ml.}$ ----, E_{260} values.

by Scherrer & Darnell (1962) on HeLa cells, by Perry (1964) with L cells, and by Georgiev *et al.* (1963). On the other hand, the heavy, rapidly labelled RNA may represent a small amount of the elusive m-RNA (Joklik & Becker, 1965a,b).

(b) 65° -RNA. On sedimentation this fraction produced the pattern shown in Fig. 1(c). Most of the material was present in the 16s region but, again, the rapidly labelled RNA sedimented considerably faster (25–40s). The same considerations apply to this rapidly labelled RNA as was mentioned above for that in the 50° extract. Data from many experiments do not indicate a significant difference in the specific activities of the entire contents of the two fractions.

Effects of actinomycin D. The addition of this inhibitor of RNA-polymerase to the incubation medium at concentrations of $2.5\text{ }\mu\text{g./ml.}$ or $0.5\text{ }\mu\text{g./ml.}$ had little effect on the incorporation of $[^{14}\text{C}]$ -orotic acid into 4s RNA (Fig. 2a). In several of

the experiments there occurred a stimulation of incorporation into cytoplasmic (transfer) RNA. This lack of inhibition of incorporation of precursors into transfer RNA by actinomycin D has been noted by Perry (1964) with L cells, by Revel & Hiatt (1964) and by Moulé & Landin (1965) with rat liver. On the other hand, actinomycin D at both these concentrations almost completely inhibited the incorporation of $[6\text{-}^{14}\text{C}]$ orotic acid into 50° -RNA (Fig. 2b) and 65° -RNA (Fig. 2c). Calculations of the specific activities of the three RNA fractions are presented in Table 4. The percentage inhibition of incorporation into the two fractions of nuclear RNA was quite similar.

Effects of Urecholine. As can be seen in Fig. 3(a), 1 mM -Urecholine in the incubation medium depressed the specific activity of the 4s RNA by a small amount. This inhibition occurred consistently but varied in amount from 20 to 50% (Table 5). An inhibition also is evident in the

Table 4. *Effects of actinomycin D on incorporation of precursors into RNA by canine pancreas slices*

The conditions of incubation and isolation of the RNA fractions are given in the text. [6-¹⁴C]Orotic acid (1 μ C/ml.) was used in the 1 hr. and 2 hr. incubations and [8-¹⁴C]adenine (0.5 μ C/ml.) in the 0.5 hr. incubation. The percentages of the control specific activities are in parentheses.

Incubation time (hr.)	Concn. of actinomycin D (μ C/ml.)	Specific radioactivity of RNA (counts/min./mg.)		
		Cytoplasmic	Nuclear	
			50°	65°
1	0	860	1135	1340
	0.5	1010 (116)	170 (15)	155 (11)
	2.5	840 (98)	150 (13)	255 (19)
2	0	1280	940	965
	0.5	2080 (163)	280 (30)	—
	2.5	1460 (114)	230 (24)	190 (20)
0.5	0	3935	—	1410
	0.5	5420 (140)	—	870 (62)

Table 5. *Effect of Urecholine on incorporation of [6-¹⁴C]orotic acid into RNA by canine pancreas slices*

The conditions of incubation and isolation of RNA fractions are given in the text. [6-¹⁴C]Orotic acid was added to produce a final concentration of 1 μ C/ml. The percentages of the control specific activities are in parentheses.

Incubation time (hr.)	Concn. of Urecholine (mM)	Specific radioactivity of RNA (counts/min./mg.)		
		Cytoplasmic	Nuclear	
			50°	65°
2	0	1625	1595	1342
	1.0	1135 (70)	966 (61)	947 (70)
1	0	1950	2140	2400
	0.001	—	1675 (80)	1970 (82)
	0.01	1295 (67)	1880 (88)	2070 (86)
	1.0	1045 (54)	865 (40)	1120 (46)

50°-RNA and 65°-RNA fractions. The values range from 40 to 88% of the controls. A decrease in specific activity of these samples was noted at μ M-Urecholine, the lowest concentration tested in this investigation. An interesting differential inhibition can be observed in Fig. 3(b) and 3(c). There was little or no decrease in incorporation of [6-¹⁴C]orotic acid into the ribosomal type RNA of these preparations. The inhibition was exerted almost exclusively on the heavier, rapidly labelled components of these fractions.

Effects of pancreozymin. Sucrose-density-gradient centrifugation of cytoplasmic RNA after pancreozymin addition to the medium produced the patterns shown in Fig. 4(a). Addition of the hormone resulted in an inhibition of incorporation of [¹⁴C]orotic acid into this fraction. Calculation of specific activity of RNA at various concentrations of pancreozymin are presented in Table 6. The minimum concentration at which an inhibition

of incorporation into 4s RNA was observed was 1.25 units/ml.

The effect of the hormone on the labelling pattern of nuclear RNA fractions is shown in Fig. 4(b) and 4(c). The radioactivity curves are remarkably similar to those obtained with Urecholine in that the major effect is exerted on the heavy, rapidly labelled RNA in both the 50°-RNA and 65°-RNA fractions. The specific activity values listed in Table 6 demonstrate an appreciable inhibition of incorporation into both fractions of nuclear RNA at concentrations of pancreozymin as low as 0.025 unit/ml.

DISCUSSION

In their development of the method of thermal fractionation of nuclear RNA samples from rat liver and other tissues, Georgiev *et al.* (1963) summarized the differences between R-RNA and D-RNA as follows.

Fig. 3. Effect of Urecholine on the incorporation of [6-¹⁴C]orotic acid into RNA. Canine pancreas slices were incubated for 60 min. and RNA was isolated as described in the text. (a) Cytoplasmic RNA. (b) Nuclear RNA, extracted at 50°. (c) Nuclear RNA, extracted at 65°. Concentrations of Urecholine in the incubation medium: ●, none; ▼, mM. ----, E_{260} values.

Fig. 4. Effects of pancreozymin on the incorporation of [6-¹⁴C]orotic acid into RNA. Canine pancreas slices were incubated for 60 min. and RNA was isolated as described in the text. (a) Cytoplasmic RNA. (b) Nuclear RNA extracted at 50°. (c) Nuclear RNA, extracted at 65°. Concentrations of pancreozymin in the incubation medium: ●, none; ▼, 1.87 units/ml. ----, E_{260} values.

Table 6. *Effects of pancreozymin on incorporation of [6-¹⁴C]orotic acid into RNA by canine pancreas slices*

Each beaker contained 4mg. of slices in 40ml. of Krebs III medium. They were preincubated at 37° for 10 min. with the stated units of pancreozymin. [6-¹⁴C]Orotic acid was added to produce a final concentration of 1 μ C/ml., and the incubation continued for 60 min. The numbers in parentheses show the percentages of the control values.

Concn. of pancreozymin (units/ml.)	Specific radioactivity of RNA (counts/min./mg.)		
	Cytoplasmic	Nuclear	
		50°	65°
0	1140	790	1130
0.025	1070 (94)	560 (72)	810 (72)
0.125	1025 (90)	535 (69)	745 (66)
0.625	1070 (94)	480 (61)	660 (58)
1.25	970 (85)	460 (58)	540 (48)
1.87	750 (66)	410 (52)	375 (33)

(1) Sedimentation: R-RNA sediments in the ultracentrifuge as two major components with similar $S_{20,w}$ to ribosomal RNA, whereas D-RNA is more heterogeneous with a maximum at about 18s.

(2) Base composition: R-RNA possesses a ribosomal composition, i.e. the G:C ratio is high and $(G+C)/(A+U) = 1.5-1.6$. D-RNA possesses a DNA type composition, and $(G+C)/(A+U) = 0.75-0.85$.

(3) Rapidity of labelling: with [³²P]phosphate

the specific activity of the D-RNA fraction from rat liver was twice that of R-RNA.

(4) Pattern of labelling: with short periods of incorporation the rapidly labelled fraction is found in the heavy (35–45s) region.

(5) Effect of actinomycin D: this substance depressed incorporation *in vivo* of [32 P]phosphate into R-RNA of rat liver to a much greater extent than D-RNA.

When a comparison is made between the results obtained with canine pancreas nuclear RNA and those cited above, certain significant differences show up. The sedimentation patterns of the two fractions are similar to those obtained by Georgiev *et al.* (1963) with rat liver, but 65°-RNA obtained from pancreas, although slightly lower in (G + C) content than 50°-RNA, is also quite similar in composition to ribosomal RNA. [14 C]Orotic acid was not incorporated significantly faster into the 65°-RNA fraction than into 50°-RNA if the specific activity of the entire fraction is used as the basis of comparison. Yet the rapidly labelled portion of both fractions did occur in the 35–45s regions. Actinomycin D was found to inhibit incorporation into the two nuclear fractions approximately equally. It appears that in pancreas, the characteristics of both fractions are similar to the R-RNA of Georgiev *et al.* (1963). The difference in sedimentation pattern of the 65°-RNA may result from the loss of secondary structure at the higher temperature. Kirby (1965) has noted a lowered $S_{20,w}$ of ribosomal RNA after heating. He attributed this effect to an 'opening up' of the molecule due to loss of secondary structure. Further work will be necessary to determine whether this phenomenon is responsible for the difference in sedimentation patterns of the samples from pancreas nuclei.

A question raised by our results concerns the inhibitory effects of the two stimulants of pancreatic secretion on the incorporation of precursor into nuclear RNA fractions. The present work demonstrates that this inhibition of RNA synthesis is at least partially specific. Thus three fractions of pancreatic RNA can be distinguished: (1) the 30–45s rapidly labelled nuclear fraction; (2) the 16–30s slowly labelled, ribosomal-type nuclear fraction; (3) the cytoplasmic 4s RNA. We see that incorporation of [14 C]orotic acid was decreased to a much greater extent into fractions (1) and (3) than into (2) by these two agents. This is not surprising in view of the much greater turnover or synthesis rate of these fractions compared to that of the ribosomal-type RNA. Hokin & Hokin (1954) reported that the administration of pilocarpine to mice decreased the incorporation of [32 P]phosphate into total pancreatic RNA by approximately 50%.

There is no reason to suspect that the two stimu-

lants act by an identical mechanism, since the action of cholinergic drugs is blocked by atropine, whereas that of pancreozymin is not (Harper & Raper, 1943). Both the hormone and the drugs result in a markedly increased turnover of phospholipid in this tissue (Hokin & Hokin, 1956). Since both phospholipid turnover and RNA synthesis require common precursors such as ATP and CTP (Kennedy & Weiss, 1956) a stimulation of the former may result in a temporarily decreased rate of synthesis of RNA.

It was not possible to collect sufficient amounts of the rapidly labelled RNA from either nuclear fraction to permit analysis of its base composition. This portion of the RNA may represent the ribosomal-precursor RNA (high G + C), which has been reported by Scherrer & Darnell (1962) and by Perry (1964), or it might be considered as messenger RNA (high A + U) (Georgiev *et al.* 1963; Sibatani, DeKloet, Allfrey & Mirsky, 1962). The isolation and characterization of these fractions as well as the 80°-RNA is being undertaken.

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REFERENCES

- Babkin, P. (1950). *Secretory Mechanism of the Digestive Glands*, 2nd ed., pp. 858–859. New York: Paul Hoeber Inc.
- Britten, R. S. & Roberts, R. B. (1960). *Science*, **131**, 32.
- Cerioti, G. (1952). *J. biol. Chem.* **198**, 297.
- Cohn, W. (1960). *J. biol. Chem.* **235**, 1488.
- Georgiev, G. P., Samarina, O. P., Lerman, M. F., Smirnov, M. N. & Severtzov, A. N. (1963). *Nature, Lond.*, **200**, 1291.
- Hallinan, T., Fleck, A. & Munro, H. N. (1963). *Biochim. biophys. Acta*, **68**, 131.
- Harper, A. A. & Raper, H. S. (1943). *J. Physiol.* **102**, 115.
- Herbert, E. (1959). *Ann. N.Y. Acad. Sci.* **81**, 679.
- Hiatt, H. H. (1962). *J. molec. Biol.* **5**, 217.
- Hokin, L. E. & Hokin, M. R. (1954). *Biochim. biophys. Acta*, **13**, 401.
- Hokin, L. E. & Hokin, M. R. (1956). *J. Physiol.* **132**, 442.
- Joklik, W. K. & Becker, Y. (1965a). *J. molec. Biol.* **13**, 496.
- Joklik, W. K. & Becker, Y. (1965b). *J. molec. Biol.* **13**, 511.
- Katz, S. & Comb, D. G. (1963). *J. biol. Chem.* **238**, 3065.
- Kennedy, E. P. & Weiss, S. B. (1956). *J. biol. Chem.* **222**, 193.
- Kirby, K. S. (1965). *Biochem. J.* **96**, 266.
- Krebs, H. A. (1950). *Biochim. biophys. Acta*, **4**, 249.
- Lerman, M. I., Mantieva, V. L. & Georgiev, G. P. (1964). *Biokhimiya*, **29**, 3065.
- Martin, R. G. & Ames, B. N. (1961). *J. biol. Chem.* **236**, 1372.
- Morris, A. J. & Dickman, S. R. (1960). *J. biol. Chem.* **235**, 1404.

- Moulé, Y. & Landin, R. M. (1965). *Biochem. biophys. Res. Commun.* **20**, 491.
- Nayyar, S. N. & Glick, D. (1954). *J. Histochem. Cytochem.* **2**, 282.
- Perry, R. P. (1964). *Monogr. nat. Cancer Inst.* **14**, 73.
- Revel, M. & Hiatt, H. (1964). *Biochem. biophys. Res. Commun.* **17**, 730.
- Scherrer, K. & Darnell, J. E. (1962). *Biochem. biophys. Res. Commun.* **7**, 486.
- Sibatani, A., DeKloet, S. R., Allfrey, V. G. & Mirsky, A. E. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 471.
- Smellie, R. M. S. (1963). *Progr. Nucleic Acid Res.* **1**, 46.
- Tamaoki, T. & Mueller, G. E. (1962). *Biochem. biophys. Res. Commun.* **9**, 451.