

these fragments occur in the intact TMV-NA preceded by any one of the three nucleotides, Cp, Up, and Gp. Of the total adenylic acid residues in TMV-NA 19.1 %, 19.6 % and 17.9 % occur "singly" as NpApCp, NpApUp and NpApGp, respectively; 12.1 %, 12.9 % and 12.8 % occur in "clusters" as NpAp(Ap) $_n$ Cp, NpAp(Ap) $_n$ Up and NpAp(Ap) $_n$ Gp, respectively (where Np is any one of the three nucleotides, Cp, Up, and Gp; $n = 1$ or more). The longest polyadenylic acid fragments so far isolated contained three adenylic acid residues. The possibility of the occurrence in TMV-NA of extremely small amounts of polyadenylic acid fragments containing more than three adenylic acid residues can not, however, be excluded.

Full details of this investigation will be published elsewhere.

I wish to thank Dr. W. M. STANLEY for his interest and encouragement during the course of this investigation. This investigation was aided by grants from the U.S. Public Health Service and the Rockefeller Foundation.

Virus Laboratory, University of California, Berkeley, Calif. (U.S.A.) K. K. REDDI*

¹ K. K. REDDI, *Proc. Natl. Acad. Sci. U.S.*, 45 (1959) 293.

² K. K. REDDI, *Biochim. Biophys. Acta*, 30 (1959) 132.

³ K. K. REDDI, *Nature*, (1960) in the press.

⁴ L. A. HEPPEL AND J. C. RABINOWITZ, *Ann. Rev. Biochem.*, 27 (1958) 613.

⁵ K. SATO AND F. EGAMI, *J. Biochem. (Japan)*, 44 (1957) 753.

Received June 7th, 1960

* Present address; Department of Biochemistry, New York University of Medicine, New York 16, N.Y. (U.S.A.).

Radiosensitivity of nuclear RNA

Very little is known of the function of RNA in the cell nucleus, but it seems not improbable that it plays an important role in the synthesis of DNA. Since this process is highly radiosensitive, we undertook an investigation of the radiosensitivity of nuclear RNA. The latter was fractionated with 1 M NaCl, the heterogeneity of nuclear RNA now being well established by several authors¹⁻⁴. Reports on the radiosensitivity of nuclear RNA have already been published⁵⁻⁸ but in these studies no fractionation was attempted.

In this communication we shall briefly report the results of experiments concerning the metabolism and radiosensitivity of total and fractionated nuclear RNA from liver and thymus.

Groups of 5 young adult male albino rats, weighing 150 g, were irradiated in a perspex cage. Control groups consisted of 5 non-irradiated rats. All further procedures were carried out in exactly the same manner for the irradiated and the control group. The dose was 700 R in the experiments with liver tissue and 300 R with thymus tissue. In the experiments on liver nuclear RNA, the rats 4 h after irradiation were injected intravenously with 0.5 ml of neutralized 0.1 % Na₂HPO₄ containing 200 μ C Na₂H³²PO₄, and killed 3.5 h later. In the studies on thymus nuclear RNA 200 μ C

Abbreviations: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; CMP, cytidine monophosphate; AMP, adenosine monophosphate; GMP, guanosine monophosphate; UMP, uridine monophosphate.

TABLE I
THE EFFECT OF TOTAL-BODY IRRADIATION ON THE INCORPORATION OF LABELLED INORGANIC PHOSPHATE INTO NUCLEAR RNA
The irradiation dose was 700 R in the experiments with liver tissue and 300 R in those with thymus tissue.

	Liver RNA						Thymus RNA									
	Total nuclear RNA			n-RNA I			Total RNA			n-RNA I						
	specific activity (counts/min/ μ mole)		$r.s.a.* \times 10^3$	specific activity (counts/min/ μ mole)		$r.s.a.* \times 10^3$	specific activity (counts/min/ μ mole)		$r.s.a.* \times 10^3$	specific activity (counts/min/ μ mole)		$r.s.a.* \times 10^3$				
	irr.	contr.		irr.	contr.		irr.	contr.		irr.	contr.					
CMP	2330	2050	285	225	1240	2780	150	305	415	955	350	775	425	1240	360	1000
AMP	4020	4000	490	435	1460	3180	180	350	910	2430	775	1935	700	1825	600	1485
GMP	3150	2540	385	290	1140	2860	140	315	605	1550	510	1260	420	1140	355	930
UMP	2000	2170	245	220	1440	3080	175	340	590	1340	500	1090	605	1300	510	1050
inorg. phosphate	8170	9110							1180	1230						

* The relative specific activity of the nucleotides, representing the quotient of the specific activities of the nucleotides and the nuclear inorganic phosphate.

$\text{Na}_2\text{H}^{32}\text{PO}_4$ was similarly injected 0.5 h after irradiation and the glands were excised 3 h later. Nuclei were isolated according to the method of HOGEBOM *et al.*⁹. The nuclear suspensions were divided into two parts. One portion was centrifuged and the pellet was washed as described by OSAWA *et al.*¹⁰ (total nuclear RNA). The other portion of the nuclear suspension was centrifuged and the pellet was homogenized with 20 vol. 1 *M* NaCl, pH 6.5. The suspension was shaken for 2 h and then centrifuged for 20 min at 15,000 rev./min. The precipitate was washed¹⁰ (*n*-RNA II). An equal volume of 96 % ethanol was added to the supernatant. The precipitate was collected by centrifugation and washed¹⁰ (*n*-RNA I). The RNA nucleotides were isolated according to the method of OSAWA *et al.*¹⁰ and separated by high-voltage electrophoresis. Nuclear inorganic phosphate was obtained from the first washing fluids (with 2 % HClO_4) from the nuclear preparations. The nucleotides in the extracts were removed with charcoal and inorganic phosphate was isolated and analysed as described by VAN BEKKUM¹¹.

The results of the experiments are shown in Table I. It will be seen that the labelling of *n*-RNA I is a radiosensitive process both in liver and thymus tissue. The effect of X-irradiation on the uptake of ^{32}P in total nuclear RNA is different for thymus and liver. We have not yet fully elucidated the effect of X-irradiation on the uptake of ^{32}P in the nucleotides of *n*-RNA II. So far, a slight increase in the uptake of ^{32}P in this RNA fraction was found in liver tissue, whereas in thymus the uptake was considerably reduced. The fact that the relative specific activity of some of the nucleotides from nuclear thymus RNA is greater than unity can be explained by assuming a relatively more rapid exchange of ^{32}P of the nuclear inorganic phosphate as compared with the exchange of ^{32}P of the RNA nucleotides during the period studied. On the other hand, the possibility cannot be excluded that one of the precursors of nuclear RNA is cytoplasmic inorganic phosphate, which has in our experiments a higher specific activity than nuclear inorganic phosphate.

*Radiobiological Institute of the
Organization for Health Research T.N.O.,
Rijswijk (Z.H.), (Netherlands)*

H. M. KLOUWEN

- ¹ W. S. VINCENT, *Science*, 126 (1957) 306.
- ² R. LOGAN, *Biochim. Biophys. Acta*, 26 (1957) 227.
- ³ Y. HOTTA AND S. OSAWA, *Biochim. Biophys. Acta*, 28 (1958) 642.
- ⁴ R. LOGAN AND J. N. DAVIDSON, *Biochim. Biophys. Acta*, 24 (1957) 196.
- ⁵ R. M. S. SMELLIE, S. F. HUMPHREY, E. R. M. KAY AND J. N. DAVIDSON, *Biochem. J.*, 60 (1955) 177.
- ⁶ A. H. PAYNE, L. S. KELLY AND C. ENTENMAN, *Proc. Soc. Exptl. Biol. Med.*, 81 (1952) 798.
- ⁷ E. HARBERS AND CH. HEIDELBERG, *J. Biol. Chem.*, 234 (1959) 1249.
- ⁸ A. SIBATANI, *Exptl. Cell Research*, 17 (1959) 131.
- ⁹ G. H. HOGEBOM, W. C. SCHNEIDER AND M. J. STRIEBICH, *J. Biol. Chem.*, 196 (1952) 111.
- ¹⁰ S. OSAWA, K. TAKATA AND Y. HOTTA, *Biochim. Biophys. Acta*, 28 (1958) 271.
- ¹¹ D. W. VAN BEKKUM, *Biochim. Biophys. Acta*, 25 (1957) 487.

Received June 1st, 1960

Biochim. Biophys. Acta, 42 (1960) 366-368