monophosphate pathways which could lead to the observed formation or disappearance of acid-labile phosphate from the nuclei. The possible function of these enzymes in nuclear phosphorylation is discussed.

It is a pleasure to thank Mr A. Morris and Mr L. Scrubb for their able assistance. Financial aid for this work has been provided by the Department of Scientific and Industrial Research, the International Atomic Energy Agency, the Rockefeller Foundation and the National Institutes of Health United States Public Health Service (Grant no. A3369).

REFERENCES

Allfrey, V. G., Mirsky, A. E. & Osawa, S. (1955). *Nature, Lond.*, **176**, 1042.
Bailey, K. & Webb, E. C. (1944). *Biochem. J.* **38**, 394.
Creasey, W. A. & Stocken, L. A. (1959). *Biochem. J.* **72**, 519.
Daly, M. M. & Mirsky, A. E. (1954–55). *Amer. J. Physiol.* **38**, 405.
Dounce, A. L. & Beyer, G. T. (1948). *J. biol. Chem.* **173**, 159.
Gornall, A. G., Bardawill, C. J. & David, M. M. (1949). *J. biol. Chem.* **177**, 751.
Hagedorn, H. C. & Jensen, B. N. (1923). *Biochem. Z.* **135**, 46.
Hogeboom, G. H. & Schneider, W. C. (1952). *J. biol. Chem.* **197**, 611.
Holton, P. W. (1959). *J. Physiol.* **145**, 494.
Hopkins, J. W. (1959). *Proc. nat. Acad. Sci., Wash.*, **45**, 1461.
Huggett, A. St G. & Nixon, D. A. (1957). *Lancet*, ii, 368.
Khym, J. & Cohn, W. E. (1953). *J. Amer. chem. Soc.* **75**, 1153.
Logan, R., Errera, M. & Ficq, A. (1959). *Biochim. biophys. Acta*, **32**, 147.
Miller, L. A. & Goldfeder, A. (1961). *Exp. Cell Res.* **23**, 311.
Mirsky, A. E. & Osawa, S. (1961). In *The Cell*, vol. 2, p. 677. Ed. by Brachet, J. & Mirsky, A. E. New York: Academic Press Inc.
Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
Ord, M. G. & Stocken, L. A. (1957). In *Advances in Radiobiology*, p. 65. Ed. by de Heversy, G., Forseberg, A. & Abbatt, J. D. Edinburgh: Oliver and Boyd Ltd.
Ord, M. G. & Stocken, L. A. (1961*a*). *Biochem. J.* **81**, 1.
Ord, M. G. & Stocken, L. A. (1961*b*). In *Mechanisms in Radiobiology*, p. 320. Ed. by Errera, M. & Forseberg, A. New York: Academic Press Inc.
Rosenberg, H., Ennor, A. H. & Morrison, J. F. (1956). *Biochem. J.* **63**, 153.
Siebert, G. (1960). In *The Cell Nucleus*, p. 176. Ed. by Mitchell, J. S. London: Butterworth and Co. Ltd.
Siebert, G. (1961). *Biochem. Z.* **334**, 369.
Siebert, G., Bässler, K. H., Hannover, R., Adloff, A. E. & Beyer, R. (1961). *Biochem. Z.* **334**, 388.
Stern, H. & Mirsky, A. E. (1953). *J. gen. Physiol.* **37**, 177.
Zubay, G. & Doty, P. (1959). *J. molec. Biol.* **1**, 1.

Biochem. J. (1962) **84**, 600

The Effects of 200r. of X-radiation *in vivo* on Phosphate-Transfer Reactions in Nuclei from Rat Thymus Gland

BY MARGERY G. ORD AND L. A. STOCKEN
Department of Biochemistry, University of Oxford

(*Received* 9 *March* 1962)

Creasey & Stocken (1959) showed that phosphorylation by nuclei from thymus and various other tissues of the rat was completely inhibited by exposures of 100r. given either *in vivo* or *in vitro*. With the elucidation of some of the factors involved in the phosphorylation (Ord & Stocken, 1962) it was decided to re-examine the radiosensitivity in an attempt to pin-point the lesion more precisely.

MATERIALS AND METHODS

These were as described by Ord & Stocken (1962).

X-Radiation. This was done either at the Medical Research Council Radiobiological Research Unit, Harwell, or at the Department of Radiotherapy, the Churchill Hospital, Oxford. The characteristics were respectively: half-value layer 2·5 mm. Cu, peak voltage 250 kv at 14 mA; or half-value layer 1·1 mm. Cu, peak voltage 220 kv at 15 mA. The animals were killed 15–50 min. after exposure.

RESULTS

When nuclei were prepared from thymus glands of normal rats that had been removed from the animals as rapidly as possible after death ('fresh' nuclei) and were then shaken for 30 min. at 0°, pyrophosphate formation occurred to a greater extent than accounted for by the apparent disappearance of ATP. It was considered that this gave evidence for phosphorylation by the isolated nuclei. When rats were exposed to 200r., and the nuclei isolated in a similar way, no increase in pyrophosphate was found (Table 1), although the initial preparation contained more pyrophosphate than did 'fresh' nuclei from control rats (Ord & Stocken, 1962). The concentration of arginine also resembled that in 'aged' nuclei from normal rats.

When 'aged' nuclei from irradiated rats were

Table 1. *Pyrophosphate formation by 'fresh' thymus nuclei from irradiated rats*

Concentrations are expressed as μm-moles/mg. of DNA phosphorus. P_i, inorganic phosphate; PP, pyrophosphate.

Initial			Change after 30 min. at 0°			
ATP	PP	Arginine	P_i	ATP	PP	Arginine
2·8	30	93	+18·3	−0·3	0	0
4·4	28	103	+23·3	−1·9	0	0

Table 2. *Adenosine triphosphate uptake by 'aged' thymus nuclei from irradiated rats*

Concentrations are expressed as μg. of phosphorus/ml. in the bound fraction. When present, the final concn. of ATP was 1 μmole/ml. P_i, inorganic phosphates; P_{15}, acid-labile phosphate.

		Initial		Change after 30 min. at 0°	
Expt.	Phosphate	Without ATP	With ATP	Without ATP	With ATP
1	P_i	0·09	0·27	0	+0·55
	P_{15}	0·91	0·39	−0·72	+0·06
	ATP	0·11	0·04	−0·09	+0·02
2	P_i	0·25	0·39	−0·06	+0·08
	P_{15}	0·51	0·32	−0·32	−0·01
	ATP	0·17	0·06	−0·17	−0·02

shaken in medium to which ATP had been added, net uptake of the triphosphate was observed above that found in nuclei shaken without added ATP (Table 2). The extent of ATP binding was comparable to that found with 'aged' nuclei from unexposed rats. There was no change in nuclear adenosine-triphosphatase activity at this time after irradiation.

When rats were given ^{32}P-labelled phosphate and the animals killed 10 min. after injection, the specific activities of the γ-phosphate groups of nuclear and cytoplasmic ATP, determined by the hexokinase method, showed no differences between control and irradiated animals, although the amount of ATP in the nuclei from the irradiated rats was only about 40 % of that in the controls. In 'fresh' nuclei obtained from normal animals and given 187 r. of γ-rays (^{60}Co) *in vitro* there was no difference in ATP disappearance in glucose-supplemented medium from that found in the controls.

DISCUSSION

The experiments reported here confirm those of Creasey & Stocken (1959) on the radiosensitivity of nuclear phosphorylation. The presence of pyrophosphate in 'fresh' nuclei from irradiated rats suggests that ATP and amino acids bound to the nuclei at the time of irradiation can be used in the normal way. This unimpaired utilization of bound ATP is substantiated by the unchanged hexokinase reaction after irradiation of 'fresh' nuclei *in vitro* and the unaltered formation of NAD from NMN after 1500 r. *in vivo* (Myers, 1960). Replacement from the cytoplasm of the ATP so utilized is not prevented but the low amount of ATP found even in 'fresh' nuclei from irradiated animals suggests that the uptake from the cytoplasm cannot be sufficient to restore the ATP to the amount usually available. This implies that nuclear phosphorylation may normally contribute to the bound ATP of the nucleus.

The interference in ATP formation by the nuclei will explain the reduced incorporation of amino acids into isolated nuclei after irradiation *in vitro* (Logan, Errera & Ficq, 1959) when no ATP is added to the medium. The site of the interference has yet to be determined.

SUMMARY

1. The radiosensitivity of phosphorylation in nuclei isolated from rat thymus gland was confirmed.

2. Activation reactions which use ATP present in the nuclei at the time of X-radiation were still detectable, and the uptake of ATP by isolated nuclei from irradiated rats was not prevented.

3. Some evidence was presented suggesting that nuclear phosphorylation contributed to the ATP content of nuclei in normal thymocytes.

We are very grateful to those who carried out the irradiations and to Mr A. Morris and Mr L. Scrubb for their assistance. Financial aid for this work has been provided by the Department of Scientific and Industrial Research, the International Atomic Energy Agency, the Rockefeller Foundation and the National Institutes of Health, United States Public Health Service (Grant no. A3369.)

REFERENCES

Creasey, W. A. & Stocken, L. A. (1959). *Biochem. J.* **72**, 519.
Logan, R., Errera, M. & Ficq, A. (1959). *Biochim. biophys. Acta*, **32**, 147.
Myers, D. K. (1960). *Nature, Lond.*, **187**, 1124.
Ord, M. G. & Stocken, L. A. (1962). *Biochem. J.* **84**, 593.