I thank Messrs Hoffmann–La Roche, Basle, for chemicals and technical information, and the ARC for a grant.

V. B. WIGGLESWORTH

Department of Zoology,
University of Cambridge.

Received November 28, 1968.

[1] Wigglesworth, V. B., *Quart. J. Microsc. Sci.*, **79**, 91 (1936).
[2] Wigglesworth, V. B., Appendix to Schmialek, P., *Z. Naturforsch.*, **16**b, 461 (1961).
[3] Wigglesworth, V. B., *Symp. Soc. Exp. Biol.*, **11**, 204 (1957).
[4] Wigglesworth, V. B., *J. Insect Physiol.* (in the press).
[5] Schneiderman, H. A., Krisnakumaran, A., Kulkarni, V. G., and Friedman, L., *J. Insect. Physiol.*, **11**, 1641 (1965).
[6] Yamamoto, R. T., and Jacobson, M., *Nature*, **196**, 208 (1962).
[7] Dahm, K. H., Röller, H., and Trost, B. M., *Life Sci.*, **7**, 129 (1968).
[8] Röller, H., and Dahm, K. H., *Recent Progress in Hormone Research*, **24** (in the press).
[9] Barrall, E. M., Porter, R. S., and Johnson, J. F., *J. Chromatog.*, **21**, 392 (1966).
[10] Bowers, W. S., and Thompson, M. J., *Science*, **142**, 1469 (1963).
[11] Willmer, E. N., *Biol. Rev.*, **36**, 368 (1961).
[12] Bowers, W. S., Thompson, M. J., and Uebel, E. C., *Life Sci.*, **4**, 2323 (1965).
[13] Baumann, G., *J. Insect Physiol.*, **14**, 1365 (1968).

Synthesis of Functional Proteins in X-irradiated Mammalian Cells

RECOVERY from X-ray induced division delay in Chinese hamster cells cultured *in vitro* requires protein synthesis but not synthesis of DNA or RNA[1,2], suggesting that the primary lesion is at the level of translation. The question then arises whether or not normal proteins are synthesized during the recovery period. Although it is well established that the rate of amino-acid incorporation into polypeptide is only slightly reduced after moderate doses of X-irradiation[3–6], it does not necessarily follow that the protein species synthesized in these conditions are functional. For example, cessation of functional protein in the presence of low doses of puromycin is almost immediate despite the fact that the rate of amino-acid incorporation is only slightly affected[7].

The ability or inability of a cell recovering from radiation damage to support synthesis of functional proteins can be established by introducing into the intact, irradiated cell an alien messenger species and determining whether or not the cellular synthetic machinery (that is ribosomes, transfer RNA species and so on) can support synthesis of the corresponding functional non-cellular protein. This experiment has been carried out by infecting irradiated Chinese hamster cells with the RNA virus mengovirus and determining the time course of production of infective virus particles. The infecting viral genome is the alien messenger species, and only particles with normal protein coats (indicating synthesis of functional protein) are able to infect cells and initiate plaque formation in the infectivity assay. Results obtained indicate that virus production proceeds normally (both in terms of the time of production and the yield of particles per cell) in cells recovering from radiation damage.

Chinese hamster cells (line CHO[8]) maintained free of PPLO were grown in F-10 medium without added calcium, supplemented with 10 per cent low "poliovirus-inhibitory" calf serum (Microbiological Associates, Bethesda, Maryland), penicillin and streptomycin. Suspension cultures were irradiated with a General Electric Maxitron X-ray therapy unit as described previously[1]. The heat-resistant 37A strain of mengovirus[9] was used throughout. Infectivity was determined by plaque formation on CHO cell monolayers essentially as described previously[10], except that plaque identification was accomplished by staining the monolayers with crystal violet solution after the method of Holland and McLaren[11].

Two suspension cultures of CHO cells in exponential growth were prepared, and one was irradiated with 600 rads which resulted in a division delay period of 7·5 h[1]. Immediately after irradiation, both cultures were infected with mengovirus at a multiplicity of infection of 6. The time required for a single cycle of mengovirus production in the CHO cell is 6·5 h[10], and so a cycle of virus replication could take place during the recovery period in the irradiated cell provided the cellular synthetic machinery was not damaged. In Fig. 1 the number of infectious particles per cell is plotted as a function of time in both the irradiated and non-irradiated control cultures. It is apparent that both the time course of virus production and the total yield of infectious particles per cell are identical in the two cultures.

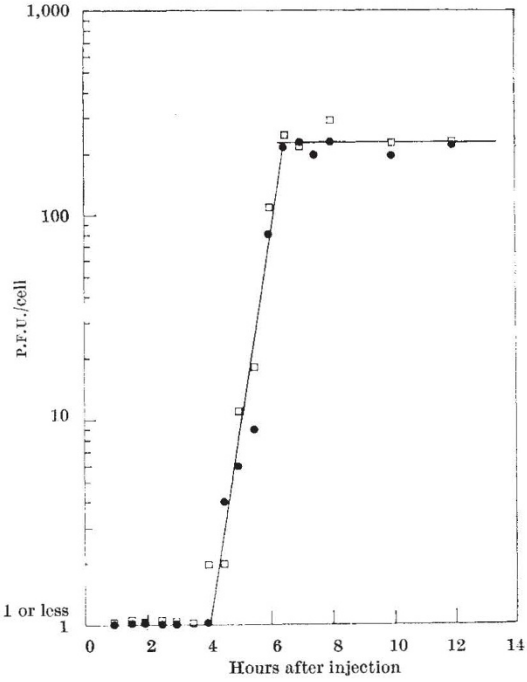

Fig. 1. Production of plaque forming units as a function of time after infection of Chinese hamster cells with mengovirus. □, Culture irradiated (600 rads of X-irradiation) immediately before infection; ●, non-irradiated control.

Thus the cellular machinery for protein synthesis in the irradiated cell is capable of synthesizing functional species because, under the direction of the viral RNA genome, faithful copies of the virus have been made. Clearly this conclusion is valid only to the extent that translation of the viral genome mimics the translation of cell messengers. Further studies of the nature of the radiation lesion or lesions are continuing.

This work was supported by the US Atomic Energy Commission.

R. A. TOBEY
D. F. PETERSEN
R. A. WALTERS

Biomedical Research Group,
Los Alamos Scientific Laboratory,
University of California.

Received October 21; revised November 29, 1968.

[1] Walters, R. A., and Petersen, D. F., *Biophys. J.*, **8**, 1475 (1968).
[2] Walters, R. A., and Petersen, D. F., *Biophys. J.*, **8**, 1487 (1968).
[3] Abrams, R., *Arch. Biochem.*, **30**, 90 (1951).
[4] Harrington, H., *Ann. NY Acad. Sci.*, **95**, 901 (1961).
[5] Painter, R. B., and Hughes, H. L., *Ann. NY Acad. Sci.*, **95**, 960 (1961).
[6] Whitmore, G. F., Stanners, C. P., Till, J. E., and Gulyas, S., *Biochim. Biophys. Acta*, **47**, 66 (1961).
[7] Taylor, E., *Exp. Cell Res.*, **40**, 316 (1965).
[8] Tjio, J. H., and Puck, T. T., *J. Exp. Med.*, **108**, 259 (1958).
[9] Brownstein, B., and Graham, A. F., *Virology*, **14**, 303 (1961).
[10] Tobey, R. A., and Campbell, E. W., *Virology*, **27**, 11 (1965).
[11] Holland, J. J., and McLaren, L. C., *J. Bact.*, **78**, 596 (1959).