21% de cellules activées et 54% de cellules témoin. Ces observations semblent montrer que l'ATP accélère le processus d'absorption du DNA-H3 exogène.

Ces résultats de même que les expériences de Adams⁸ et les travaux de Gropp¹¹ sur l'effet de l'ATP semblent appuyer l'hypothèse d'une relation étroite entre pinocytose et absorption du DNA¹⁴.

	Cellules activéesª	Cellules témoin
Fort marquage $(n > 60)$	42,7%	1%
Faible marquage $(n < 60)$	21,6%	54,4%
	64,3%	55,4%

a Plus de 800 cellules ont été examinées pour chaque cas. n = nombrede grains par noyau. Un marquage inférieur à 10 grains par cellule n'a pas été pris en considération.

Summary. Cultures of HeLa cells treated with ATP show an increased uptake of DNA-H3 for a short period of treatment. In the light of GROPP's findings on pinocytosis activation, it is tempting to suppose that the stimulation of DNA-uptake by ATP is mediated through an activity of the latter on pinocytosis.

M. C. Loni

Section de Biochimie Cellulaire, Département de Radiobiologie, C.E.N.-S.C.K, Mol (Belgique), June 21, 1966.

Enhancement of Oxidation Products of Lipids in Liver Mitochondria of Whole-Body Irradiated Rats

There have been many reports on the early and mostly reversible damage of the structural and morphological integrity of rat liver mitochondria after whole-body Xirradiation. The observed changes appear very early, are of transient character, and are soon reconstituted up to the pre-irradiation level. The irradiated mitochondria still keep their repair ability. Thus the significant depression in total mitochondrial count per liver was observed very early in post-irradiation and returned to the normal level within 6h and kept the normal range through 48 h post-irradiation¹. Similarly the increased rate of oxidative phosphorylation was restored to the normal level 24 h after whole-body irradiation2. The elevated lability of isolated mitochondria is demonstrated by swelling, lysis, and disintegration. From this point of view, the observed correlation between swelling, lysis and mitochondrial lipid peroxidation³⁻⁵ is very interesting. It is very probable that the primary cause of mitochondrial disintegration is the peroxidation of unsaturated fatty acids, which are present in the mitochondria in a relatively high concentration and form the special functional component of phospholipid-protein complex of mitochondrial membranes and crista.

According to the well-known toxic effects of peroxides, their higher solubility and their many inhibitory properties to various enzymes, it is possible to suppose an important role in primary radiation changes 6.

We have estimated the effect of whole-body X-irradiation on the oxidation products of lipid mitochondria at various time intervals after exposure to 1400 r (Macrophos 250, 235 kV, 14 mA, 0.5 mm Cu, 1 mm Al, dose rate 70 r/min). The groups of male rats weighing 160-180 g were irradiated and killed at time intervals of 0, 3, 6, 12, 24 and 48 h after the irradiation. For 12 h before they were killed, the animals were left without food. The isolation of mitochondria was performed according to

Hogeboom⁷, and the final suspension was washed twice with isotonic NaCl (0.15M NaCl + 0.02M Tris pH 7.4). The concentration of the mitochondrial suspension was done at a protein content (bovine albumin - Mann) of 1 mg/ml of final mitochondrial suspension. Protein was determined by the biuret method⁸. Lipid peroxide was measured by the thiobarbituric acid colour test (TBA₅₂₅) after aerobic incubation in a Dubnoff shaker in the following manner: an amount of 4 ml mitochondrial suspension was put into a flask of about 25 mm diameter and incubated at 37 °C and simultaneously shaken. At time intervals of 15, 30, 60, 120, and 180 min, a sample of 0.5 ml was taken for TBA colour reaction in the following solution: 0.5 ml of incubated suspension + 0.5 ml isotonic saline + 1 ml 5% TCA + 1 ml 1% TBA. The mixture was heated for 10 min in a boiling water bath, cooled, centrifuged, and the clear supernatant directly measured (spectrophotometer CF 4 SB Optica, 525 nm).

The following observations were made. At zero time interval after the whole-body irradiation, the amount of TBA chromogen in mitochondria was elevated. The enhancement was statistically significant up to 6 h after irradiation; after this period the level returned to normal control value (Figure 1).

The production of TBA chromogen is linear, depending on the incubation time. The initial values after 15 min

- ¹ P. P. Noyes and R. E. Smith, Expl Cell Res. 16, 15 (1959).
- ² J. C. Hall, A. L. Goldstein, and B. P. Sonnenblick, J. biol. Chem. 238, 1137 (1963).
- ³ F. E. Hunter, J. Weinstein, A. A. Scott, and A. K. Schneider, Biochem. biophys. Res. Commun. 11, 456 (1963).
- A. A. Scott and F. E. Hunter, Biochem. biophys. Res. Commun. 11, 461 (1963).
- F. E. Hunter, A. Scott, P. E. Hoffsten, J. M. Gebicki, J. Wein-STEIN, and A. Schneider, J. biol. Chem. 239, 614 (1964).
- E. D. Wills, Biochem. Pharmacol. 7, 7 (1961).
- ⁷ G. H. Hogeboom, in Methods in Enzymology (Ed. S. P. Colowick and N. O. Kaplan; Academic Press, New York 1955).

 8 E. E. Jacobs and M. Jacob, J. biol. Chem. 223, 147 (1956).

¹⁴ Tous nos remerciements vont au Dr A. Billau et à Mademoiselle F. Cornette (Rega Institut, Louvain) qui nous ont procuré les cellules HeLa utilisées dans ces expériences et à Mr. P. Charles (Département de Radiobiologie, C.E.N.-S.C.K., Mol) pour le DNA-H3. Travail effectué dans le cadre du contrat Euratom-CEN N° 053-64-3 BIOB.

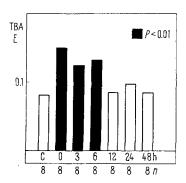


Fig. 1. TBA chromogen values in irradiated rat liver mitochondria after 180 min aerobic incubation at 37 °C.

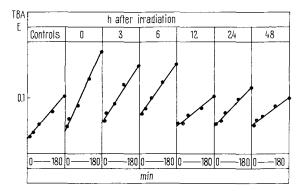


Fig. 2. The dependence of TBA chromogen production on the irradiation time in irradiated rat liver mitochondria.

incubation were higher in irradiated mitochondria, but only up to 6 h post-irradiation (Figure 2).

The higher level of TBA chromogen is not, of course, an evidence of elevated lipid peroxides after irradiation in vivo. The cell has a large number of stabilizing factors, some of which may be damaged by irradiation. The irradiation may influence these factors. In this connection it seems to be very interesting that a lipid fraction with a strong swelling effect has been isolated from the liver of irradiated rats. The results of this experiment show that although the mitochondria are transiently damaged by irradiation in vivo, they are soon reconstituted to reach pre-irradiation level according to the recovery of other morphological and functional changes.

Zusammenfassung. Der Einfluss der ionisierenden Strahlung auf die Bildung der Oxydationsprodukte von ungesättigten Fettsäuren in Lebermitochondrien ganzkörperbestrahlter Tiere wurde untersucht. Die TBS-Chromogenwerte erhöhen sich in bestrahlten Mitochondrien statistisch signifikant und sinken in 12 h nach der Bestrahlung wieder zur Norm ab.

B. Zícha, K. Lejsek, J. Beneš, and Z. Dienstbier

Department of Biophysics, Faculty of Medicine, Charles University, Prague (Czechoslovakia), March 2, 1966.

⁹ H. Urakami, J. Okayama med. Soc. 74, 307 (1962).

Direct Action of Extracellular Ca Ions on Skeletal Muscle

It is known that the intracellular Ca⁺⁺ regulates the behaviour of the contractile structures of muscle fibre and is also involved in excitation-contraction coupling. The frog skeletal muscle in vivo contains a total of $1.4-2\cdot 10^{-3}$ M Ca/kg of wet tissue. The intracellular free Ca⁺⁺ concentration is maintained at a level of about $10^{-7}M$ by the calcium binding and storing systems. In frog blood plasma the total calcium concentration was found to be about $1.8\cdot 10^{-3}M^1$. CaCl₂ is added to the physiological salunt solution in a concentration between 1 and $2\cdot 10^{-3}M$ in order to simulate the extracellular ionic conditions of cold-blooded animals.

The lowest Ca⁺⁺ concentration necessary to maintain the physiological properties of muscle membrane and end plate is about $10^{-4}\,M^{2,3}$. By storing the muscles in Ringer's solution with 'normal' Ca content $(1-2\cdot 10^{-3}\,M)$, the amount of intracellularly accumulated Ca essentially increases ^{4–6}. This fact leads Shanes and Bianchi to the conclusion that a CaCl₂ concentration of $1\cdot 10^{-3}M$ corresponds better to physiological conditions than higher values.

In spite of the observed Ca influx in skeletal muscle it was found that only a very high Ca concentration in the external medium induces contractures of low tension? These experiments were performed with muscles prebathed for various lengths of time in Ringer's solution with $1.8 \cdot 10^{-3} M$ CaCl₂. In most recent investigations, the authors found that freshly dissected muscles of Rana esculenta go into contracture after being dipped into Ringer's solution. The tension development depends on the Ca concentration of the external solution. The Figure shows the contracture tension at the various Ca concentrations expressed in the percentage of the maximal tension development of individual muscle in isotonic CaCl₂

- $^{\rm 1}~$ B. Merrem and G. Küchler, Acta biol. med. germ. 14, 669 (1965).
- ² H. C. Lüttgau, J. Physiol. 168, 679 (1963).
- ³ D. Elmquist and D. S. Feldmann, J. Physiol. 181, 487 (1965).
- D. L. GILBERT and W. O. FENN, J. Gen. Physiol. 40, 3, 393 (1957).
 A. M. SHANES and C. P. BIANCHI, J. Gen. Physiol. 42, 5, 1123
- ⁶ E. Cosmos and E. J. Harris, J. Gen. Physiol. 44, 1121 (1961).
- W. Kutscha and K. Brecht, Pflügers Arch. ges. Physiol. 272, 479 (1961).