EFFECT OF GAMMA IRRADIATION IN VITRO ON THE CYTOPLASMIC PARTICULATES OF MOUSE LIVER 1. MITOCHONDRIA

By

Keiji HORI and Yasusada FUJINO

From the Department of Radiology, Osaka University Medical School

(Director: Prof. H. Tachiiri)

Changes in functions of several enzyme systems in mouse spleen or liver following whole body irradiation have been previously reported[1)~6)]. Such changes have been observed initially in the enzyme systems of respiration and of phosphorylation and later in those of nucleic acid and protein synthesis. But much seems left to be seen before the interrelations between the such changes are fully clarified.

In the present study an attempt was made to clarify what reaction system might be initially affected by radiation followed by subsequent functional changes. Respiration and phosphorylation were pursued on the basis of mutual enzymic reactions, subject to various dosage of radiation. Morphological changes were studied with a electron microscope.

Materials and Methods

DDO male mice weighing an average 20 gm. were used. The animals were starved overnight and then decapitated. The liver was placed in cold 0.9% KCl; liver connective tissue was removed and suspended in 10 ml. of a cold 0.013 M kalium phosphate buffer solution containing 0.123 M KCl, 0.005 M NaCl, and 0.0012 M $MgCl_2$, and then homogenized in a glass homogenizer. Mitochondria were isolated from the homogenate with a differential centrifugation (Schneider, 1950[7)]). After the final centrifugation, mitochondria were suspended in 2 ml. of the same buffer solution per gm. of the original liver. All experiments were carried out in Warburg's apparatus at 30°C. For irradiation about 30 mC of ^{65}Zn was put into a chamber which surrounded the center well of Warburg flask as shown in Figure 1; an average intensity of gamma irradiation was about 18r per minute. For the estimation of a series of respiratory enzyme systems succinate, fumarate, malate, citrate or α-ketoglutarate was used as substrate. Readings of oxygen uptake were done every 15 minutes for one and half hours. Oxidative phosphorylation was measured by the ratio of P : O following a modified method of Maruo[8)]. using citrate, malate, etc. as substrates. The main compartment of the chamber contained 100 μM KCl, 20 μM MgCl, 80 μM Cytochrome C, 40 μM NaF, and 1 ml. of a mitochondrial suspension. The center well held 0.2 ml. 20% KOH, and side arm 20 mg. of hexokinase. Readings of oxgen uptake were done every 15 minutes for one and half hours, after which time the reactions were stopped with 78% TCA and the amounts of phosphate were determined by the Fisk and Subbarow method[9)]. ATP or ADP was analysed with the anion exchange method of Cohn and Carter[10)]. The column was about 1 cm. in diameter containing 3 cm. high Dowex-1, 400 mesh. ATP-ase and pyridine nucleotide were measured with the method of

Fig. 1. Warburg flask

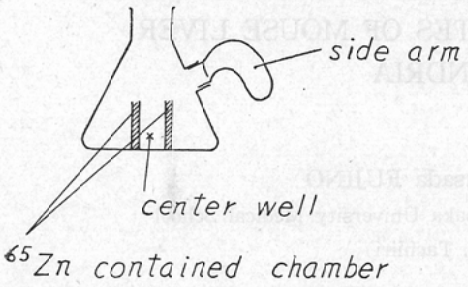
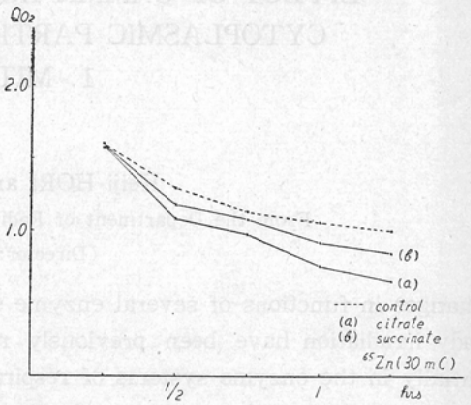


Fig. 2. Respiratory enzyme systems inactivation curve



Potter[11] and the method of Colowick[12] respectively. Each value was studied statistically.

Results

Figure 2 shows inactivation curves of respiratory enzyme systems of mitochondria. The Q_{O_2} value of the control reveals a slight decrease in the course of irradiation, but that of the irradiated (about 1000 to 1700r) shows a considerable decrease. Inactivation curves when malate, fumarate or α-ketoglutarate is used as substrate are almost identical with those when citrate (curve I(a)) is used. But the use of succinate results in a slightly more decrease.

Fig. 3. Pyridine nucleotide value

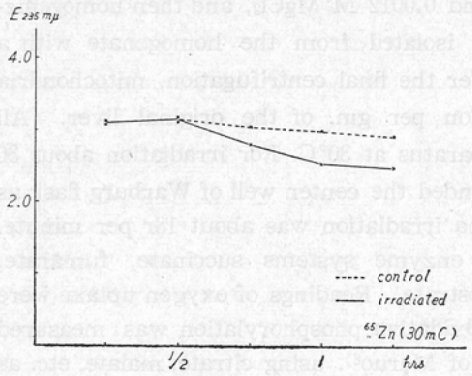
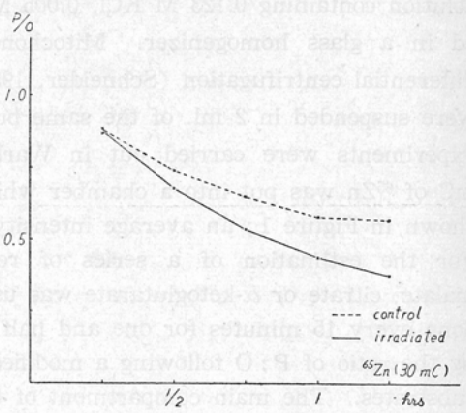


Fig. 4. Oxidative phosphorylation value



The differences in the value of succinate and other substrates are statistically non-significant where $P=0.02$. Figure 3 shows the amounts of pyridine nucleotide of mitochondria following irradiation. These amounts of the irradiated are slightly decreased in about one and half hours disclosing a statistical significance, where $P=0.02$. In oxidative phosphorylation the P : O value is decreased following irradiation (Figure 4), using fumarate, but a reduction of the irradiated is lower and delayed to less extent than that in

Fig. 5. Adenosine nucleoside value

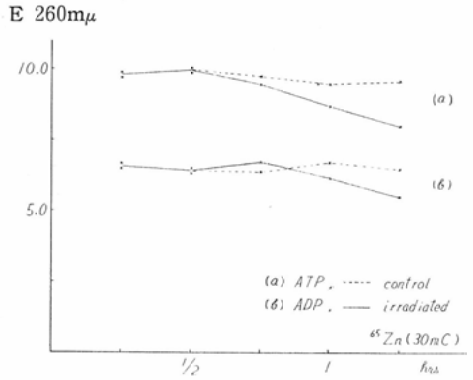


Fig. 6. ATP-ase activity

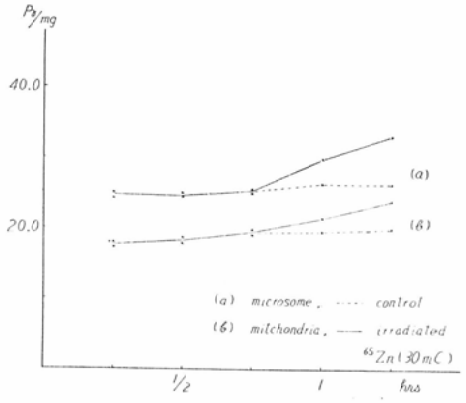
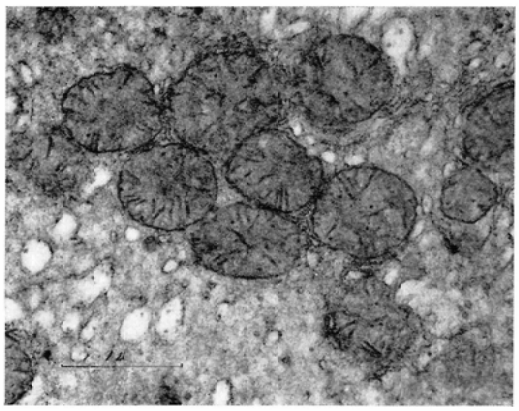
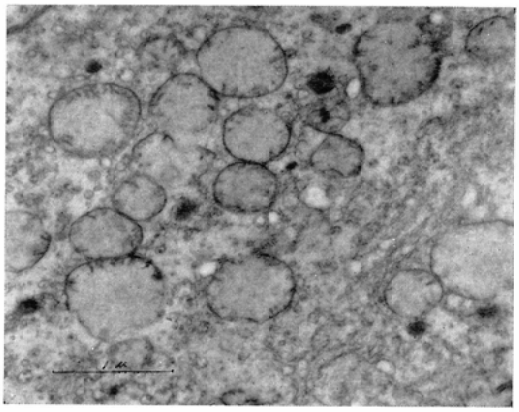


Fig. 7. Normal mitochondria in situ. ×40,000



Irradiated mitochondria in situ (15 minutes following irradiation). ×40,000



respiratory enzyme systemes.

The amounts of ATP and ADP in mitochondria following irradiation are shown in Figure 5. ATP is obviously decreased when oxidative phosphorylation is inhibited by irradiation. ADP is also decreased, although in somewhat less degree.

Following irradiation the activity of ATP-ase shows a slight increase in about one and half hours. An increase in the activity of mitochondria is less than that in the microsome.

A morphorogical structure of mitochondria is shown in Figure 7 electron microscopically. Even 15 minutes after whole body irradiation an obvious swelling and dilution in the matrix are seen in mitochondria. Cristae mitochondriales are few in number, being shortened and of irregular in form. A trend of vesiculation is observed. Morphological these findings reveal that mitochondria are easily affected by irradiation.

Discussion

The present experimental results indicate that initially the respiratory enzymic systems

in vitro in mitochondria are found suppressed following irradiation. Cohen et al[13] postulate that the radiosensitive site is located somewhere in the oxido-reduction chain of cellular metabolism.

Following 15 minutes' irradiation histologically there appear a swelling of mitochondria, a dilution of the matrix and deformity of crista. There also observed a decrease in the amount of pyridine nucleotide, a slight difference in the inactivation curve between the usage as substrate of citrate and succinate, and almost a constant succinic dehydrogenase activity maintained throughout irradiation. These findings suggest disturbances in an oxidation process that seem to have developed during a course of electron transportation. Uncoupling of oxidative phosphorylation seems to be delayed; oxidation was relatively suppressed. Thus, it is considered that the suppression precedes phosphorylation.

Yost et al[14] reported that isolated rat liver mitochondria required a dose of over 10,000r to produce inactivation of phosphorylation. But a decrease in the amount of ATP or ADP observed in the present experiment that the uncoupling of phosphorylation does not require so large a dose.

Early histological changes observed following whole body irradiation also suggest that mitochondrial inactivation may result mainly from indirect action. It seems necessary to discuss the problem of direct or indirect inactivation of mitochondria from various points of view. But a stress should be given to disturbances of oxidation and of uncoupling of phosphorylation on an metabolic basis.

An increase of ATP-ase activity following irradiation is interpreted to show no marked influence on oxidative phosphorylation or respiratory enzyme inactivation. But further study seems necessary before such interpretation is fully confirmed in reference to a metobolic pahtway. Interrelations between cellular inactivation following irradiation in vitro seem to require further study with cell culture, isotope techniques, etc.. The metabolism of nucleotide, protein and nucleic acid in cellular particulates and in nucleus is also to be studied.

Summary

Effects of gamma irradiation in vitro for isolated cytoplasmic particulates (mitochondria) studied are as follws:

1. Disturbances of respiratory enzyme systems in mitochondria develop in the early stage.
2. Uncoupling of oxidative phosphorylation seems to be slightly delayed; oxidation is relatively suppressed.
3. Pyridine nucleotide is slightly decreased.
4. An increase of ATP-ase activity in mitochondria is slight, appearing in a more or less late stage.

References

1) Ashwell, G., and Hickmann, J.: J. Biol. Chem. 201, 651 (1953). —2) Kurnick, N.B., Massey, B.W.,

and Saudeen, G.: Rad. Research 9, 141 (1958). —3) Maxwell, E., and Ashwell, G.: Arch. Biochem. Biophys. 43, 389 (1953). —4) Van Bekkum, D.W.: Liege Radiology Symposium 201 (1954). —5) Potter, R.L., and Bethell, F.H.: Fed. Proc. Proc. 11, 270 (1952). —6) Hori, K.: Symposia cell. Chem Vol. 9, 109 (1959). —7) Schneider, W.C., and Hodgeboom, G.H.: J. Biol. Chem. 183, 123 (1950). —8) Maruo, B.: Standard biochemical experiments, 1ed, Bunkodo, Tokyo, 392, (1955). —9) Fiske, C.H., and Subbarow, Y.: J. Biol. Chem. 80, 407 (1952). —10) Cohn, W.E., and Carter, C.E.: J. Am. Chem. Soc., 72, 4273 (1950). —11) Potter, V.R., Manometric techniques and tissue metabolism, 6ed, Burgess Co., Minneapolis, Minn: 139 (1951). —12) Colowick, S.P., Kaplan, N.O., and Ciotti, M.M.: J. Biol. Chem. 191, 447 (1951). —13) Cohen, J.A., Vos., O., and van Bekkum, D.W.: Advances in Radiology, Oliver and Boyd Co.: Edinburgh, London (1956). —14) Yost, H.T., Jr., and Robson, H.H.: Biol. Bull., 113, 198 (1957).

マウス肝細胞質顆粒に及ぼす in vitro γ 線照射の影響

1. ミトコンドリア

大阪大学医学部放射線医学教室（主任　立入教授）

堀　　啓　二　　藤　野　保　定

（昭和36年6月19日受付）

抄　録

放射線の細胞質顆粒に対する作用はエネルギー系及び蛋白，核酸代謝系ならびにこれらの関連性の検討において追求さるべきである．

本実験はマウス肝より遠心分劃したミトコンドリアを用いて恒温槽中30℃でγ線を（^{65}Zn を照射線源として）照射しながら燐酸化機転，呼吸酵素系，ＡＴＰ，ＤＰＮ等の補酵素への影響ならびにこれらの関連性について検討を行い次の結果をえた．

1. 呼吸酵素系は早期に影響を蒙むる．

2. 酸化的燐酸化の共転化への障害は酸化への障害におくれるようである．

3. ピリヂンヌクレオチイドは軽度に低下を示す．

4. ＡＴＰアーゼ活性度の亢進はミトコンドリアにおいては軽度で，時期的に現れ方がおそい．