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# Effects of Ionizing Radiations on Oxygen Uptake by Rat Liver Slices, Mitochondria, and Mitochondrial Membranes

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It has been shown that irradiation causes a decrease in oxygen consumption of rat liver slices. Electron microscopy of the irradiated tissue reveals marked swelling of mitochondria accompanied by a decrease of the density of the matrix, as well as by vesiculation of the rough and smooth endoplasmic reticulum. In jejunum irradiation causes a shortening of the microvilli and a reinforcement of the microfilaments of the terminal web. It was also found that in suspensions of mitochondria from irradiated rat liver slices, irradiated mitochondria and irradiated inner mitochondrial membranes there was a faster onset of swelling and lysis provoked by ascorbate. It has been also shown that irradiation of inner mitochondrial membranes causes the formation of lipid peroxides.

In previous reports from our laboratory (10) we have shown that doses of 20,000 r, *in vitro*, on strips of rat jejunum with or without added substrate cause a marked inhibition of oxygen uptake, as well as a decrease of glucose utilization when this sugar is present in the medium. Under those conditions the amount of lactate production was unaffected. These results could be interpreted as if a higher proportion of the glucose metabolized by the irradiated strips had been converted into lactate. These metabolic alterations could be explained by an inhibition of the oxidative mechanism of the irradiated tissue.

On the other hand, *in vivo* irradiation of rats with 700 r (2) inhibits oxidative phosphorylation in spleen and thymus; the activity of succinate dehydrogenase and cytochrome oxydase of rat liver mitochondria are also inhibited (4).

CLARK has also shown that irradiation of isolated rat liver mitochondria with doses of 100,000 r decreases both the oxidative phosphorylation and respiratory control (3). These biochemical alterations are usually attributed to an indirect action of the radiation yielding free radicals which would in turn cause the formation of peroxides of organic molecules. This effect

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has also been extensively studied by WILLS and ROTBLAT (21) in animal tissues and by WILLS and WILKINSON (22) in mitochondrial-lysosomal fractions.

HUNTER *et al.* (7, 9) have found that the formation of peroxides results a disaggregation of the mitochondrial structure, WILLS and WILKINSON has also suggested the same effect on the lysosomal structure (22).

In our Laboratory (13, 14) we have found that both ascorbate and cysteine effect the phospholipids of mitochondrial membranes, due to peroxidation of their unsaturated fatty acids, bringing about the disaggregation of the membrane structure.

In the work here reported, our aim has been to study if the oxygen consumption by irradiated rat liver slices is also inhibited in the same manner as it had been found using strips of jejunum (10); and also to observe the effects of X-ray on liver mitochondria. Assuming that the mechanism of action of irradiation on mitochondria could be explained as indicated above, we decided to carry out different types of experiments to study the behaviour of mitochondria of irradiated liver and isolated irradiated mitochondria in the presence of ascorbate, substance which is known to cause peroxidation of structural lipids.

## Materials and Methods

Male Wistar rats, weighing between 150 and 200 g, were starved for 48 hours and killed by decapitation: jejunum, and liver were rapidly removed, washed and chilled in Krebs-Ringer-Tris solution. Slices of 0.5 mm were prepared with a hand microtome, and washed in the Krebs-Ringer solution as described by UMBREIT *et al.* (19), using Tris-HCl as buffer.

Liver mitochondria were prepared as described by HOGEBOOM (6). Inner mitochondrial membranes were prepared following the method of PARSONS *et al.* (12), using the «low speed pellet», which had been washed three times in 1 mM buffered phosphate.

Irradiation was carried out in a Siemens radiotherapy apparatus at 200 KV, 15 m A with an Aluminium filter of 2 mm, which gives 1,000 r/min at 14 cm from the focus. The irradiation time was 20 minutes and the samples were kept at 25° C.

Oxygen uptake was measured by the direct method of Warburg (20) trapping to $CO_2$ with 10 % KOH.

After irradiation with 20,000 r mitochondria or inner mitochondrial membranes aliquots were taken and resuspended in a medium containing 0.02 M Tris-HCl, pH 7.4, in 0.25 M sucrose, with or without added ascorbate (final concentration, 1 mM). The final protein concentration in the suspension was 2 mg per ml in the case of mitochondria from irradiated liver; and 1.35 mg per ml in the case of irradiated mitochondria and inner mitochondrial membranes. Incubations were carried out at 30° C, with continuous shaking, and changes in optical density of the suspensions were followed at 520 m$\mu$ with a Zeiss PMQ II spectrophotometer at different intervals. The decrease in optical density reflects the degree of mitochondrial swelling and structural disaggregation.

Electron microscopy of jejunum and liver was carried out on this sections embedded in Epon. Fragments about 1 $mm^3$ were fixed from 1 to 3 hours at 4° C with buffered glutaraldehyde and postfixed in Veronal buffered 2 % osmium tetroxide for 2 hours. The fragments were dehydrated in ethanol, soaked in propylene oxide and embedded in Epon 812. Thin sections were stained with uranyl acetate and/or lead hydroxyde and examined with a Siemens Elmiskop IA electron microscope.

## Results

Irradiation of rat liver slices caused an inhibition of oxygen uptake which was as

Table I. *Effect of the irradiation (20,000 r) on the respiratory metabolism by rat liver slices.*

Data in $\mu$M $O_2$/100 mg w.w./hr. The number of experiments carried out is given in parentheses. Statistical calculations according to Student's $t$ method. $P < 0.001$ in all experiments.

| | TIME (hours) | | |
|---|---|---|---|
| | 1st | 2nd | 3rd |
| Control | 3.70 ± 0.13 (29) | 3.14 ± 0.12 (31) | 2.71 ± 0.12 (28) |
| Irradiated | 2.78 ± 0.11 (27) | 2.14 ± 0.11 (31) | 1.55 ± 0.13 (28) |
| Inhibition % | 24.86 | 31.84 | 42.80 |

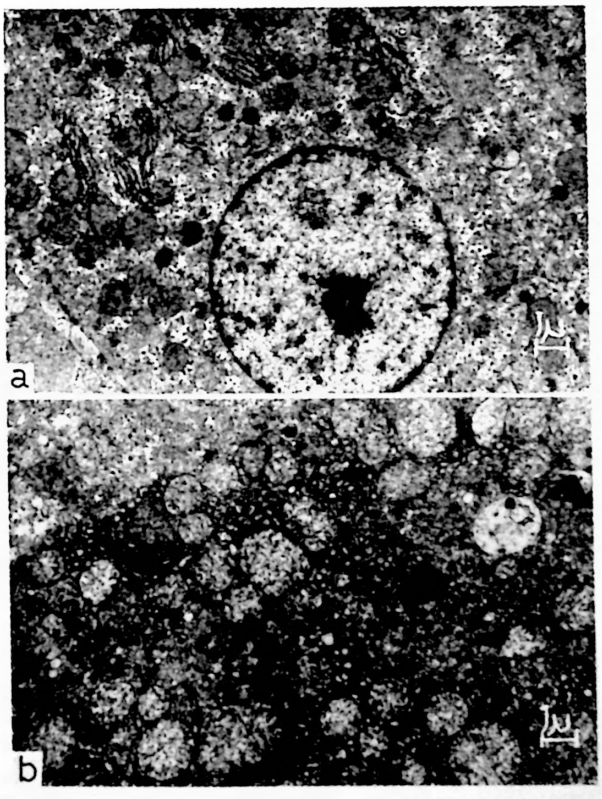


Fig. 1. *Electron micrograph of a) a normal and b) an irradiated liver cell.*

high as 24 % in the first hour and reached 42 % in the third hour (Table I). It should be noted that this inhibition increases with time. These results are comparable to

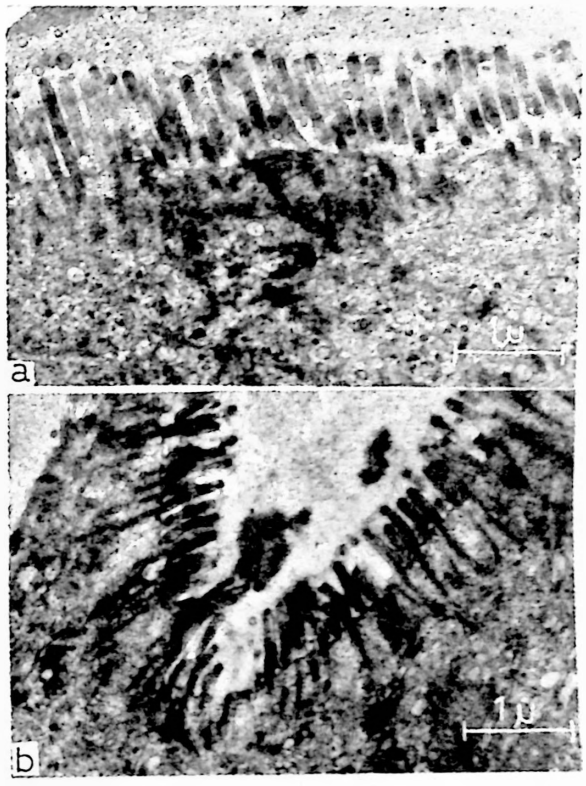


Fig. 2. *Electron micrograph of epithelial cells of a) normal, and b) irradiated jejunum.*

those found in our Laboratory with rat jejunum strips (10).

When the irradiated tissue, liver or jejunum, was studied with the electron microscope several histologic alterations were found. Figure 1 *a* shows the electron micrograph of a normal liver cell; a moderate density of the mitochondrial matrix can be observed. This picture is quite different after X-ray irradiation as it can be seen in Figure 1 *b*; a marked swelling of the mitochondria together with a great decrease of the density of the mitochondrial matrix, as well as some vesiculation of the rough and smooth endoplasmic reticulum. These findings confirm those of GOLDFEDER in tumor cells (5). Similar pictures were observed with irradiated jejunum strips. Besides, in this tissue a shortening of the microvilli of the epithelium was also found after irradiation (Fig. 2).

In the apical part of the cell appeared the microfilaments of the terminal web more reinforced and much longer in the irradiated jejunum than in the normal intestine.

After having observed these alterations of the cellular structure it was decided to study the effect of the X-ray irradiation on the swelling and lysis of mitochondria provoked by the ascorbate. These experiments were carried ut with mitochondria isolated from irradiated liver slices and with mitochondria which had been directly irradiated *in vitro*. In both types of experiments it was found that irradiation causes a slow decrease in optical density of the suspensions, as well as a faster onset of swelling and lysis provoked by the ascorbate (Figs. 3 and 4). Similar results were obtained with isolated inner mitochondrial membranes (Fig. 5). In this case lipid peroxide formation was determined with the TBA test (8) after 1 hour of incubation, in irradiated and non irradiated controls; these values expressed in O.D. units were 0.074 ± 0.010 in non irradiated controls and 0.151 ± 0.013, per ml of suspension, in the irradiated inner mitochondrial membranes.

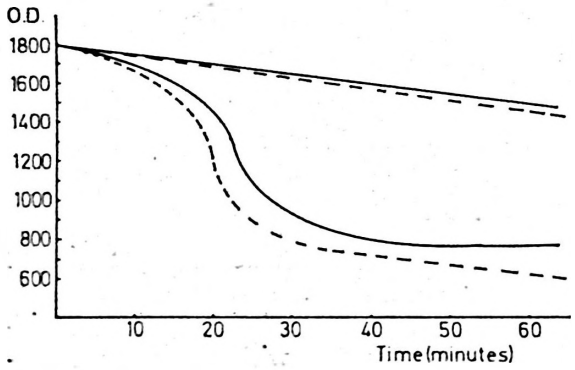


Fig. 3. *Optical density changes of mitochondrial suspensions from irradiated (---) and controls (—) liver slices.*
Upper, without ascorbate; lower, with 1 mM ascorbate.

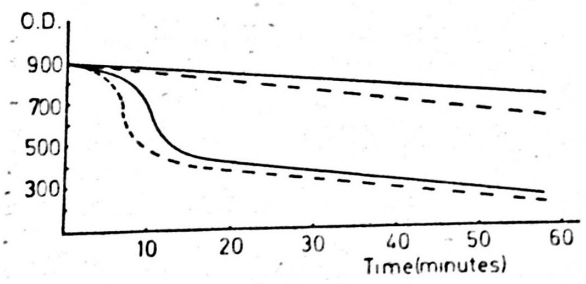


Fig. 4. *Irradiation of the mitochondrial suspensions.*
As an figure 3.

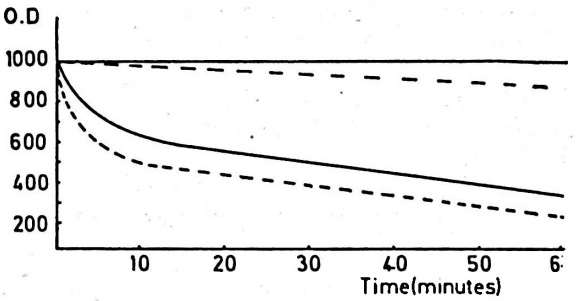


Fig. 5. *Optical density changes of control (—) and irradiated (---) mitochondrial inner membranes.*
Upper, without ascorbate; lower, with 1 M ascorbate.

## Discussion

Irradiation of jejunum strips and liver slices with 20,000 r inhibits the oxygen uptake. This alteration of the oxidative metabolism had been interpreted in previous reports (10) as a consequence of a lesion at the level of the oxidative cell systems. These results are in agreement with those found by other authors, who have observed an inhibition of oxidative phosphorylation in mitochondria (1, 15, 16), alterations of the electron transport chain in thymus mitochondria (15, 16) a transient decrease in the amount of protein in mitochondria of irradiated animals, and an acceleration of lipid peroxide formation induced by ferrous ions (11).

The results shown in this paper seem to point out that the action of X-ray irradiation is in some way similar to that of the ascorbate on mitochondrial membranes,

i.e., peroxidation of structural lipids. Irradiation by itself would not cause lysis, of the mitochondrial membranes, at heart under the conditions of our experiments, but it would render those membranes more sensitive to the action of ascorbate.

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