the change in ionic conductance leads to an increase of the net influx of calcium ions due to the reduction of a backward flux of calcium during the time the papillary muscle is depolarized.

Zusammenfassung. Eine Verringerung der extrazellulären Kaliumkonzentration im Bereich zwischen 9,6 und 2,4 m*M* führt zu einer Zunahme der positiv inotropen Glykosidwirkung am Meerschweinchenpapillarmuskel; die Wirkung des Adrenalins wird nicht beeinflusst. Die Vergrösserung der inotropen Wirkung geht parallel einer starken Zunahme der Verkürzung der Aktionspotentialdauer, besonders in Höhe des Plateaus (30% Repolarisation).

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Post-Irradiation Induced Sensitization of Inhibition of Oxidative Phosphorylation by Iodoacetamide

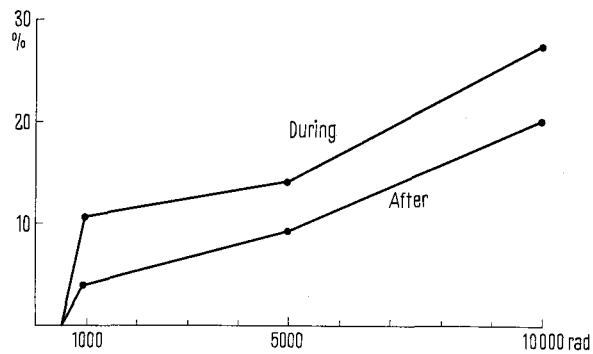
The potent sulphydryl poison iodoacetamide (IAA) and the related iodoacetate are known as radiosensitizers on death of mammals[1], inactivation of bacteria[2], loss of intracellular potassium and hemolysis of erythrocytes[3], etc. The mechanism of action is still largely unknown. With the aim of investigating the possibility that iodoacetamide inhibits post-irradiation repair processes[2], we investigated the effect of IAA on the oxidative phosphorylation during and after irradiation of rat liver mitochondria in vitro.

Method. The mitochondria of rat liver were isolated in the manner described earlier[4], diluted in tris buffer and irradiated by a Philips-MG 150 apparatus (6670 rad/min). After irradiation the mitochondria were reconcentrated and placed immediately in the incubation mixture. (Each flask contained in 3.95 ml: 24 μ moles $MgCl_2$; 39 μ moles KF; 5.76 μ moles DPN; 6.0 μ moles tris buffer, pH 7.4–7.5; 59 μ moles phosphate buffer; 30 μ moles AMP; 0.6 mg cytochrome c; 30 μ moles α-ketoglutarate; 0.5 mg hexokinase; 38.9 μ moles glucose; 5 μ moles ATP.) In one part of the experiments iodoacetamide was added during the irradiation at different concentrations to the mitochondrial suspension in buffer (pH 7.4 or 7.8). In another part IAA was given after irradiation, either immediately in the buffer with mitochondria or in the reaction mixture of the Warburg vessels. Oxygen consumption and loss of inorganic phosphate[4] was tested for 30 min at 20 °C (equilibration time 10 min).

A marked inhibition of oxidative phosphorylation can be seen when IAA is present during the irradiation of the mitochondrial suspension diluted in tris buffer and after removal from the suspension (total exposition time: 15 min) (Table I). IAA is known, like the iodoacetate, as an uncoupler of oxidative phosphorylation[5] and an inhibitor of oxygen uptake[6], but, with the concentration used of 1 m*M* IAA, the P:O ratios are not deeper than the ratios of non-treated controls. Irradiation with 10,000 rad alone alters only slightly the P:O ratio (inhibition 3–4%). The sensitization is largely dependent on the irradiation dose (Figure 1). When the IAA was given immediately after irradiation in the diluted suspension and removed after 10 min, we also observed a depression of the P:O ratios (Table II). The effect still remains when IAA is added 10 min after irradiation, but the P:O ratio of unirradiated sample is also depressed. The sensitizing effect is rever-

Table I. Application of IAA (concentration 1 m*M*, total 12 ml suspension) during the irradiation

Dose (rad)	No. of measurements	Change (%) in O_2 uptake	P:O ratio		Change (%) in P:O ratio
			Unirradiated	Irradiated	
500	6	− 35.9	2.35	2.36	–
1,000	6	+ 13.5	2.39	2.14	− 10.5
5,000	6	− 14.4	2.85	2.45	− 14
10,000 without IAA	18	− 12	2.08	1.53	− 27.4
10,000	18	− 16	2.25	2.17	− 3.6



Irradiation dose dependency of the sensitizing effect of IAA during (Table I) and after (Table II) irradiation.

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Table II. Application of IAA after irradiation in the irradiated suspension (concentration 0.1 m*M*) and in incubation medium. Each flask contained 0.125 m*M*

Dose (rad)	Treatment with IAA	No. of measurements	Change (%) in O_2 uptake	P:O ratio		Change (%) in P:O ratio
				Unirradiated	Irradiated	
10,000	Immediately after irradiation for 10 min, 0.1 m*M*	18	− 35.6	1.97	1.42	− 27.9
10,000	10 min after irradiation for 10 min, 0.1 m*M*	18	− 35.1	1.52	1.27	− 16.5
10,000	0	6	− 4.5	2.35	2.28	− 3
500	During incubation, 10 min after irradiation, 0.125 m*M*	6	− 14.2	1.60	1.85	+ 15.6
1,000	During incubation, 10 min after irradiation, 0.125 m*M*	6	− 11.1	1.81	1.74	− 3.9
5,000	During incubation, 10 min after irradiation, 0.125 m*M*	6	− 11.8	1.84	1.67	− 9.2
10,000	During incubation, 10 min after irradiation, 0.125 m*M*	6	+ 15.8	2.28	1.82	− 20.2

sible, because after two careful washings in saccharose-versene solution the P:O ratio becomes normal again. To investigate whether a minimum amount of IAA present during measurement can inhibit oxidative phosphorylation, 0.125 m*M* of IAA were added to the reaction mixtures (10 min after irradiation). The same enhanced radiosensitivity and dose dependency was found (Table II); 0.125 and 0.25 m*M* IAA alone depresses only oxygen uptake (Table III).

These investigations show that one of the most important functions of the cell, the formation of adenosine triphosphate, can be strongly influenced by treatment after irradiation. The sensitizer iodoacetamide damages the irradiated multi-enzyme system of oxidative phosphorylation even in the small amount of 0.125 m*M* given 10 min after irradiation. This can explain some sensitizing effects on microorganisms[2,7] as an inhibition of post-irradiation repair processes. The lack of ATP enhances the irradiation damage. Since, in cellular damage, the support of ATP should presumably be there immediately after irradiation, a post-irradiation treatment of microorganism can be without effect on the cell survival[7].

We considered tentatively the possibility of explaining the radiosensitizing effect of IAA by supposing that IAA releases latent radio-induced ATPase in mitochondria, by reacting with an ATPase-inhibitor complex (see discussion on latent ATPase and ATPase inhibitor complex in [5] and [8]). IAA unmasks a latent irradiation damage which may be a defect in the membrane. The possibility not excluded is that IAA reacts with DNA and RNA present in the mitochondria, although the function of nucleic acid in mitochondria is unknown. Further work is in progress.

Table III. Effect of iodacetamide on oxygen uptake and oxidative phosphorylation

Concentration of IAA in incubation medium	P:O ratio	Change in O_2 uptake	Change in P:O ratio
–	2.07	–	–
0.125 m*M*	2.28	− 50.56	+ 10.14
0.25 m*M*	2.09	− 43.33	+ 0.97
0.31 m*M*	1.84	− 32.56	− 11.1

Zusammenfassung. Zugabe von Iodazetamid während und nach der Bestrahlung von isolierten Lebermitochondrien der Ratte sensibilisiert die strahleninduzierte Hemmung der oxydativen Phosphorylierung. Die Nachbehandlung lässt einen latenten Strahlenschaden sichtbar werden, der selber vermutlich in einer Produktion latenter ATPase besteht. Es ist wahrscheinlich, dass die bekannte strahlensensibilisierende Wirkung von Iodacetamid bei Lebewesen und Zellen auf dieser Hemmung der ATP-Produktion beruht, welche Erholungsprozesse erschwert.

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