# Nd:YAG LASER RADIATION MODIFIES THE PROTEOLYTIC RESISTANCE OF RAT LIVER TISSUE PROTEINS

**R. R. Machyulaitis, G. L. Zel'tser, A. A. Zhloba, M. S. Pluzhnikov, and A. I. Nevorotin**

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Nd:YAG laser radiation ($\lambda$ = 1.06 $\mu$) is absorbed mainly in the tissues by macromolecules, and if present in high intensity it causes their denaturation [9, 10]. Under those conditions, necrosis with inactivation of histochemically detectable enzymes, followed by substantial slowing of resorption of the necrotic irradiated tissue, are observed [2-4, 8]. Meanwhile cellular infiltration around the necrotic tissue was found to exceed somewhat that observed after other forms of intervention, including laser radiation with lower intensity [3]. The mechanisms of delayed resorption of dying tissue with a simultaneous intensification of infiltration around it are not yet clear. The aim of the present investigation was to study the biochemical and structural nature of components of the irradiated cell responsible for these reactions.

## EXPERIMENTAL METHOD

Strips of tissue about 2 mm thick were excised from the liver of noninbred albino rats. Each strip was irradiated by an Nd:YAG laser beam through a quartz light guide 1 mm in diameter at a distance of 15 mm from the object to the end of the light guide, and with a power of 15 W acting for 45 sec [2, 3]. Homogenates of the irradiated and unirradiated (control) samples were prepared (20 mg/ml calculated as mass of intact tissue). The residue after centrifugation (10,000*g*, 40 min, 20°C) was washed twice, and the content of protein material in the supernatant was determined at 280 nm. The results were expressed after calibration as tyrosine [1]. Absorption spectra were determined on a "Specord M-40" spectrophotometer. The concentration of products soluble in 5% TCA in the supernatant and residues was studied. Proteolytic resistance of the homogenates and washed residues was studied with the use of trypsin (25 $\mu$g/ml), and also of a mixture of trypsin with chymotrypsin (50 $\mu$g/ml of each) at 37°C, for between 30 min and 24 h. The residual material after proteolysis (24 h) was solubilized in 5 N KOH.

Electron-microscopic investigation of the samples also was carried out. For this purpose fragments of liver tissue 4 and 24 h after the beginning of incubation in the proteinases, were fixed with aldehydes [3], postfixed with osmic acid, and after subsequent treatment in the usual way, including ultramicrotomy, they were examined in the IEM-7A electron microscope.

## EXPERIMENTAL RESULTS

Homogenates of native liver tissue contained only traces of TCA-soluble products of peptide nature. Their concentration was increased by many times 30 min after the beginning of proteolysis with trypsin (46.3 ± 3.5 and 175.4 ± 3.1 respectively, $n = 3$, $p < 0.001$). The material irradiated under identical conditions had higher initial (87.3 ± 1.2, $n = 3$, $p < 0.05$) and final (236.4 ± 4.6, $n = 3$, $p < 0.05$) values. These data were expressed in nanomoles tyrosin per milligram mass of liver. The release of proteolytic products was most rapid during the next 2.5 h, especially in the irradiated specimens (Fig. 1a). The fraction of solubilized proteins in the homogenates from these specimens was much lower than in the control series, but the concentration

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TABLE 1. Content of Protein and Products of Proteolysis (in nmoles, tyrosine/mg tissue) in TCA-Soluble Fraction (A) and TCA-Insoluble Fraction, Solubilized by 5 N KOH (B) (M ± m, n = 3)

| Fraction of homogenate | Unirradiated specimens | | Irradiated specimens | |
|---|---|---|---|---|
| | A | B | A | B |
| Aqueous extract | 29,4±1,5* | 245,4±8,6* | 60,2±2,7* | 26,6±3,3* |
| Suspension of residue after proteolysis for 24 h | 139,0±5,1* | 22,7±1,0** | 385±59,5* | 38,3±1,0* |

**Legend.** *$p < 0.05$.

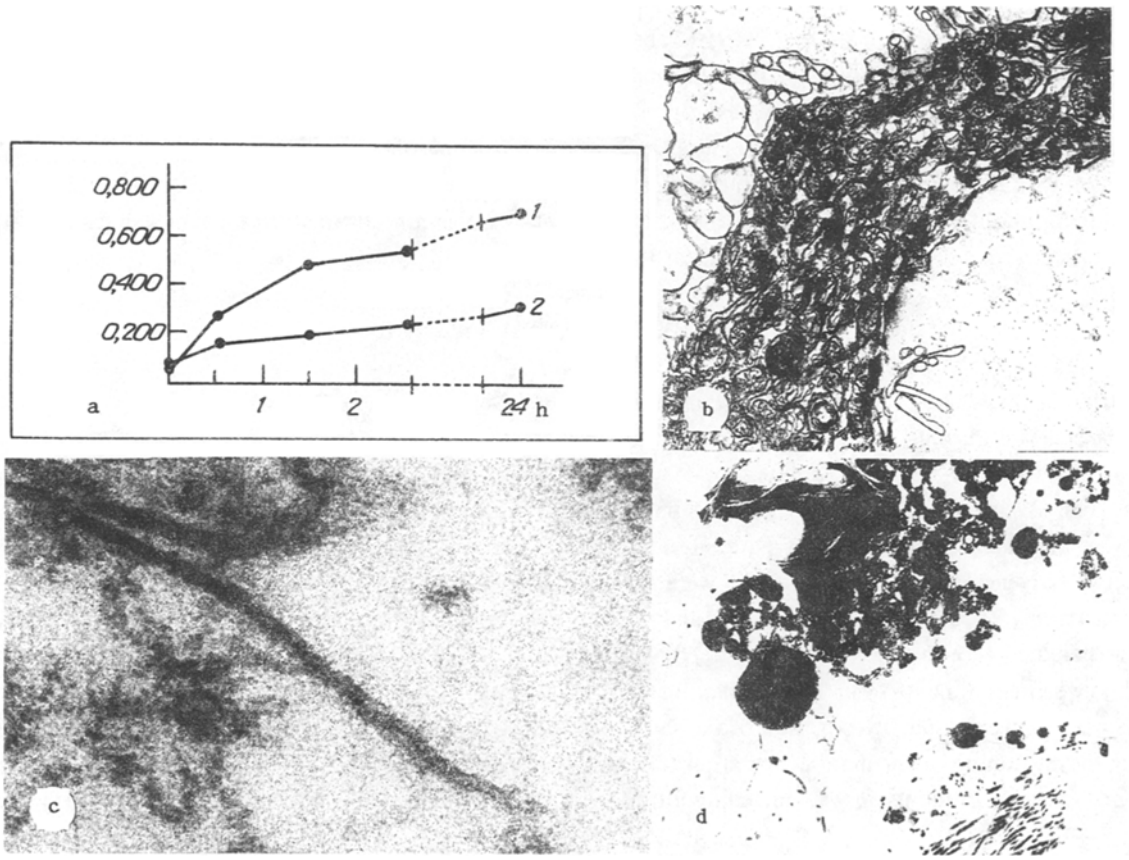


Fig. 1. Proteolytic resistance of rat liver tissue proteins after irradiation with Nd:YAG laser. a) Release of proteolytic products soluble in 5% TCA, from irradiated (1) and control (2) liver tissue homogenates, 0.5, 1.5, 2.5, and 24 h after the beginning of proteolysis with trypsin. Results expressed in nanomoles tyrosine/mg tissue. Abscissa, optical density at 280 nm (relative units); b) ultrastructure of cell (presumed to be a hepatocyte) 24 h after beginning of incubation of irradiated liver fragment in mixture of proteinases. Despite marked injuries, general organization of cell, nucleus, and some mitochondria preserved due to integrity of membranes. 10,740×; c) lysis of membranes observed in unirradiated cell 4 h after beginning of proteolysis. 248,900×; d) 24 h after beginning of proteolysis only debris, bundles of collagen fibers, and membrane fragments can be seen in unirradiated specimen (compare with Fig. 1b). 7170×.

of TCA-soluble products was considerably higher, both before and after treatment with the two proteinases, but after irradiation, more of the proteolytically resistant material was preserved (Table 1).

The increased proteolytic resistance of the residue corresponded to a higher degree of structural integrity of the irradiated material. For instance, 24 h after the beginning of proteolysis of the liver fragment, individual hepatocytes, despite severe injury, nevertheless preserved both the general organization of the cell and of certain organelles characteristic of the normal state, and also the typical structure and continuity of the cell membranes (Fig. 1b). Conversely, in the unirradiated fragments the membranes were found to be the structures least resistant to proteolysis, and they were quickly ruptured (Fig. 1c); the cells, moreover, underwent total disorganization and destruction, with the formation of debris containing unidentifiable amorphous formations, collagen fibers, and only detached fragments of membranes (Fig. 1d).

The quantities of TCA-soluble products of proteolysis isolated from the irradiated tissue being greater than those isolated from intact tissue, this indicates intensive destruction of the protein molecules, evidently under the influence of intensive Nd:YAG laser radiation. Catalytic hydrolysis (autolysis) seems an unlikely cause of this destruction because of inactivation of the enzymes under the conditions of irradiation [2]. Since the addition of proteinase released an additional portion of oligopeptides, this suggests not only a destructive action but also a simultaneous denaturing action of the radiation on molecules spared from direct destruction, changing their conformation abruptly to a state of increased sensitivity to proteolysis. The isolation of yet another type of material, namely a reserve protein fraction with increased proteolytic resistance, from the irradiated tissue is very interesting. The most likely cause of this qualitative diversity of changes in the protein molecules under the influence of irradiation is generation of free radicals by them [5]. Their ability to induce simultaneously a wide range of intramolecular and intermolecular modifications could be explained both by the release of oligopeptides (rupture of covalent bonds) and by increased or, conversely, reduced sensitivity to proteolysis (the formation of all possible kinds of abnormal conformations and/or connections). It is the resistance of the membranes that is most likely due to "cross-linkages" between the membrane proteins and hydrophobic components of the lipid bilayer, which would make the peptide bonds sterically inaccessible for proteinases. The molecular mechanisms of these changes require special investigation. Nevertheless, the results shed considerable light on the causes of the unusual reactions in a surgical wound applied by Nd:YAG laser [3, 8]. For instance, delayed resorption of the wound debris is evidently due to the proteolytic resistance of its membrane components to the action of proteinases of phagocytic cells, whereas increased infiltration can be explained by secretion of products of protein hydrolysis after irradiation, many of which have a strong immunogenic action [6, 7]. Although both these reactions reflect negative aspects of laser surgery, prolonged healing in the presence of severe local inflammation [3, 11], and the explanation of their true nature has already contributed [2], and will undoubtedly contribute in the future, to the further development of alternative approaches to the surgical use of the Nd:YAG laser under biologically optimal conditions.

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