ACTION OF RADIATION OF RUBY AND NEODYMIUM LASERS ON NONPIGMENTED CELLS IN TISSUE CULTURE

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Irradiation of monolayer HeLa cultures by a pulsed ruby laser causes destruction of the cell membranes with the formation of foci consisting of radially arranged fused cytoplasmic bands. The character of the foci of injury suggests that two different effects participate in it: thermal and kinetic. An important role in the action of radiation from the neodymium laser is played by the kinetic factor, evidently connected with the cavitation process.

The possibility of destruction of pigmented tissue culture cells by the action of radiation from pulsed lasers was demonstrated by the experiments of Rounds and co-workers [10,11]. Introduction of dyes into a nonpigmented culture of cells is known to increase the traumatizing action of laser radiation [4,10].

The object of this investigation was to study the character of injury to nonpigmented transplantable cultures of human tumor cells by pulsed ruby and neodymium lasers and to shed light on the mechanism of action of laser radiation on a cell monolayer.

EXPERIMENTAL METHOD

Transplantable cultures of human tumor cells (HeLa, HEP-2, KB) grown on cover slips for 3-5 days in wide-necked Carrel flasks were used in the experiments. The cover slips with the cell monolayer were placed on a slide in a drop of Hanks's solution and the edges of the specimen were sealed with petrolatum. The specimens irradiated by the laser were investigated under the phase-contrast microscope. Focused and nonfocused radiation from pulsed ruby (wavelength 6943 Å) and neodymium (wavelength 10,600 Å) lasers were used in the experiments (the pulse duration for both lasers was the same, 250 μsec). The radiation energy varied from 5-10 to 50-60 J for both lasers. To detect damage to the cell monolayer when a low emission energy was used, a method of photographic marking was used in which the laser beam, having passed through the specimen, left a track on photographic paper placed underneath the specimen, and a mirror image of the track on the undersurface of the slide, outlining the zone of maximum irradiation. To rule out the possibility of additional effects of reflected light on the specimen, irradiation was given against a "straight-through background" (the hole in the support was placed opposite the monolayer) [1,2].

Parallel with monolayer tissue cultures, protein-rich biological fluids (calf serum and egg albumin) were also irradiated.

EXPERIMENTAL RESULTS

The experiments showed that with an output energy of the ruby laser of the order of 10 J and above, death of some of the cells was observed with injury to the surface membrane and liberation of intracellular contents to the exterior in the form of transparent vesicles, indicating the absence of thermal coagulation of the cytoplasm at the moment of destruction of the cell membrane (Fig. 1a).

With an increase in output energy to 40-50 J or more, or with the introduction of dyes into the specimen increasing the absorption of the laser radiation (for example, Janus green in a concentration of 0.002%), the changes in the cell monolayer were more severe in nature. Instead of single destroyed cells alternating with intact, the formation of one or several isolated foci of injury, all the cells of which were destroyed and grossly deformed, was observed in the monolayer. The structure of these foci was very

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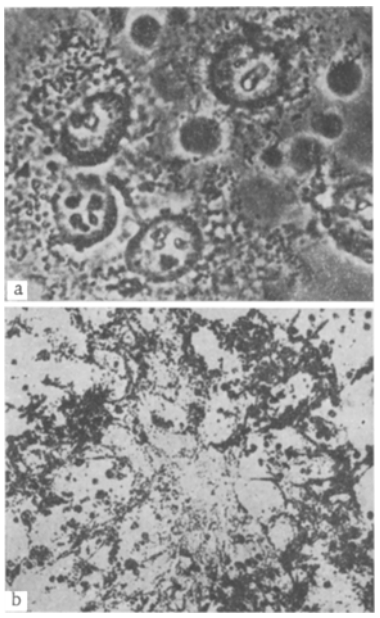

Fig. 1. Destroyed cells of a monolayer HeLa culture without dye (a) and focus of destroyed cells of a monolayer in the presence of 0.002% Janus green after irradiation by a ruby laser. Phase contrast. a) 600 ×, b) 63 ×.

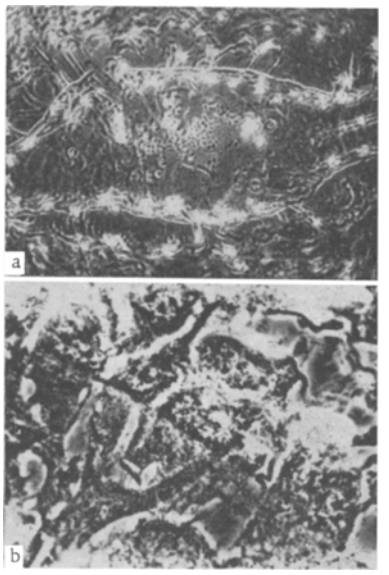

Fig. 2. Focus of injury to a cell monolayer after irradiation by a neodymium laser (a), distinct phase-dark border around the focus of injury (b). Phase contrast. a) 70 ×, b) 400 ×.

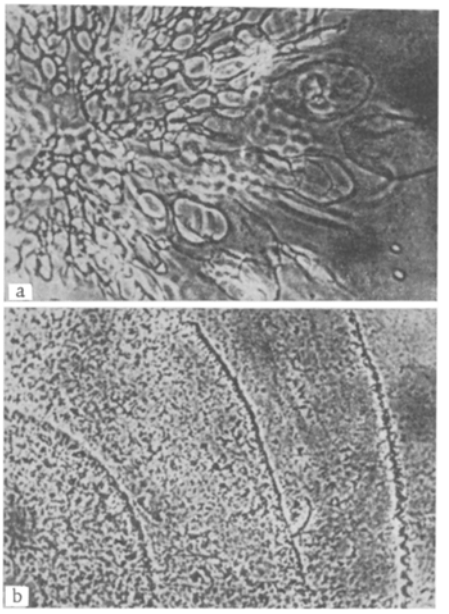

Fig. 3. Irradiation of cell-free substrates by a neodymium laser. a) center of coagulation zone of egg albumin; double-layered azure structure; b) concentric curved lines in the coagulation zone of calf serum. Phase contrast, 140 ×.

characteristic, so much so that their analysis was used as one method of studying the mechanism of tissue damage by laser radiation. The focus resembled a network of fused and interwoven cytoplasmic bands, and its most specific feature was its radial structure, all the bands running toward the same center (Fig. 1b). Inside the focus the distribution of cell material was irregular, with the formation of areas of condensation and rarefaction. The focus was usually surrounded by a zone completely devoid of cell material, outside which was a region of the monolayer with injury to its cells of the milder type as described above (destruction of membranes of individual cells), then giving way to the intact part of the monolayer.

If cell material was irradiated on the surface of a dish containing Hanks's solution with 0.002% Janus green, the irradiated zone contained no foci with the characteristic structure and single center, but consisted of a collection of microfoci, each formed apparently by the "bursting" of a single cell.

These structural features of the foci of injury to the cell monolayer arising after exposure to ruby laser radiation suggest that at least two different effects are concerned in the formation of these foci: a) thermal, whose role is indicated by the "welding" of the cytoplasmic bands and cell residues together, and b) kinetic, leading to the radial distribution of the cell material around the epicenter of injury, and to the formation of a sharp boundary between the focus and the empty zone separating it from the rest of the monolayer. The possible role of these effects in the traumatic action of laser radiation is indicated by data in the literature [3,7,9,12].

The role of the kinetic factor in injury to tissue cultures by laser radiation becomes more evident still when foci of injury formed in the cell monolayer under the influence of neodymium laser radiation are examined. As Fig. 2a shows, the central part of these foci is filled by a honeycomb structure, containing no cells whatever but, probably, related to the interweaving of bands in the focus of injury produced by the action of ruby laser radiation. In the middle of this zone a small area of complete devastation was frequently observed, without even the remnants of cells. Next followed a zone of the monolayer with cells elongated in a radial direction, from center to periphery, and frequently separated from the surrounding monolayer by a zone of rupture. In the case of formation of a zone of rupture, the edges of the elongated cells were folded downward. On both sides of the zone of rupture, borders could be observed to surround the focus, consisting

of condensed cells. Sometimes two or three such borders could be observed in one focus, with zones of rupture between them.

If the radiation energy was insufficient to cause the formation of a zone of rupture around the focus, frequently a very clear, phase-dark (phase-contrast microscope) border to the focus could be seen, as if drawn by compasses, and evidently formed by condensation of the cytoplasm inside the cells (Fig. 2b).

Judging from the structure of the focus of injury produced by radiation from the neodymium laser in the monolayer, two kinetic effects, opposite in direction, participate in its formation. The border of the focus consisting of condensed cell material could only develop under the influence of pressure forces directed from the center of the zone of injury toward the periphery, causing the appearance of a compressive stress in the monolayer at a certain distance from the geometric center of the focus. The zone of elongated cells is evidence that a compressive stress was in fact present there (this could have been a secondary process superposed on the primary compression), evidently directed toward the center of the focus.

In that case the direction of the force from the center of the focus toward the periphery could have been considered probable were it not for the cells at the edges of the zone of stretching, which had separated from the slide and were turned with their free end downward and toward the center of the focus.

Zones of coagulation in the irradiated specimens of calf serum and egg albumin were similar in structure to the foci just described in the monolayer. An azure structure, very similar to the central part of the foci of injury to the cell monolayer was found at the center of the zones of coagulation. A double-layered azure structure is clearly visible in Fig. 3a, with its lower layer on the surface of the slide and its upper layer on that of the cover slip. Concentric curved lines, formed by condensation of coagulated protein globules, run toward the periphery from the azure center of the coagulation zone (Fig. 3b). The number of these lines varied (from 1 to 5 or more), as also did their extent. They were found around the center, and sometimes they reached the size of a semicircle, although none were ever observed to form a complete circle.

It can be concluded from the results described above that the honeycomb structure is not specific for the cell monolayer. The double-layered azure structure is evidently connected with the appearance of cavitation under the conditions present in the specimen at the border separating the glass slide and the liquid. The formation of periodically repeated curved lines in the irradiated layer of egg albumin and calf serum is conclusive proof of the generation of a shock wave in the irradiated zone. In the course of the experiments facts were obtained confirming the importance of the kinetic effect, evidently associated with a cavitation process. Cavitation is known to develop only in a fluid medium, and ultrasound is one of the main causes of its appearance [6]. The possibility of the appearance of ultrasonic waves in material irradiated by a laser has been demonstrated in principle [5]. Cavitation is known to develop most easily at points of local weakening within a fluid which are the boundaries separating the liquid from solid particles or gas bubbles contained in it. In the present experiments, foreign particles, accidentally contaminating the specimen, were frequently found at the center of the foci of injury formed in the cell monolayer by the action of laser beams. Hence it follows that the formation of cavities and, consequently, centers of the foci of injury, are determined not only by the hot points of the beam, but also by the presence of reduced bursting strength in the specimen. The formation of gas bubbles in the center of the focus can be attributed to the degassing effect of cavitation [8].

Many of the facts thus established can be explained not only by the cavitation hypothesis, but also by postulating the generation of a shock wave in the irradiated zone, caused by the appearance of areas with a sharp temperature drop. Further investigations will help to reveal the true mechanism of formation of foci of injury in monolayer cultures irradiated by lasers.

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