the enzyme was estimated by preincubation of the membranes for 3 h with samples of the 2 fractions in quantities corresponding to 1×10^{-6} M–5×10^{-6} M cholesterol. It is of interest that the changes are produced only by the monomeric cholesterol existing in the fraction B. Control experiments, using cholesterol-free residual proteins of the fraction B, had no effect on the enzymatic activity. These proteins free of cholesterol were obtained by exhaustive dialysis of the fraction B as described in the experimental section. Lipoproteins fail to produce any changes within that period of preincubation time. The results agree with those obtainable with the exogenously added aqueous solutions of cholesterol at concentrations up to 5×10^{-6} M (see fig.).

A test of the state of this nonesterified cholesterol was applied by detecting it gas-chromatographically after extraction, etc., in the urine (up to 1.7×10^{-6} M). The free (monomeric) material may, probably, partly be filtered at the glomeruli of the kidneys and then reabsorbed at subsequent portions of the nephron. The data are limited at present to 16 cases, of which 8, more thoroughly studied, are presented here. Six of these patients, known not to suffer from any vascular disease, were used for the derivation of a mean value of the ratio of the nonesterified cholesterol in fraction (B) over that of the nonesterified cholesterol remaining in lipoprotein ($x=0.17\pm0.03$). Patient No. 2 had a history of coronary disease and patient No. 6 had well recognized symptoms of vascular disease. Although, at this point, high ratios may be a coincidence, the problem warrants further studies in normal subjects and patients. It is early yet to make pronouncements on the factor(s) influencing the ratio (table, column VI). Triglycerides, fatty acids, etc., which all show a certain statistical relation with atherosclerosis, may be responsible.

Such a study is now in progress. The fact remains that 'free' monomeric nonesterified cholesterol, not carried by lipoproteins, may evoke changes of cellular membrane proteins, capable ultimately of harming those cells, and similar material is present is serum, often at concentrations very much higher than those permitted by the 'solubility' of the free compound in aqueous media. At such concentrations, cholesterol continuously present in the circulation would eventually incorporate into the phospholipid bilayer and produce changes similar to those shown in the figure, where the ouabain-sensitive ATPase was selected as a parameter. Such an increase of the nonesterifed cholesterol in the membranes of aortic endothelial cells could be involved as an initiator, in association with other factors (mechanical, etc.) of preatherotic lesions[17].

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Superoxide dismutase activity in the skin of rats irradiated by He-Ne laser[1]

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Summary. The activity of the enzyme superoxide dismutase has been measured in the skin of rats irradiated by a low-power He-Ne laser. The irradiation was performed at the doses of 4, 8 and 20 J/cm^2 in a single or repeated treatment. The increase in activity of superoxide dismutase in the skin of irradiated animals was not statistically significant.

In the present work the activity of the enzyme superoxide dismutase (SOD) was measured in the skin of rats irradiated by a He-Ne laser. The choice of this enzyme was suggested by the following considerations: a) the SOD is responsible for the neutralization of the toxic O_2^- radicals which are a product of oxidative processes which normally occur in the cell[3,4]; b) these O_2^- radicals increase notably in inflammatory processes[5,6]; c) the copper zinc SOD (Cu, Zn-SOD) has 2 cupric ions in the active site which are responsible for the peak of absorbance at 680 nm[7,8]; at 632.8 nm, which is the wavelength of the He-Ne laser, the Cu, Zn-SOD shows 80% of the maximum absorbance[7].

Materials and methods. Male Sprague-Dawley rats (250–300 g) were used for the experiment. Before and during the treatment they were allowed water and food ad libitum. The day before laser irradiation 2 areas (1.5×1.5 cm) of the lumbar region, symmetrically located on the right and on the left of the dorsal midline were depilated, keeping the animals under ether anesthesia. Of the 2 depilated areas, the one on the right side was irradiated and the other on the left side served as contralateral control. The irradiation was performed with a continuous wave He-Ne laser (Valfivre, Italy, 25 mW nominal output, wavelength 632.8 nm). A spot of about 1 cm in diameter with a minimum scanning

Level of superoxide dismutase activity in the skin of rats irradiated or not by He-Ne laser

Group	Number of rats	Daily dose (J/cm^2)	Number of treatments	SOD activity (μg/mg protein) ± SE Skin at right* (irradiated)	Skin at left* (nonirradiated)
1	3	4	1	3.4±0.4	3.8±0.8
2	9	8	1	3.2±0.6	2.4±0.4
3	7	20	1	5.7±0.5	5.2±0.6
4	6	8	4	3.6±0.5	3.4±0.3
5	7	20	4	3.1±0.2	2.5±0.2
6**	7	0	0	5.8±0.4	5.5±0.6
7**	7	0	0	2.8±0.2	2.9±0.4

*The terms right and left refer to the lumbar region on the right side and on the left side of the dorsal midline. **Rats of groups 6 and 7 were from the same nest as rats from groups 3 and 5 respectively. Groups 3 and 6 received only 1 ether anesthesia; groups 5 and 7 received 4 anesthetic treatments.

was delivered. The output was measured by a power meter (PM 25 Valfivre) and the dose of radiation was determined by the time of irradiation. During the treatment the animals were anesthetized by ether. Groups of rats were also depilated under ether anesthesia but not irradiated, and they served as independent controls. The animals were divided into groups and irradiated as reported in the table. Immediately after the last laser treatment, the skin was excised from both the irradiated and nonirradiated areas. For the nonirradiated animals (groups 6 and 7) the samples were removed after the last ether anesthesia. The samples of skin (150 mg) were washed twice with 5 ml of ice cold 50 mM Tris-HCl pH 7.8 in 0.1 mM EDTA, homogenized in 2 ml of the same solution and then centrifuged at 15,000 rpm for 20 min. The activity of SOD was measured in the supernatant by the method of Misra and Fridovich[9]; a standard curve was constructed using highly purified Cu, Zn-SOD from bovine erythrocytes; the enzyme activity was expressed as μg SOD per mg protein. Proteins were estimated by the method of Lowry et al.[10] using bovine serum albumin as standard. The content of Cu, Zn-SOD was determined by potassium cyanide inhibition[11].

Results. The levels of SOD in the skin of rats irradiated or not with a He-Ne laser are reported in the table. In the animals which underwent only ether anesthesia (groups 6 and 7) the level of SOD was in the range 2.8–5.8 μg/mg protein. This rather large variability of the basal levels of SOD was also observed in the epidermis, and was related to the age of the rats[12]; in the present work other parameters, not easily identifiable, were probably involved. The method employed to measure the activity of SOD was excluded since within a group the SE was quite low; in addition, samples of skin excised from the left and the right areas of the same not irradiated animals showed identical values (see groups 6 and 7). The effect of laser irradiation was evaluated by comparing the activity of SOD in the irradiated skin vs the activity of SOD in the skin of a contralateral area, not exposed to radiation. Except for group 1, the apparent increase of SOD activity in the irradiated skin (6–33%) was not statistically significant after any laser treatment. In fact, the increase of 33% observed for group 2 had a $p < 0.3$, as calculated according to Student's t-test; for group 5 the increase was 24% with $p < 0.1$.

Laser irradiation did not alter the level of SOD in the nonirradiated skin, as indicated by the fact that the enzyme activity remained unchanged in rats from the same nest (see groups 3–6 and 5–7).

Discussion. Of the total SOD activity present in the skin of rat a large amount, equal to 88%, was found to be the Cu, Zn-SOD. This high level of Cu, Zn-SOD, which has an absorption spectrum with a peak at 680 nm, made it reasonable to suppose that irradiation of the skin with He-Ne laser (632.8 nm) might produce a variation in the activity of total SOD.

The irradiation by He-Ne laser was performed at different doses, and as single or repeated treatments, in order to distinguish between immediate and later effects. The results show that laser treatment did not damage the SOD activity in the skin of rats under any conditions. The increase observed under certain conditions (group 2 and group 5 in the table) did not reflect a dose-effect relationship. The overall results indicate that following He-Ne laser irradiation in vivo, superoxide dismutase fully retains the ability to display its antitoxic action as scavenger of O_2^- radicals.

The stability of the Cu, Zn-SOD following He-Ne laser irradiation was confirmed also by in vitro experiments in which solutions of enzyme purified from bovine erythrocytes were exposed to radiation (unpublished results). It is worthy to mention that the plasma metallo-protein ferroxidase, which contains 3 cupric ions per molecule and shows an absorption spectrum similar to that of the Cu, Zn-SOD, did not change its spectral properties when irradiated in vitro with a He-Ne laser; spectral changes were instead observed after treatment with laser radiation of a lower wavelength[13].

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