Shot report

MORPHOLOGICAL CHANGES OF QUINEA PIGS' SKIN EXPOSED TO X-RAYS

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The analysis of experimental animals' (guinea pigs) different focalization skin derma structures' morphological changes on 1- 60 days after X-rays exposure finish was carried out.

In the comprehensible literature the available data about skin derma changes at the X-ray radiation action are extremely few and contradictory, due to what it becomes interesting to carry out an investigation dedicated to the cells' factors changes study at their being exposed to X-ray radiation.

The investigation was carried out on 81 mature male guinea-pigs weighing 400-450 g, 51 of which were used in the experiment and 30 – served as the control. Maintenance and work with the experimental animals were carried out in accordance with the rules accepted by the European Convention about the defense of vertebrate animals used for experimental and other scientific purposes (Strassburg, 1986). Before the experiment the guinea-pigs 3-5 times were subject to a "false" effect with the apparatus on, but the irradiation off, to exclude the stress factor. The experimental guinea-pigs were exposed to single general irradiation (dosage – 5 Gy, 0,64 Gy/min, filter – 0,5 mm SI, voltage – 180 kV, amperage – 10 mA, focal distance – 40 cm). The radiological apparatus "RUM-17" was used as a radiation source. The irradiation took place at one and the same time of the day – from 10 to 11 o'clock in autumn-winter period. Excluding the animals from the experiment and sampling the materials were done immediately, in 6 hours, on the 1^{st}, 5^{th}, 10^{th}, 25^{th} and 60^{th} days after finishing the exposure. The flaps of skin were taken from different areas (head (cheek), back, stomach). For the histological examination the material fixed in 12% neutral formaline and then poured into wax, from which 7 mcm thick sections were made, which were stained by means of the traditional method – with hematoxylin and eosin according to van Gieson in Weigert modification, - was used. Also a range of histochemical methods was used. For proteins the skin sections were stained with 0,1% water or saturated sulem solutions of bromphenol blue (BPB). Glycosaminoglycans were detected by sections' staining by 1% solution of alcian blue with pH -1,0 and pH -2,5 and the corresponding controls performance and 0,5% solution of toluidine blue for metachromatism detection. For glycoproteins and neutral mucoplysaccharides the sections were stained by means of carrying out the periodic acid Schiff reaction according to MacManus. The control for glycoproteins and neutral mucopolysaccharides was carried out by the sections' treatment with amylase. For the RNA detection the method of Brache sections' staining was used. A part of the objects for the RNA and DNA detection was stained with the help of chromic aluminous gallocyanine according to Einarson. For submicroscopy the skin flaps were fixed in 2,5% glutaraldehyde in 0,2 M cocadylate buffer (pH-7,2) and post-fixed in 1% solution of osmic acid. All the objects were poured with araldite. Sectioning was carried out on an ultratome LKB-III. Semifine sections were stained with toluidine blue, ultrafine ones - contrasted with uranyl acetate and plumbum citrate, observed and photographed through electronic microscope JEM-100 CX-II (Japan). Hematological control (total count of erythrocytes and leucocytes) was carried out during the experiment.

At the microscopic investigation of the histologic specimen during the first day after finishing the X-ray effect on the part of all focalization regions skin derma cells the decrease, compared to the control, of plasma sensitivity to acid stains is registered. The nuclei of separate cells, including fibroblasts, are rounded off, and in the karyoplasm 1, more rarely 2, hyperchromatic nucleoli become apparent. At submicroscopic investigation from a part of all focalization regions skin derma collagen fibers the osmophility degree lowering is registered (fig.1). On the 5^{th} day after the X-ray effect finishing the plasma acid stain intensity of most papillary and reticular dermis cells decreases. A part of the specified cells were expanded, with blurred limits. In the given cells a decrease of the pyroninophilia intensity is observed, that manifests itself as faintly red diffuse coloration, when stained according to Brache. In separate fibroblasts the pyroninophilic substance is detected only near the plasmolemma. Compared to the control, the bromphenol blue water and saturated sulem solutions stain intensity of the most skin derma cells' plasma decreases, that testifies to the decrease of basic and total proteins content in the specified cells. On the 10^{th} day after the X-ray radiation effect finishing at submicroscopy in a range of visual fields changes on the part of collagen fibrils come under notice, which manifest themselves as non-uniform optical density, the fibrils' thinning and their lysis locus (fig.2).

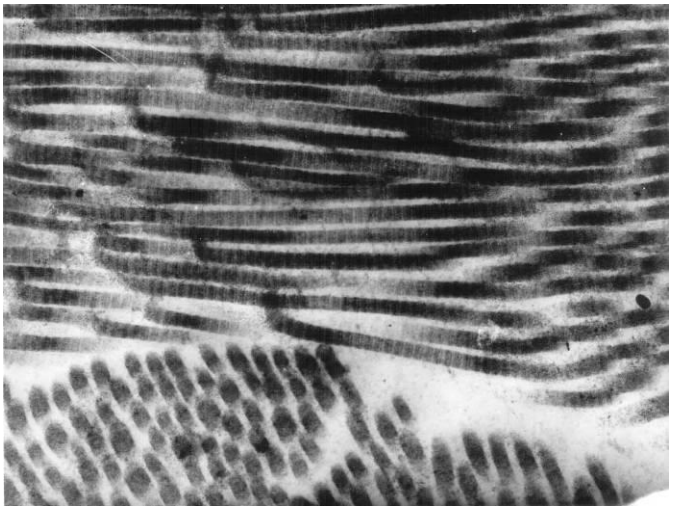

Figure. 1. Fine structure of a guinea-pig's head skin derma collagen fibers. Control. Blown-up 48000 times.

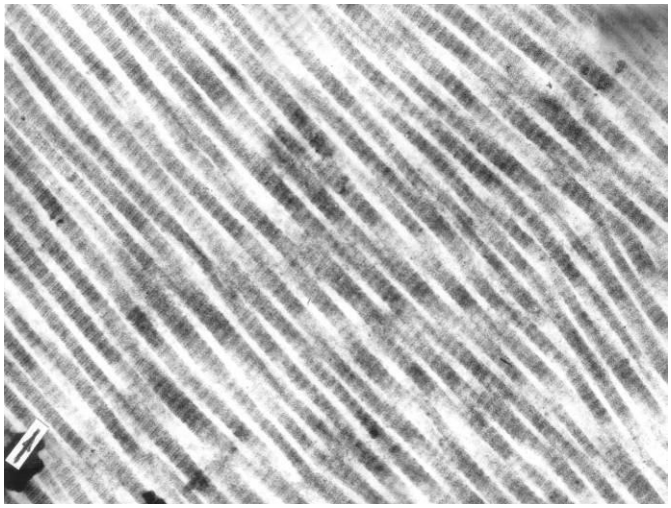

Figure 2. A guinea-pig's head skin derma collagen fibers' osmophility degree lowering on the first day after X-ray radiation effect finishing (arrow). Blown-up 48000 times.

In separate fibroblasts located near the specified regions of fibrils intracellularly collagen inspissation forms appear, in particular in the form of globules in the developed cytoplasmic cytoplasmic cavities, which most probably are the cistern of cytoplasmic reticulum (CPR), alongside with this actin-measin cytofilaments are detected. Histochemically the regions of the fibrils' intense synthesis are characterized with fuchsinophilia, while in the collagen fibers disorganization places the pikrynophilia and γ-metachromasia become apparent, which testify to the accumulation of acid glycosaminoglycans (GAG) between the decomposing collagen fibrils. On the 25th day after the X-ray radiation effect finishing in the reticular dermis the increase of acid magenta affinity of a considerable part of collagen fibers is registered, and large, reaching 60-65 mcm, fibroblasts are also detected. In the nuclei of the specified fibroblasts chromatin globules are diffused, more often 1-2 nucleoli become apparent, one of which in many cases is dislocated to karyolemma. The plasma of the specified cells is faintly basophilic. In oil glands the plasma of the most cells of cambial layer is faintly stained with eosin, and their nuclei – with hematoxylin. The nuclei of the specified cells are enlarged in size; often have an oval shape, in many cases contain hyperchromatic enlarged in size nucleoli intensively stained with hematoxylin and methyl green, when stained according to Brache. On the skin specimen stained according to van Gieson in separate regions of subcutaneous fat thin collagen fibers are detected. Round single lipocytes macrophage clump is registered. On the 60th day after the X-ray radiation effect finishing a considerable part of collagen fibers is intensively stained with fuchsin. The affinity of nuclei and plasma of the most part of fibroblasts to hematoxilin and eosin and also gallocyanine, when stained according to Einarson, is enhanced, in separate cells expressed so significantly, that it is not possible to detect their detailed structure. In the conjunctive tissue of papillary and reticular dermis, sepa-

rate cells with plasma vacuolization and hyperchromatic nucleoli phenomena are observed.

The findings testify to vivid morphofunctional changes of different localization regions' skin derma cellular elements observed during the whole observation period (60 days), when exposed to X-ray radiation.