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THE CHARACTERISTICS OF THE LOCAL (SKIN) REACTION IN THE OFFSPRING OF IRRADIATED DOGS

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It is known that the offspring of animals as well as of man exposed to X-irradiation during the period of embryogenesis are different from the offspring of healthy parents with respect to their development (2, 3, 4, 6, 8, 10 and 11). After internal irradiation of a female animal with long-lived radioactive isotopes part of the activity is transmitted to the offspring through the placenta and in the mother's milk (1, 4, 7, 9, 12). Therefore, the offspring is subjected to irradiation from activity obtained before birth and because of activity entering the organism additionally after birth.

The specific importance of the activity which is transmitted to the offspring by a female animal which had been injured long before gravidity varies within the limits of from several fractions of a percent to 1.5-3 percent (of the radioactivity of the female animal), that is, it is comparatively small. However, the sensitivity of tissues and organs to irradiation during the course of development is considerably greater than the sensitivity of maturing tissues, which cannot

help but be reflected on the acquisition of some new properties by such offspring which are different from the properties of the intact organism. Actually, the resistance of newborn animals to the effect of the environment is reduced, the course of a number of diseases is distinguished by the rate of development of the process, and sometimes by the unusualness of the symptom complex. A reduction in viability is evidently brought about by special immunobiological properties of the organism. The problems of what these characteristics are, how they are characterized, and by which mechanisms they are accomplished have not yet been discussed in the literature.

In the present report, which represents a part of the investigations on the study of the reactive properties of the offspring of irradiated parent animals the results are presented of the macroscopic and microscopic studies of the skin of such offspring (young dogs) after the influence of a chemical irritant on it.

The experiments were performed on the offspring of male and female animals which had been given uranium fission products according to the calculation of one mC/kg in the food 10-18 months before copulation. As has been reported previously (1), uranium fission products at these calculated levels did not exert any essential influence on the general condition of the dog when administered by mouth, although they led to a temporary impairment of the sexual function.

Puppies from the affected animals lagged in growth and development, were less resistant to pathogens of infectious (spontaneous) diseases and died in much larger numbers than in the controls.

In the experiments the strongest puppies were selected, which had reached the age of eight to 10 months. In weight, general physical development and in age the individual selected was no different from the control dogs.

In searching for the most readily dosage irritant, providing reactions of identical nature, a number of physical agents (wounds, heat, cold) and chemical agents (acids, alkalis, certain complex ether derivatives and others) were tested. The most suitable for our purposes was chlorvinyldichlorarsine (CVDA). The application of one mg/square centimeter of CVDA to the skin of young control dogs led to the necrosis of the upper and middle layers of the dermis, a an irreversible injury of the superficial vascular network and to a mild injury of the deep system of vessels (blood and lymphatic). With the application of 0.2 milligrams/square centimeter of CVDA necrosis of the epidermis occurred, with necrosis of the papillary layer of the dermis and injury of the vessels of the superficial network without any essential changes in the deep vascular network. The healing of injuries with a dose of one mg/square centimeter lasted about four months; after the application of 0.2 mg/square centimeter, about two weeks.

Thirty-eight animals were used in the experiments -- eight from the healthy parents and 30 from injured parents. After a careful shaving of six sections of the skin of the back the CVDA was applied to all the dogs. Each injured area was no less than nine to 10 centimeters away from the neck, which, to a certain degree, excluded an interaction between the affected foci. The CVDA was applied with a graduated pipette in 0.01 cubic centimeters of acetone and was distributed over an area of one square centimeter. Twelve dogs (three from the healthy group and nine

from the injured parents) were given one mg/square centimeter on each of the six sections of skin; 26 animals, five from healthy and 20 from injured dogs, 0.2 mg/square centimeter.

One, three, five, seven, ten and 15 days after the application of the CVDA, in addition to a daily examination of the animals, the injured sections were measured, thereby taking into consideration the area of the injury and the thickness of the fold of skin injured. On the third, seventh and 15th day after the application of the irritant a biopsy was performed (for each area at each interval); the other three areas were left for clinical observation of the course of the healing. The excised sections of skin were smoothed out and dissected according to a definite standard for the part, after which it was fixed in formalin, a mixture of formalin and alcohol, and by the Bouin method. Part of the material was embedded in celloidin; another portion was cut in a freezing microtome. The sections were stained by the generally accepted methods.

After both of the doses of CVDA used the symptoms of incipient inflammation (hyperemia, edema) appeared in the experimental dogs earlier and were more pronounced than in the healthy ones; the hemorrhages were spread over a wider area of skin; the scab fell off later, and the epithelialization of the defects was delayed; healing of the injured areas after the application of one mg/square centimeter of CVDA occurred one to one and a half weeks later, and with the use of 0.2 mg/square centimeter healing was delayed for two or three days in the majority of cases. It is characteristic that epithelialization of the defects in the offspring of the injured parents was accompanied by a more prolonged separation of

the scales than in healthy dogs. The nature of the desquamation was different; in the experimental dogs large scales fell off; in the control dogs, small ones.

On microscopic examination the degree of injury of the deep tissues attracted attention primarily. Thus, after the effect of one mg/square centimeter of CVDA foci of necrosis were found in all the skin layers, sometimes down to the subcutaneous muscles, whereby the argentophilic stroma of the muscle fibers lost its characteristic small-loop structure and was converted into thick coarse pieces of tissue arranged without any order. A much greater degree of disturbance was observed also in the vascular and lymphatic vessels of the deep skin and network. Here, in the best case a marked separation and disarrangement of the fibers of the vascular membranes was noted with the exudation of plasma and formed blood elements into the surrounding tissue (Fig. 1). However, not uncommonly changes were also encountered which were accompanied by the formation of a thrombus and complete necrosis of the vascular wall. Necrosis of the vascular membranes was noted also with respect to the veins of smaller and moderate sized caliber and in the arteries.

Three days after the application of CVDA a multiplication of cells of the areolar connective tissue, endothelium began in the skin of the control dogs, and there was a formation of new capillaries. In the offspring of the injured dogs the recovery was extremely slight; by the seventh day part of the capillaries and young cells formed died, as a result of which the filling in of the defects and the sloughing off of the necrotic masses were decidedly delayed.

The difference in the picture of the changes after the effect of 0.2 mg/

square centimeter of CVDA was no less distinct. The underlying muscles, subcutaneous tissue and deep vessels, as has been noted above, remained intact in the control animals. In dogs from injured parents a swelling and separation of the layers of the blood vessels of the deep system was found, which led to an increase in permeability, edema of the surrounding tissues and overfilling of the lymphatic vessels with fluid, rich in protein. In the veins of the subcutaneous fatty tissue the formation of "purulent" thrombi was observed, and in certain cases there was necrobiosis and necrosis of the blood vessel walls. The papillary and partly the middle layer of the dermis was injured in both the control and experiment dogs; however, the degree of change was different. In the control dogs the collage fibers swelled up comparatively slightly, and no fragmentation of them was noted; in the offspring of the injured dogs, in addition to a swelling and separation of the layers, a pronounced degeneration of the fibers occurred into separate clumps. Ede and cellular infiltration extended over a more extensive area in both the vertical and horizontal directions. Finally, not uncommonly small vesicles occurre which were located under the epidermis and inside it (Fig.2). The contents of the vesicles consisted either of a protein fluid with an admixture of fibrin, erythrocytes and leucocytes, or of leucocytes and fragments of them, which wa observed less commonly.

By the 15th day after the application of 0.2 mg/square centimeter of CVDA in the control dogs the injured area of skin had been cleansed of its necrotic masses and was colvered with a newly regenerated epithelium; in the experimental offspring, in the majority of cases, epithelialization had not occurred, and the

necrotic masses remained firmly adherent to the underlying tissues. However, even in these cases, where the newly formed epithelial layer covered the defect, the condition of the tissues was different from the normal. We are referring to the appearance of microscopic vesicles. These secondary vesicles were localized, like the primary ones, either under the epidermis or directly in the epidermis itself (Fig.3). The contents of the secondary vesicles, in contrast to the primary ones, were never purulent, but rather consisted of a protein fluid with a small admixture of fibrin and solitary tissue cells. The newly formed epithelial layer of the epidermis in such cases was notably edematous; the lymphatic vessels of the papillar layer of the dermis were distended with fluid which sometimes accumulated in considerable quantity (Fig.4).

Therefore, with both doses of CVDA investigated a distinct difference was observed in the picture of the course of the chemical injury of the skin of control young dogs and animals from experimental parents. After the application of one mg/square centimeter of CVDA the epidermis, papillary and middle layer of the dermis was injured in the control dogs; in dogs from the experimental parents the injuries in a number of cases extended to the lower layers of the dermis, subcutaneous fatty tissue and even the subcutaneous muscles. An essential characteristic of the course of the skin injuries in the experimental offspring consists of the serious disturbances in the blood vessels of the deep system. The separation and the disarrangement of fibers of the elastic, argentophilic and collagenous frameworks of the blood vessel layers involved an exudation of plasma and formed blood elements into the surrounding tissue, a subsequent edematous

impregnation of the tissue, and naturally, a disturbance in its nutrition. The normal blood supply of the injured sections was disturbed to an even greater degree with plugging of the blood vessels and necrosis of their walls.

It is known that the deep vascular network has a direct bearing on the nutrition of the arcolar connective tissue of the skin, where the restorative multiplication of cells begins first. In our cases a delay in the formation of granulation tissue and necrosis of newly formed capillaries can, we believe be directly related to the disturbances occurring in the deep vascular system.

Experiments with the application of a dose of CVDA five times smaller (0.2 mg/square centimeter) make it possible to note a more considerable degree of injury to the superficial skin layers. This is shown not only by the structural abnormalities occurring in the blood vessels but also by the degree of edematous and cellular infiltration as well as by the dimensions of the foci of injury. The sloughing off of necrotic masses with this dose of substance is very slightly delay on an average, by two or three days. However, the epithelialization of the injured areas occurs under less favorable conditions because of the edema of the epithelial layer with the formation of secondary vesicles in it or under it and edema of the underlying maturing granulation tissue. The edematous impregnation of the tissues apparently disturbs the interrelationship of the "epithelium--connective tissue" to some degree, . which provides strong epithelialization of the defect.

As a result, a desquamation of epithelial cells occurs as a group in the form of large scales, which in its turn makes the skin more vulnerable to environmental influences in these sections.

The mechanisms accounting for the edematous impregnation of newly formed tissues are unclear.

It is possible that a combination of two factors occurs here: increased permeability of the newly regenerated blood vessels and capillaries and an imperfection of the lymphatic outflow.

Conclusions

1. Chlorvinyldichlorarsine in doses of 0.2 and one mg/square centimeter produces a more severe injury of blood vessels of the superficial and deep systems in the offspring of irradiated dogs than in the control animals.

2. The course of the chemical skin burns produced by this substance in young dogs of injured parents is different from the normal in the following characteristics: a) the early appearance of signs of inflammation; b) injury to the deeper layers of the skin; c) delayed formation of granulation tissue ; d) later healing.

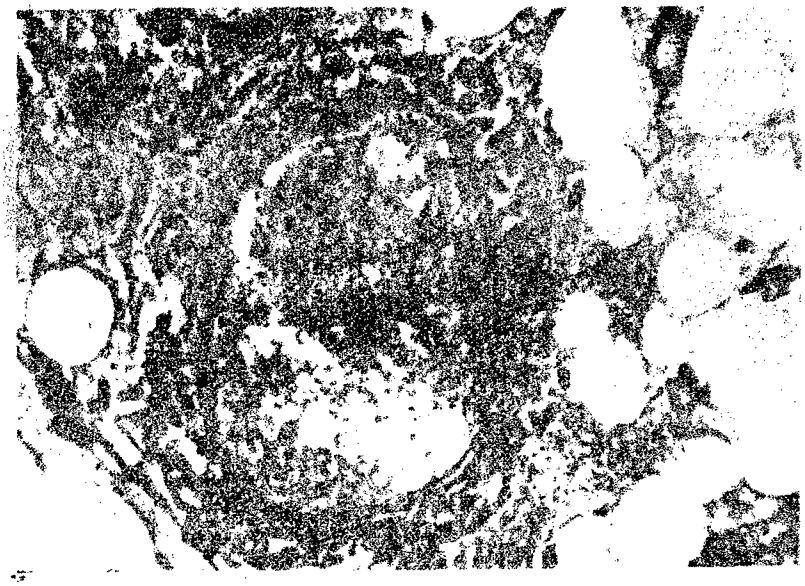

Fig. 1. Skin of dog from injured parents seven days after the application of 1 mg/square cm of CVDA. Disarrangement of vein layers in the deep network, exudation of plasma and formed elements. Silvering according to the Bielschowsky method with hematoxylin-eosin counterstaining. Magnification, 10 x 20.

Fig. 2. Skin of dog from injured parents 3 days after application of 0.2 mg/square cm of CVDA. Subepidermal microscopic vesicles, edema, and fragmentation of collagen fibers. Silvering according to the method of T. Pap. Magnification, 1- x 20.

Fig. 3. Skin of dog from injured parents 15 days after the application of 0.2 mg/square cm of CVDA. Edema of the newly formed epithelium of the epidermis. "Secondary" intraepidermal microscopic vesicles. Staining with hematoxylin-eosin. Magnification 10x40.

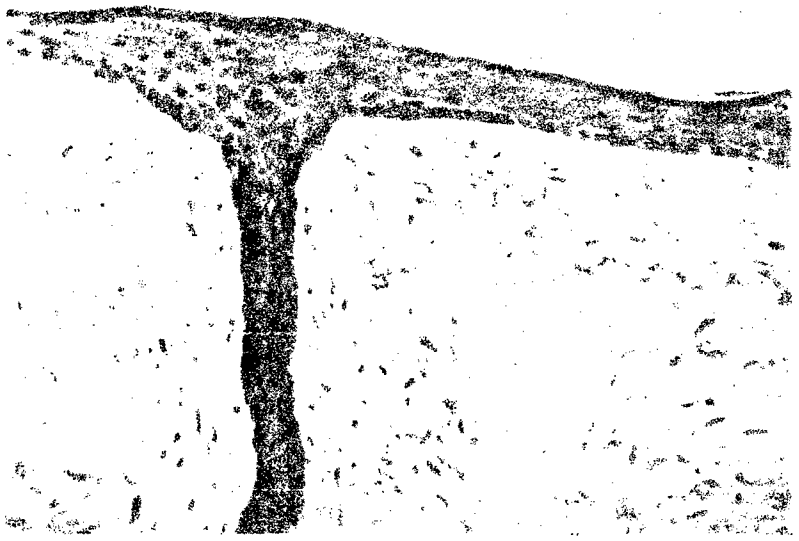

Fig. 4. Skin of dog from injured parents 15 days after the application of 0.2 mg/square cm of CVDA. Edema of the maturing granulation tissue, marked dilatation of a lymphatic vessel. Staining with picrofuchsin. Magnification, 10 x 10.

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