Effect of Irradiation on Some Portions of Head of Young Axolotl (*Siredon mexicanum*)*† (24423)

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In our previous reports concerning influence of x-ray irradiation upon development of the head(1,2), the great sensitivity of teeth and eyes was demonstrated. We are interested now in comparative sensitivity of various portions of the head of these animals.

Materials and methods. A total of 163 young axolotls (25-30 days after hatching) were used in this experiment. Forty-three animals served as untreated controls, 105 were irradiated with a dose of 3,000 r and one group of 15 animals was irradiated with a dose of 6,000 r. The conditions of irradiation were as follows: 100 kv, 30 ma, 0.5 mm Al filter, beryllium window tube; half value layer 1.25 mm Al; target object distance 18 cm. The animals were anesthetized before irradiation by placing them in an aqueous solution of M. S. 222 (Tricaine methanesulfonate), until they were anesthetized. During irradiation the animals were arranged in radial position within a circle on moistened filter paper. The posterior portions of the animals were covered with moist cotton. The nonirradiated portions were shielded with a lead covering with a round radiation localizer about 10 cm in diameter (Brunst, 3). Fixations were made using a saturated solution of bichloride of mercury with 5% acetic acid and in acetic mercuric chloride formalin (Stieve). 70 irradiated animals and 10 controls were examined histologically.

Results. Definite growth suppression of the irradiated portion of the head was observed 80-110 days after treatment. A dose of 3,000 r was lethal in all cases if the whole head was irradiated (all animals died within 6 months after treatment). However, if only the anterior portion of the head was irradiated with 3,000 r, all of the animals were not killed; some animals lived more than 20 months, when suppression of growth became especially obvious.

As a result of the disturbance of development, the visceral arches are exposed. In normal animals these are always covered. In most animals the eyes had disappeared or rudimentary eyes were covered by skin several months after irradiation.

Graphic reconstructions show that the most typical effect is suppression of development of the entire anterior portion of the head and complete disappearance of teeth. While growth of olfactory chambers is much suppressed, they never disappear completely. Owing to suppression of growth of the olfactory chambers, the anterior portion of the brain is reduced. Growth of the lower jaw is not only suppressed, but the whole anterior portion of the jaw has disappeared and as a result, the visceral (branchial) arches supporting the gills are shifted forward.

Comparison of the reconstruction of an animal on the day of treatment, 30 days after hatching, with an experimental animal 174 days after irradiation of the anterior portion of the head with 3,000 r, 204 days after hatching, shows that growth of the irradiated portion of the head is almost completely suppressed and that the anterior portion of the head has almost the same size as on the day of irradiation. The posterior portion of head of the same animal that was not irradiated is greatly enlarged. For example, the size of lymph nodes and branchial arches is 3-4 times larger than on the day of treatment.

Cornea. The normal cornea of axolotl consists of substantia propria and epithelium of cornea. The pigment cells and Leydig's cells that can be found in normal skin epithelium are absent in normal epithelium of cornea. Following irradiation, the whole cornea never disappeared. In some cases the substantia propria was almost normal in appearance and thickness; in other cases, it was 2 to 4 times thicker than normal. As a result of abnormal

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thickness or development of pigment cells the substantia propria lost their transparency in some cases. Rohrschneider(4) observed the same phenomenon in irradiated rabbit cornea. Damage of corneal epithelium was observed in 38% of all irradiated animals. In some cases the reduction of the number of layers of this epithelium was typical; in cases of extreme damage only 2 layers of cells were observed with simultaneous development of giant cells. In other cases, the epithelium was thicker than normal, but Leydig's cells, and giant cells, never present in normal epithelium, were found. In cases of the most severe corneal damage, observed in 30% of the irradiated animals, corneal epithelium disappeared completely and was replaced by typical skin epithelium with many Leydig's cells. The entire cornea was normal in 32% of the irradiated animals.

Lens. The normal lens of control animals has almost homogeneous structure with slight concentric layers. The lens is covered with a capsule, a cuticular membrane. Cracks of the lens are very typical. The formation of these cracks obviously is artefact, but at the same time is a response of definite type of lens structure to fixation. They are mostly observed in a definite direction—parallel to the direction of lens fibers. These cracks were observed consistently in the lens of non-irradiated animals, but they disappeared almost completely even after slight roentgen damage. In some cases, cracks appeared only in the central portion of irradiated lens, when the peripheral portion is entirely changed. Damage of the lens was observed in 56% of irradiated animals. Slight damage was observed in 5%, characterized by many typical granules, vacuoles, and spheres. Moderate damage was observed in 22%, characterized by great disturbances in all peripheral portions. More than half of the lens was completely transformed into a globular mass having various sizes of granules, vacuoles, and spheres. The central portion alone retained its original structure. Serious damage was observed in 29%, characterized by almost complete disappearance of normal lens substance as a result of dissolving of the lens fibers; only remnants of the lens were noted in most cases. The lens membrane, however, was the most resistant structure, although many small folds were typically found. In many cases, after total disappearance of the lens, a wrinkled lens membrane alone was found. A most significant observation was the presence of a great number of large cells in the body of the seriously damaged lens. These were wandering giant cells, macrophages, *i.e.* phagocytes destroying the substance of the irradiated lens. They partially phagocytized and partially dissolved the lens fibers, both these activities being typical of these cells. Cells are never found in the normal lens. The lens was entirely absent in 28% of the irradiated animals.

Retina. In the retina it is necessary to distinguish between damage of the rods and cones layer and damage of the other layers. According to our previous observations the rods and cones layer of the retina is the most sensitive. The sensitivity of this layer is almost the same as the sensitivity of the epithelium of the cornea. In 31% of all cases the rods and cones layer was completely absent, in 33% it was damaged. Serious damage was found in 9% of the animals, where only remnants of this layer were preserved; moderate damage in 15%, where about half of the layer was badly damaged; partial damage in 9%, where only portions of the layer were partially damaged. Finally, this layer was normal in appearance in 36% of the irradiated animals. Retinal layers other than the rods and cones are much more resistant to irradiation. Complete absence of retina was not observed and in only 6% of the irradiated animals was damage of all retinal layers so severe that only small rudiments of retina were noted. In 4% about half of the retinal layers were destroyed and in 6% only portions of the layers were damaged. In most cases (84%) of the retinal layers appeared entirely normal.

Teeth. Teeth were entirely absent in 89% of irradiated animals. However, teeth were still found in animals fixed within 30 days after irradiation, that is, during the latent period for the disappearance of teeth. With these exceptions, teeth were absent in 100% of the animals. In the head of the axolotl

teeth may be the structure most sensitive to roentgen irradiation.

Lymph nodes. In normal axolotls several pairs of large lymph nodes are situated in the boundary region between the head and trunk. After irradiation of the whole head, lymph nodes disappeared in 100% of the animals. After irradiation of only the anterior portion of the head, lymph nodes appeared normal in 95% of the animals; in the remaining 5% they were slightly abnormal and, being in the boundary zone of the irradiated area, obviously had been partially irradiated. Such partial irradiation may have been the result of slight movement of the animals during treatment, or even of slight error in their positioning for treatment—if they had been placed too near the center of the irradiated field, the posterior portion of the head, including the region of the lymph nodes, would have received some irradiation. The lymph nodes, as well as other types of lymphoid tissue are highly sensitive to irradiation.

Gills. In evaluation of damage to gill epithelium it is important to consider time of fixation. When gills were fixed during the primary acute reaction (20-60 days postirradiation) severe damage is usually found, as evidenced by the formation of giant cells. When gills were fixed later, the appearance of gill epithelium was almost normal. In such epithelium it is often possible to see the Leydig's cells which are typical of old skin epithelium but not gill epithelium. However, in some cases the epithelium of the gills appeared normal even within 20-60 days after the animals were irradiated. In all probability, such gills themselves were not irradiated. It is obvious that local irradiation of even the whole head of the axolotl will not result in consistent irradiation of the gills, because of the location of the gills at the boundary between head and trunk.

Olfactory chambers. The olfactory chambers are large organs taking up considerable space in the anterior portion of the head of the normal axolotl. Normal olfactory epithelium has a typical structure and many sensitive olfactory buds which are modifications of the sensitive skin buds distributed in some regions of the skin. Normal olfactory epithelium appears distinctly different from normal skin, and Leydig's cells are not found in it. Normal olfactory epithelium was never found after irradiation. However, in 17% of the irradiated animals the epithelium was only slightly damaged; typical orientation of olfactory cells disappeared. With one exception, such slightly damaged epithelium was discovered only after early fixation (13-36 days postirradiation). This suggests that olfactory epithelium reacts with comparative slowness to irradiation. All late fixations give a clear picture of damage of the olfactory epithelium. In 28% of these cases the olfactory epithelium disappeared completely and was replaced by typical skin epithelium. In 55% the olfactory epithelium had not disappeared, but was greatly damaged; cells similar to those of olfactory epithelium were found but without any special structures. Sensitive olfactory buds disappeared and the distribution of cells in the epithelial layer was completely abnormal. We conclude that olfactory epithelium is one of the tissues of the head most sensitive to irradiation.

Discussion. With respect to sensitivity to irradiation, tissues and organs of the head may be arranged in 5 groups: (I) Teeth and lymph nodes (disappeared completely in 100% of animals). (II) Olfactory epithelium (disappeared in 28%, damaged in 55%, slightly damaged in 17%), and the lens (disappeared in 28%, damaged in 56%, normal in 16%. (III) Epithelium of the cornea (disappeared in 30%, damaged in 38%, normal in 32%), rods and cones of the retina (disappeared in 31%, damaged in 33%, and normal in 36%), and the gills. (IV) Other layers of retina (damaged in 16%, normal in 84%). (V) Pigment cells of choroid, and other tissues of the head (comparatively resistant, no apparent damage after irradiation with 3,000-6,000 r under our conditions).

Characteristic damage was observed in several organs: specialized but not completely differentiated tissues destroyed by x-ray irradiation were never restored. These tissues include oral epithelium forming enamel organs of the teeth, olfactory epithelium of the nasal chambers, corneal epithelium, and possibly, gill epithelium. In all of these tissues the spe-

cialized epithelium which had disappeared completely was replaced by skin epithelium. A peculiarity of skin epithelium is the presence of Leydig's cells, never observed in differentiated epitheliums of enamel organs, olfactory chambers, cornea or gills. Therefore, specialized but not completely differentiated epithelium must be more sensitive to irradiation than skin epithelium.

Summary. 1) Definite suppression of growth of treated portion of the head was observed 80-110 days after irradiation with 3,000 or 6,000 r. In irradiated portions: teeth disappeared completely, growth of olfactory chambers suppressed greatly, but they never disappeared completely, anterior portion of the lower jaw had disappeared, visceral arches all shifted in rostral direction, development of the eyes was suppressed. According to sensitivity, it is possible to distribute the tissues and organs among 5 groups. (I) The teeth and lymph nodes (disappeared completely in 100% of animals). (II) Olfactory epithelium and the lens (disappeared in 28%, damaged in 55% and normal in 17%). (III) Epithelium of the cornea (disappeared in 30%, damaged in 38% and normal in 32%) and the rods and cones layer of the retina (disappeared in 31%, damaged in 33% and normal in 36%). The gills. (IV) Other layers of retina (damaged in 16% and normal in 84%). (V) Pigment cells of choroid and other tissues of the head, which are comparatively resistant and did not show any clear signs of damage. 2) Specialized but not completely differentiated tissues can be destroyed by X-ray irradiation and after this never restored, being replaced by typical skin epithelium with Leydig's cells. This was observed in oral epithelium, olfactory epithelium and epithelium of the cornea.

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Myasthenia Gravis III: Lack of Inhibitory Action of Fractionated Thymus Extracts.* (24424)

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Several investigators(1-5) have studied the effects of thymus extracts on nerve muscle preparations from various animals. However, the reported neuro-muscular blocking activity of isolated frog sartorius muscle by acetone extracts of thymus glands from myasthenic patients(5), was probably due to large amounts of potassium in the extracts(6). The present study was undertaken to assay neuromuscular blocking activity of fractionated thymus extracts free of excess potassium.

Methods. The specimen studied was a surgically resected thymoma from a 30-year-old woman with myasthenia gravis. The tumor was roughly H-shaped and weighed 48.5 g. One lobe measured 14 cm in length and contained a 5 x 7.5 x 3 cm mass covered by a firm fibrous capsule. The other lobe measured 9 x 1.5 x 1 cm. The cut surface of the tumor showed lobules of soft yellow-white tissue separated by firm fibrous trabeculae. Microscopic examination of several blocks fixed in Helly's fluid and stained with hematoxylin and eosin showed a solid tumor composed of almost equal proportions of lymphocytic and epithelial cells. Used as a control was a sample of gastrocnemius muscle obtained at autopsy from a patient who died of cardiac disease. These tissues were extracted and fractionated† by the low temperature, low

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