X-Rays as Causative Factors of Sex Reversal in the Developing Chick

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Introduction

Experiments dealing with the effects of X-rays on the origin and differentiation of germ cells and the formation of gonads have involved many departures from the normal course of development. Difficulties have often been encountered as to the proper interpretation and evaluation of such anomalies. Structures resembling young seminiferous tubules were found in the cortex of X-rayed ovaries. Findings of this nature have led to a closer study of the accumulated material from the point of view of sex reversal. The data are recorded in this publication.

Materials and Methods

The X-ray machine used was a Type C model, made by the Standard X-ray Company. It was mechanically rectified and provided with a Landauer roentgenometer. A Universal Coolidge therapy tube was used. The set-up of the machine for the entire experiment was as follows: The kilowatt meter was set at 96, which delivered 112 peak kilovolts as measured by the sphere gap; the milliameter was set at 6 milliamperes; the focal distance was 10 inches; the filter was equivalent to 4 mm aluminum; the roentgenometer was kept at 3.2 microamperes, which, by calculation, gave 0.6 r per second. The desired r, or dosage, was obtained by varying the time of exposure.

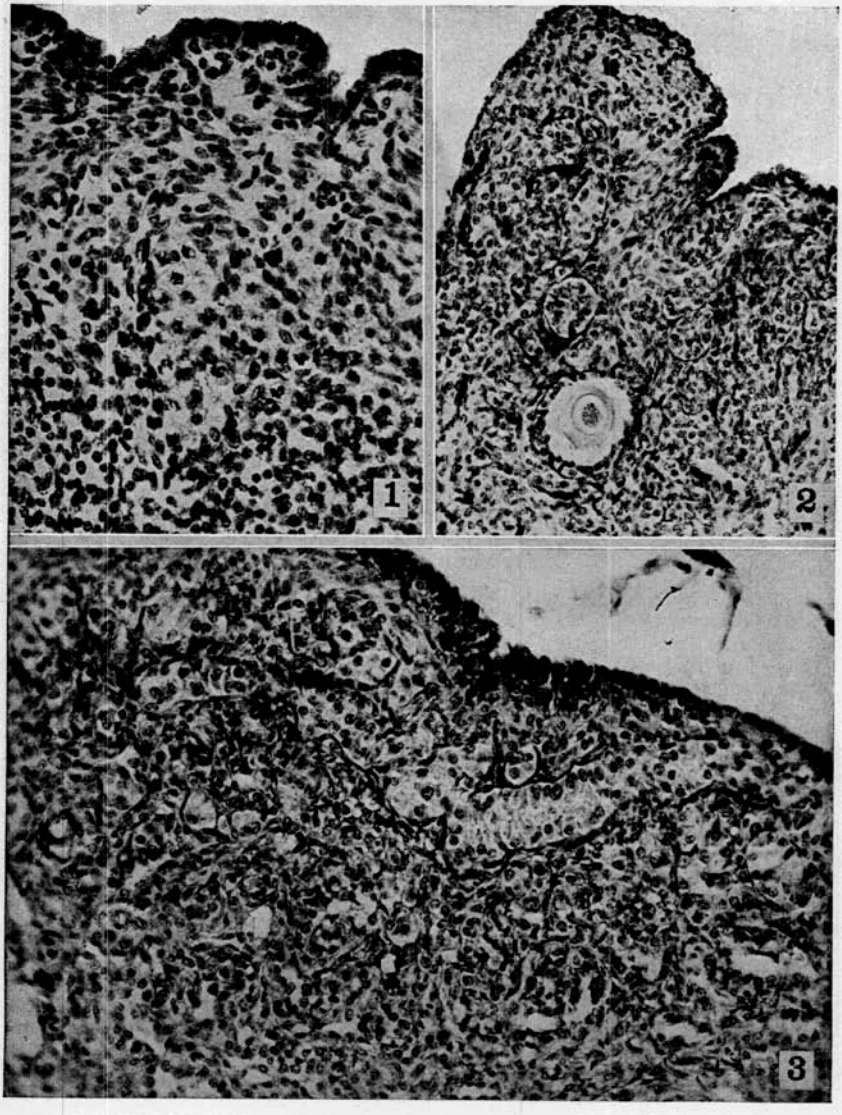
The number of specimens used for these experiments was 121. Of these, 28 were controls and 93 were X-rayed. Of the latter there were 18 eggs irradiated prior to incubation, 26 eggs treated between 5 and 12 days of incubation and 49 chicks X-rayed from the time of hatching to 21 days of age. Fourteen of the specimens irradiated during incubation received a second dose of X-rays after hatching. The dosage of X-rays varied from 40 to 600 Roentgen units. The time of preservation of the material for study ranged from the 18th day of incubation to the 22nd day after hatching.

All of the material was fixed in a solution of four parts of Kleinenberg's picro-sulphuric acid reagent and one part of 10 per cent formaldehyde. It was embedded in paraffin and stained with eosin and hematoxylin.

Results

Mitotic activity is perhaps one of the most sensitive indicators of the effects of X-rays on living tissues. In all of our material so far studied, no definite injuries on mitosis were observed with dosages between 60 to 80 r. Recovery from a temporary arrest takes place in a few hours. On the contrary, there are indications that mitotic activity is accelerated in the embryonic chick with dosages less than 80 r.

The next most sensitive structure in the chick embryo to the effects of X-rays appears to be the germinal epithelium. Initial injuries to the germinal epithelium were noted with dosages between 80 and 100 r. The proliferation of germ cells is inhibited and the fully formed germinal epithelium of two or three layers of cells in thickness returns to the original structure of the peritoneal epithelium. The germ cell content of the cor-



EXPLANATION OF FIGURES

Fig. 1. Section of an ovary of an 18-day-old chick embryo irradiated with 400 r on the fifth day of incubation. Note the scarcity of germ cells and the excessive tunica albuginea (x488).

Fig. 2. Section of an ovary of a 21-day-old chick irradiated at the seventh day after hatching with 400 r. Note atrophy of primary follicles and the abundance of testicular cords (x275).

Fig. 3. Section of an ovary of a 25-day-old chick X-rayed with 240 r at 70 hours incubation and again on the seventh day after hatching with 120 r. Note anovular follicles, testicular cords, testicular tubules and excessive tunica albuginea (x450).

tex was drastically reduced. Its place is occupied by fibroblasts in the formation of an exaggerated tunica albuginea (Fig. 1).

With dosages of 200 r or more the ova of the young follicles are so severely injured that atrophy follows. The resulting anovular follicles were found in all the ovaries of this experiment. Their further development can be divided into two stages: (1) testicular cords and (2) testicular tubules.

Testicular Cords.—During the degeneration of the ovum, the follicular cells continue to multiply and invade the space occupied by the ovum. It is likely that the absorption of the contents of the ovum is accomplished by the follicular cells. In due time, the follicle becomes a spherical mass of cells (Fig. 2). There is reason to believe that such cell masses may also originate from germ cells prior to follicle formation. This is indicated by the presence of cell masses in ovaries in which primary follicles had not formed at the time of killing of the specimen. The degeneration of ova in growing follicles has been noted in many ovaries. Their fate remains, for the present, uncertain. It is likely that they degenerate completely.

The spherical shape of the cell masses is apparently not retained for any length of time. By growth, mostly in length, the cellular masses become cords of various sizes and shapes. The testicular cords are surrounded by a connective tissue sheath which is derived from the former ovarian follicles (Fig. 2).

Testicular Tubules.—The next stage in the development of the original anovular follicles is the formation of lumina in the testicular cords. Apparently, lumina result by rearrangement of cells constituting the cords. The cells assume an epithelium-like position along-side the connective tissue sheath. In the formation of lumina, liquifaction of cells has not been noted. The testicular tubules were found almost exclusively in ovaries that received a double innervation (Fig. 3).

Discussion

Testicular cords have been found in all of the ovaries that were severely injured by X-rays. Definitely formed testicular tubules occur in double irradiated ovaries that had received at least 300 Roentgen units of X-ray. These structures were found in the cortex of the definitive ovary.

By histological criteria the testicular tubules are readily comparable to the seminiferous tubules of the chick shortly after hatching. The developmental history of the testicular tubules resembles that of the seminiferous tubules. A third male characteristic is the greatly enlarged tunica albuginea found in severely injured ovaries.

It is possible that sex reversal in the developing chick can be induced experimentally by means of X-rays. However, the problems cannot be solved on histological data alone. The findings must be verified in the living chick before any definite statement can be made.

That sex in animals is not irrevocably set at the time of fertilization is now recognized by all students of sex problems. Partial sex reversal occurs spontaneously in many groups of animals including man. The literature dealing with sex reversal in the domestic fowl is abundant but only a few publications can be mentioned here.

Sporadic sex reversal in the chicken has been reported by Dent (1), Berner (2), Crew (3) and Hartman and Hamilton (4). These reports deal with adult chickens in which the changes are in the female-male direction. The factors underlying such changes have not been definitely determined. In some instances pathological destruction of the ovary has been found and thus interpreted as the cause of sex reversal. Histological studies of such birds have revealed the formation of seminiferous tubules in the diseased ovary or the rudimentary right ovary: Boring and Pearl (5), Fell (6) and Mackling (7).

Attempts have been made to induce sex reversal in the domestic fowl experimentally. Two methods have been used: ovariotomy and sex hormones. Ovariotomized young female chickens have developed testis on one or both sides. In some instances apparently normal spermatogenesis was observed: Goodale (8), Zawadowsky (9), Benoit (10) and Domm (11). By the use of the male sex hormone partial sex reversal has been induced in the chick embryo. Seminiferous tubules were obtained in the definitive as well as the rudimentary ovary, Willier (12).

Summary

1. Initial injuries to the ovarian follicle result in all ovaries irradiated with 200 or more Roentgen units.
2. Major damage to the ovary is caused by double irradiation.
3. The ovum of the young follicle is affected first, it atrophies and the cavity is filled with follicular cells.
4. The resulting cell masses are found in the cortex of ovaries irradiated with 200 or more r units.
5. Testicular cords develop from the spherical cell masses.
6. Testicular cords become tubules by the formation of a lumen.
7. The tubules so formed have a marked resemblance to the seminiferous tubules of the young chick. The developmental history of the two structures is similar.
8. The development of an extensive tunica albuginea adds a third male characteristic to the X-ray injured ovary.

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