injury at the subcellular level is discussed.

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Morphology and Hatching Power of the Developing Chick Embryo Following X-Irradiation.* (28597)

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Gilman and Baetjar(1) published some of the earliest observations on the effects of irradiation upon the chick embryo. These workers described various conditions following irradiation, such as deformities of growth, patchy distribution of feathers, and hemorrhages in the occipital region. Wilson(2), using radium rays determined the amount necessary to cause the death of the chicken embryo at various stages of development. It was found that a diminishing radiosensitivity from the first to the sixth day of incubation occurred. Results of extensive studies were reported by Stearner *et al*(3), who determined that the periods of radiosensitivity following irradiation treatment fall roughly into 3 intervals: 0 to 1 day, 2 to 5 days and 6 to 12 days. Particular types of responses accompanied each of these divisions.

Karnofsky *et al*(4) determined that roentgen rays exerted both an acute and a delayed toxic effect upon the chick embryo. Acute death occurred within 20 hours post-irradiation while delayed death occurred approximately 10 days after irradiation. It was further determined that young embryos are not highly susceptible to the acute effects, but that they develop their maximum sensitivity during the 8 to 10 days of incubation. Developmental abnormalities were common, especially those of the beak, limbs, head and eyes. Result of studies on many kinds of organisms, by Landauer(5), revealed that there are definite and well-defined stages of embryonic development during which certain environmental agencies will change the fate of particular parts. Muller *et al*(6) in a study of $LD_{50}/22$ days of incubation dose curves for the chick embryo reported that sensitivity to the combined injury mechanisms of X-rays increases through the first 3 days of development, then decreases through the 12th day where it plateaus at approximately 750 roentgens. Irradiation through the 12th day of development also evoked a progressive depression in growth rates of the surviving chicks.

The present studies were designed to demonstrate the effects of X-irradiation upon the developing chick embryo, the resulting abnormalities and their degree of occurrence. The chicken embryo was chosen for these studies as it offers a number of advantages which enhance its application to X-irradiation experiments. Since the chick is a vertebrate, it might be expected to respond in a manner similar to other vertebrates. The size

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and symmetry of the avian egg make it a convenient biological material for exposure to X-irradiation, affording more uniform density distribution of the rays, and eliminating the need for shielding since no maternal side effects are present.

Methods. Eggs from Single Comb White Leghorns of the Colorado State University experimental flock were selected for use as a source of stock with a uniform genetic background. The eggs were collected daily, sorted according to uniformity of size, and cleaned. They were stored in a refrigerated room at 45°F for approximately 10 days.

Groups of 48 eggs per treatment were selected and identified by code for exposure to X-irradiation beginning on 0 day of incubation. The eggs were exposed in groups of 16 each. The source of X-irradiation was a Westinghouse 150 KV deep therapy unit, operating at 100 KV and 10 MA, providing a dose rate to the embryo site of 85.3 r/min. (6). To insure uniform distribution of the irradiation, the corner eggs were interchanged with the center eggs when one-half the exposure time had elapsed (Fig. 1). This procedure involved an exposure interruption of approximately 50 seconds. The unit was calibrated using a Victoreen condenser-r-meter placed in embryo position to determine the amount of irradiation received at embryo site.

1	2	3	4
5	6	7	8
9	10	11	12
13	14	15	16

FIG. 1. Egg plot for irradiation treatment. Egg position changes at one-half irradiation time: 1-11; 6-16; 4-10; 7-13.

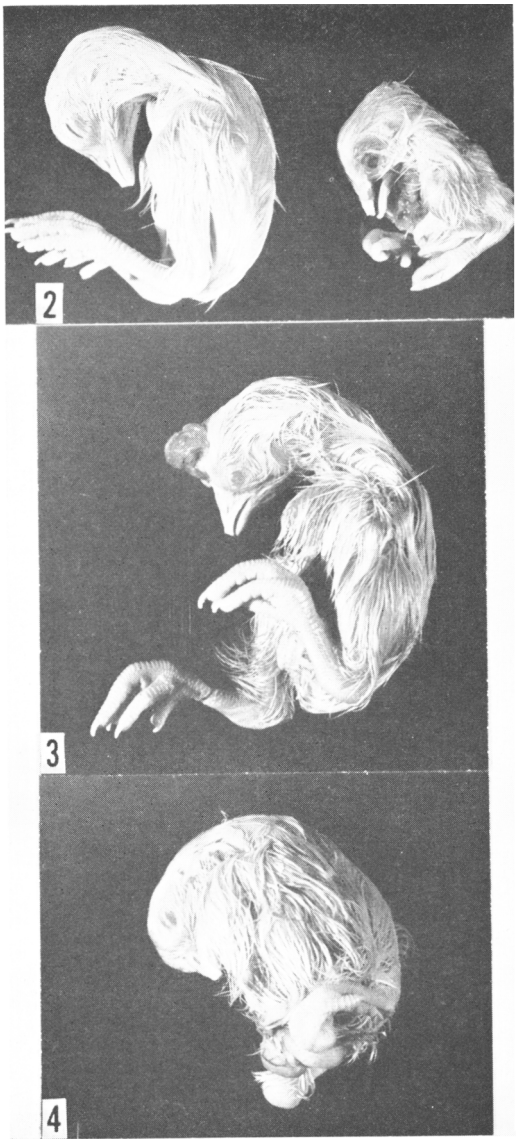

FIG. 2. Twenty-day embryos. A. Normal appearing nonirradiated, and B, midget which received 550 r first day of incubation.

FIG. 3. A 20-day embryo. Brain exposed, cranial roof incomplete. Embryo received 450 r third day of incubation.

FIG. 4. A 20-day embryo. Taliped deformity. Embryo received 450 r fourth day of incubation.

It was determined that by placing the Victoreen meter within the egg a penetration of 60.5% was realized. The half value layer for the X-ray beam was calculated at 1.38 mm of aluminum. Doses were selected to cover the lethal range. Single dose X-irradiation was administered to embryos during each of

TABLE I. X-ray Dosages* in Roentgens Administered to Embryos on Successive Days of Embryonic Development.†

Age of embryo (days)	Dosages in roentgens				
	Group I	Group II‡	Group III	Group IV	Group V
0	400	500	600	700	800
1	250	350	450	550	650
2	275	375	475	575	675
3	250	350	450	550	650
4	375	475	575	675	775
5	450	550	650	750	850
6	500	600	700	800	900
7	550	650	750	850	950
8	500	600	700	800	900
9	550	650	750	850	950
10	625	725	825	925	1025
11	700	800	900	1000	1100
12	725	825	925	1025	1125
13	700	800	900	1000	1100

* A dosage rate at embryo position of 85.3 roentgens per min at settings of 10 MA and 100 KVP.

† Group size varied on the basis of fertility of eggs treated, from 30 to 32 embryos, except in groups 11, IV, V, and 12 and 13, I through V, where group size varied from 25 to 26 embryos.

‡ Previously determined to produce LD_{50}/22 days of incubation(6).

the first 13 days of embryonic development (Table I).

Treatments were applied in a specially designed lead-lined exposure chamber constructed to maintain a temperature equivalent to that of the incubator. Eggs were removed from the incubator just prior to treatment and returned immediately after treatment. The incubator was operated at accepted humidity and temperature settings for optimum embryo development.

Survival during embryonic development was determined by candling the eggs on the 3rd day, the 10th day and also preceding the transfer to pedigree baskets on the 19th day of incubation, prior to hatching. Eggs containing dead embryos were broken out on glass plate, and the stage of development of the embryo compared to a standard set of fixed embryos in order to observe nonviable embryos and determine embryo age at time of death. Treatment histories of abnormalities were noted and the embryos preserved in a 10% formalin solution.

Since ability to emerge from the shell is a sensitive indicator of viability, data on hatchability were included as an additional observation. Individual body weights of chicks were recorded on the day of hatching as a means of providing additional evidence concerning possible alterations in growth rate.

Results. Of 2169 fertile eggs irradiated, 200 abnormal embryos were observed, which accounted for 9.2% of the treated groups, while there were no abnormal embryos observed in the non-irradiated control group. Mortality following irradiation was classified according to a total figure as well as by acute deaths which were determined to have occurred within 48 hours post irradiation. It was noted that embryonic mortality following irradiation rose as the dose received was increased (Groups IV and V), and with limited exceptions acute deaths were substantially below 50% of the total deaths. It is recognized that time of death in embryos of various ages may introduce a bias that influences the extent of the deformities observed. Thus, those that die within 2 days may not live long enough to develop deformities. It is interesting that there were no observed deformities or anomalies among the controls and that there was only one death in this group; this occurred on the 11th day of incubation. Morphological abnormalities were classified as to particular type (Table II), midgets, deformities of feet and legs, mandibles, and miscellaneous modifications which included edema, exencephalia, external body cysts, protrusion of body organs and hydrocephalia (Fig. 2, 3, 4). A strong similarity exists between the morphological changes observed and those commonly found in the normal embryo of the domestic fowl(7); however, the degree of occurrence was increased by the irradiation. Abnormal developmental patterns noted at 20 days of incubation were as follows: Embryo B 8597F — femurs and digits present, remainder of pelvic limbs undeveloped, no other visible abnormalities; embryo received 425 r second day of incubation. Embryo B 8597E — extra digit attached to cranial aspect of tarsus; treatment of 850 r on second day of incubation. Embryo B 8597C — hydrocephalus, malformed and missing digits; embryo received 525 r sixth day of incubation. Embryo B 8597A — left pelvic limb incomplete from tarsus distally; right pelvic limb presented a single

TABLE II. Distribution of Deformities, Expressed as Percent of Embryos Exhibiting Deformities.

Gross observations	Number	Distribution according to dose level (%) Group I*	Group II	Group III	Group IV	Group V
Midgets	80	5.0	7.5	2.5	31.3	53.7
Legs and feet	53	.0	.0	.0	49.0	51.0
Mandibles	28	3.6	3.6	7.2	25.0	60.6
Miscellaneous modifications	9	.0	.0	22.2	44.4	33.4
Hemorrhage	30	10.0	20.0	26.7	10.0	33.3

* See Table I for group dose level.

digit craniodorsal aspect of tarsus; embryo received 425 r on second day of incubation.

The 2 highest doses, 200 and 300 r above the previously determined LD_{50}, were responsible for the majority of the cases of abnormalities. The groups treated between 1 and 6 days of embryonic development displayed 62.5% of the midgets, 73.6% of the foot and leg deformities, and 86.2% of the abnormal mandibles. Gross observation of the appearance of hemorrhage revealed somewhat uniform distribution from zero through 12-day embryos and accounted for 15% of all observed abnormalities.

TABLE III. Deformities of the Mandibles.

	No.	% of occurrence and embryonic age (days) at time of irradiation 4	5	6	7
Crossed or deformed mandibles	8	37.5	37.5	.0	25.0
Parrot beak	20	.0	55.0	45.0	.0

Parrot-type beaks accounted for 71.4% of the mandibular abnormalities (Table III) and were concentrated in the 5- and 6-day group, while crossed or deformed mandibles (cross beak) were produced from treatment at 4, 5 and 7 days of incubation.

A comparison of the effects of irradiation upon hatching weight of the chick is shown in Table IV. The chicks hatched from irradiated embryos in treatments II, III and IV appear to have a slightly greater average body weight. The overall results do not indicate any apparent relationship between hatching weights and the dosage received during the various stages of embryonic development.

The chick embryo was found to be most resistant to the effects of irradiation prior to incubation. In considering the treatment between 1 and 11 days, the data present a uniform pattern of hatchability (Table V). In the majority of cases the percentage of treated embryos capable of hatching decreased as irradiation dose increased. In all situations the experimental group did not hatch as well as the controls.

Discussion. The formation of the upper and lower beaks is complete in the 7-day chick embryo. Irradiation previous to this period interferes with normal beak development. Those embryos which displayed signs of beak damage had been irradiated between 4 and 7 days of development. Of the 28 embryos with deformed beaks, 75% had sur-

TABLE IV. Average Weight of Chicks (Grams), when Hatched from Embryos Treated with Varying Amounts of X-Irradiation* During Progressive Stages of Embryonic Development.

Group	Age of embryo (days) at time of irradiation 0	1	2	3	4	5	6	7	8	9	10	11	12	13
I	37.8	38.7	37.6	38.8	38.6	38.7	38.4	38.0	37.0	38.4	37.1	37.9	37.7	39.0
II†	41.1	38.4	38.3	39.3	38.9	39.7	38.8	38.9	39.5	37.9	38.7	38.4	39.2	39.8
III	40.7	40.1	39.7	39.9	39.9	38.3	39.1	39.1	38.2	38.2	40.1	39.1	39.0	39.9
IV	39.7	40.2	39.8	37.6	39.0	40.1	38.2	40.1	39.1	38.0	37.6	39.2	37.8	38.2
V	38.7	39.8	37.0	39.5	38.0	37.7	38.8	38.2	36.7	38.6	37.9	39.0	38.0	38.9
Controls	39.6	—	—	—	—	—	—	—	—	—	—	—	—	—

* See Table I for group dose level.

† Previously determined to produce LD_{50}/22 days of incubation(6).

TABLE V. Percent Hatchability of Treated Embryos.

	Age of embryo (days) at time of irradiation													
Group*	0	1	2	3	4	5	6	7	8	9	10	11	12	13
I	68.8	66.7	76.7	78.1	75.9	74.2	84.4	79.3	65.6	75.0	81.3	84.4	81.3	76.9
II	48.4	62.5	76.7	75.9	62.5	71.4	70.0	65.5	71.0	65.6	90.6	87.5	72.4	88.0
III	63.3	36.7	48.4	50.0	25.0	33.3	61.3	50.0	56.3	59.4	71.9	56.3	76.9	84.0
IV	61.3	16.7	36.7	33.3	9.4	21.9	43.8	43.8	43.8	31.3	40.6	46.1	53.8	61.5
V	78.1	16.1	12.5	12.5	3.1	18.8	43.8	33.3	9.4	15.6	31.3	19.2	46.2	46.2
Controls	93.5	—	—	—	—	—	—	—	—	—	—	—	—	—

* See Table I for group dose level.

vived until hatching but in all cases were unable to break out of the shell, apparently as a result of a hindrance to the pipping process due to the deformity.

Occurrence of hemorrhage was not readily correlated with dose level or time of exposure. As indicated by areas appearing to be cyanotic, increased vascular permeability apparently reduced the circulation sufficiently, in some instances, to cause vascular distention and eventual death. This apparent damage to the circulatory system of the chick embryo was distributed uniformly from 0 day through 12 days of embryonic development, with all abnormalities being quite well distributed throughout this period. In other embryos, evidence of edema was especially noticeable in the cranial region.

As previously suggested by Muller *et al* (6), the embryo is most sentitive to X-ray irradiation during 1 through 6 days as measured by hatchability. A direct relationship between dose level and hatchability has been established in the present study, indicating a reduction in the viability of surviving embryos. In some groups the embryos which survived irradiation and hatched exhibited the greater body weights, when compared with the controls. This may be due to the fact that the weaker or less developed embryos succumbed to the detrimental effects of irradiation administered during development, and the survivors represented the stronger and more vigorous individuals. Further investigations to determine if these chicks also possess the ability to transmit their higher resistance would be most interesting.

Summary. Single dose, whole body X-irradiation of Single Comb White Leghorn embryos from 0 through 13 days of development resulted in an increased incidence of morphological abnormalities similar to those commonly observed among embryos of the domestic fowl. The two highest doses, 200 and 300 r above the previously determined LD_{50}, were responsible for the majority of the abnormalities which included midgets, foot and leg deformities and abnormal mandibles. Hatching percentage of treated embryos decreased as the dose increased; no apparent relationship existed between hatching weight and dose received.

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