

## EMBRYONIC MORTALITY RESULTING FROM GAMMA IRRADIATION<sup>1</sup>

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It is generally accepted that irradiation can cause death of biological materials. Animals vary in their degree of tolerance in terms of age, size and species. The chick embryo has been used extensively in irradiation studies because embryonic age can be easily controlled and embryonic viability is easily determined by candling the egg. The purpose of this study was to determine the lethal effect of varying dosages of gamma radiation on developing chick embryos, to attempt to identify significant sex alterations and to observe the treated embryos and chicks for morphological abnormalities.

### MATERIALS

One-hundred-fourteen White Leghorn eggs were incubated prior to treatment with irradiation. The eggs were placed in the incubator so that there would be one and one-half dozen at 9 days embryonic age and two dozen each for embryonic ages of 4 days, 3 days, 2 days and 1 day, at the time of exposure. Two dozen eggs were unincubated prior to treatment. All eggs were incubated the day after they were laid. Of the 138 eggs used, nine were lost because of infertility or cracking.

A cobalt<sup>60</sup> source was employed for irradiating the eggs. Treated eggs were exposed for one hour; control eggs were removed from and returned to the incubator with the treated eggs. All eggs were out of the incubator for approximately two hours. Irradiation dosage was controlled by calibrating the intensity at varying distances from the source. Three levels of treatment were employed: 1000r (11½" from source), 500r (16½") and 200r (26½").

For purpose of exposure, two groups of ten eggs each constituted the experimental groups at each age level except for the 9-day group. Treatments of 1000r and 500r were given to all except the 9-day groups, which received dosages of 500r and 200r. When the eggs were first candled, which was on the morning that the eggs were irradiated, all eggs in excess of the 20 needed for the two treatment groups at each age level were used as controls. Consequently in Table 1 there are ten eggs represented in most of the treatment groups and there is a maximum of four eggs in the groups which received no treatment.

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TABLE 1. HATCHABILITY AS INFLUENCED BY IRRADIATION  
DOSAGE AT DIFFERING EMBRYONIC AGES

Embryonic Age (days)	1000r				500r				200r				Control	
	Eggs Set	Chicks Hatched	Eggs Set	Chicks Hatched	Eggs Set	Chicks Hatched	Eggs Set	Chicks Hatched	Eggs Set	Chicks Hatched	Eggs Set	Chicks Hatched	Eggs Set	Chicks Hatched
9	...	...	9	9	9	9	7	7	2	2	2	2	2	2
4	9	0	8	7	8	7	...	...	2	2	2	2	2	2
3	10	0	10	10	10	10	...	...	4	4	4	4	4	4
2	10	0	9	9	9	9	...	...	3	3	3	3	3	3
1	10	0	10	9	10	9	...	...	4	4	4	4	4	4
0	10	8	10	9	10	9	...	...	2	2	2	2	2	2
Total	49	8	56	53	7	7	7	7	17	17	17	17	17	17

## RESULTS

Data for the 129 fertile eggs are presented in Table 1. Dosages of 500r and 200r at nine days embryonic age were not deleterious to the developing embryos. However, the 1000r dosage was lethal to all of the eggs which had been artificially incubated. The unincubated irradiated eggs which were treated with 1000r took longer to hatch than did the non-irradiated eggs. Because hatchability of all other groups fell within an expected normal range, it seemed important to examine the mortality caused by 1000r. An obvious question is "Did the exposure to gamma rays cause immediate death, or was there a latent survival period?" Embryos which died were individually examined. Twenty-eight of the 41 dead, appeared to have succumbed at the time of treatment (Table 2). However, at no age level was 1000r immediately lethal to all treated embryos.

TABLE 2. LETHALITY OF 1000r OF GAMMA RADIATION  
TO DEVELOPING CHICK EMBRYOS

Embryonic Age (days)	Eggs Set	Number of Dead Embryos		Chicks Hatched
		When Treated	Post- Treatment	
4	9	5	*4	0
3	10	8	2	0
2	10	8	2	0
1	10	7	3	0
0	10	0	2	8

\* A live chick which had not broken the shell was found in one of the eggs when examined on the 22nd day of incubation.

In commercial operations, a 90% hatch of all eggs set is considered very good. The hatchability of the eggs in the control, 200r and 500r groups was above 90%.

Baby chicks which hatched from the control and experimental groups appeared healthy and normal except for #8682M which hatched from an egg treated with 1000r prior to incubation. That chick had aberrant digits and metatarsus on the left leg. The first digit, which normally is relatively short and projects posteriad, was elongated and was enlarged in the metatarsal area. It measured 10 mm. without toenail; the normal appearing first digit on the right foot measured 8 mm. including toenail. The other three digits on the left side measured 18 mm., 7 mm., and 4 mm. Corresponding lengths of digits on the right side were 19 mm., 23 mm. and 14 mm.

## DISCUSSION

Chick embryos have been used as a biological system for evaluating radiation effects since 1904 (1). Loken *et al* (2) reported that the LD<sub>50</sub> for their unincubated White Leghorn eggs was approximately 700 rads. A number of studies (2, 3, 4, 5, 6, 7) have considered the lethal effects of irradiation on the developing embryo. However, most of these experiments have terminated prior to the hatching date. Perhaps the trials which most nearly parallel the current study were those reported by Sandvik (8). Although the experimental procedure differed in some respects (dosage range was 270r to 3700r, time of exposure was 6 hours and all eggs were not incubated before 24 hours old), two observational results in common were limb anomalies and prolonging of incubation period. Sandvik reported an LD<sub>50</sub> of approximately 1000r.

Kushner (9) associated improved hatchability and reproductivity with small doses of gamma radiation. Although his hypothesis has not been tested with the current study, there does not appear to be a reduction of hatchability with doses of 500r and less.

The lethal response of chick embryos from 2-16 days of incubation has been reported for X-irradiation (3). Guided by the studies of Goff, who reported an extreme sensitivity of 8-9 day embryos, we substituted a lower (200r) dosage for the 1000r dosage at the 9-day level. All of the treated embryos in our small population hatched after the treatment at 9 days, so we were unable to demonstrate a critical embryonic age during this stage of development for gamma irradiation. However, of the 16 chicks that hatched, 14 proved to be males.

Stearner *et al* have studied radiation mortality of chicks (10) and of chick embryos (7). Two related phenomena, as suggested by their work, by the current study, and by the work of others are: (1) Lethality varies directly with dosage and (2) older embryos, ie. during the second and third weeks of development, are less susceptible to the lethal effect of a given dosage than are young (first week) embryos.

## SUMMARY

White Leghorn eggs were treated with Cobalt<sup>60</sup> gamma rays. A dosage of 1000r proved fatal to all embryos which were from one to four days old when treated. Death was not instantaneous for all treated embryos. A dosage of 500r had no measurable effect on the survival of chick embryos. Dosages of 500r and 200r were not deleterious to 9-day old chick embryos.

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