THE EFFECT OF X-IRRADIATION ON THE DEVELOPMENT OF CHICK EMBRYO*

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INTRODUCTION

Ten years ago, Stearner *et al.* (1960) studied the mortality and modes of death of chick embryo after exposure to Co^{60} gamma-ray (1,2). Later experiments by Stearner *et al.* (1968) described the early mortality following various doses of X-irradiation and the cause of death(3,4). Their electron-microscope study (1969) revealed that early mortality of the chick embryo was due to early vascular injury after irradiation (5). Murakami *et al.* (1963) reported the relation of X-ray dose to the malformation in the mouse foetus (6,7). Robert (1971) studied the growth depression, malformation and mortality response of rat embryos to variations in exposure rate of 150R X-irradiation (8). Kohn *et al.* (1954, 1955) observed the weight changes of mice testes after treatment of X-irradiation (9,10). Kallman *et al.* (1955) used X-ray and Jordon *et al.* (1956) used Co^{60} gamma-ray to treat mice and the reaction of the mice's spleens to radiation was observed (11,12). The present study referred to the researches described above. Various doses (100-1100R) of X-ray were used to treat 3 to 11-day chick embryos. The effect of X-irradiation on the time of death, part and percentage of deformity, and the growth rate of chick embryos were studied; the changes in the body weight, liver, kidney, spleen and testis of chickens were also studied.

MATERIALS AND METHODS

For our experiment, a total of 600 fertilized eggs of Shaver 288# were selected and incubated in an egg incubator. Incubator temperature was maintained at 38°-38.5°C, relative humidity about 60% and the eggs were turned automatically.

Starting from the third day of incubation, five batches of 100 each, of well incubated eggs were chosen every other day consecutively, i.e. on the 3, 5, 7, 9 and 11th day after incubation. Each batch was then divided into 5 groups, i.e. 20 eggs per group. One of the five groups was treated as a control and the remaining four were radiated with 100R, 300R, 700R and 1100R X-ray respectively. Conditions of irradiation were: 250 KVp, 10 mA deep X-rays; filter, 0.25mm Cu+1mm Al; H.V.L.=1.09mm Cu; T.S.D.=35cm; open field, 22cm dia. circle; and exposure rate, 103R per minute.

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RESULTS

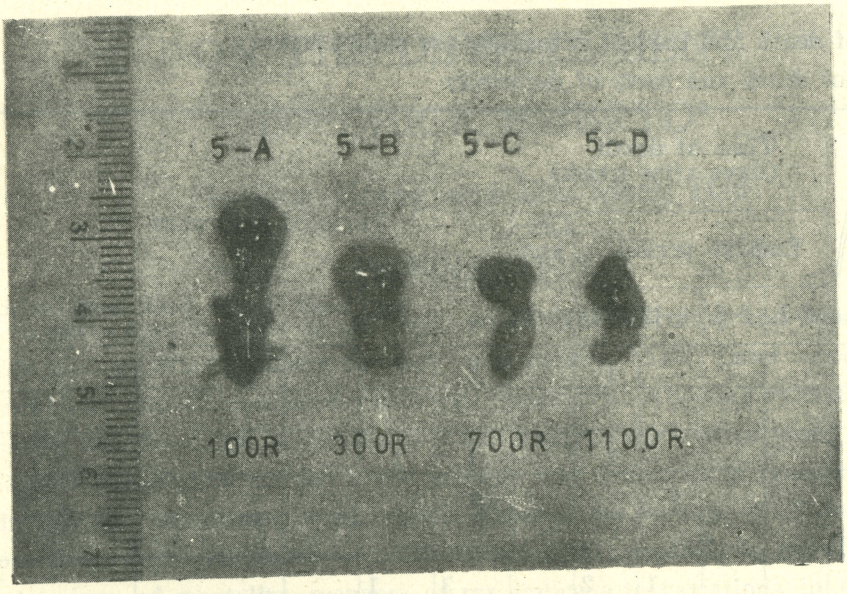
The time of death and part of deformity are shown below:

Table 1. Time of death and parts of deformity

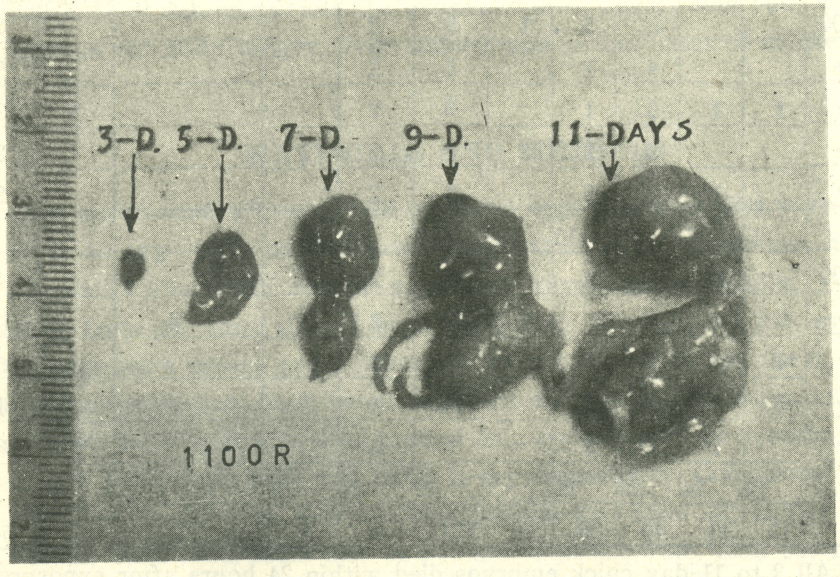
Item / No. / Dose (R) / Age at irr. (days) / Group			Time of death (days after irrad.)						Parts of deformity				
			Embryo period				Chi-ken p.	subt.	leg	beak	leg & beak	neck	subt.
			0-1	2-5	6-12	13-19	20-30						
Contr.	3	0				1		1					
	5	〃											
	7	〃				1		1	1				1
	9	〃			1			1					
	11	〃											
	Subtot.				1	2		3	1				1
A	3	100		2		4	2	8	1				1
	5	〃		1	1			2	3				3
	7	〃		2	1			3	2				2
	9	〃			3	2		5					
	11	〃			2			2					
	Subtot.			5	7	6	2	20	6				6
B	3	300			5	3	1	9	2				2
	5	〃			4	1		5	5				5
	7	〃	1	1	3	1		6	2			1	3
	9	〃			2	3	2	7	2				2
	11	〃		1	2	2		5	2				2
	Subtot.		1	2	16	10	3	32	13			1	14
C	3	700		16		4		20	2	2			4
	5	〃		4	16			20	8	7	2		17
	7	〃	4		2	2		8	5				5
	9	〃	8	3	2	1		14	1				1
	11	〃		1	2	4		7	5			1	6
	Subtot.		12	24	22	11		69	21	9	2	1	33
Total			13	31	46	29	5	124	41	9	2	2	54

* Group D: All 3 to 11-day chick embryos died within 24 hours after exposure to 1100R, as shown in Fig. 1 (b)

Fig. 1



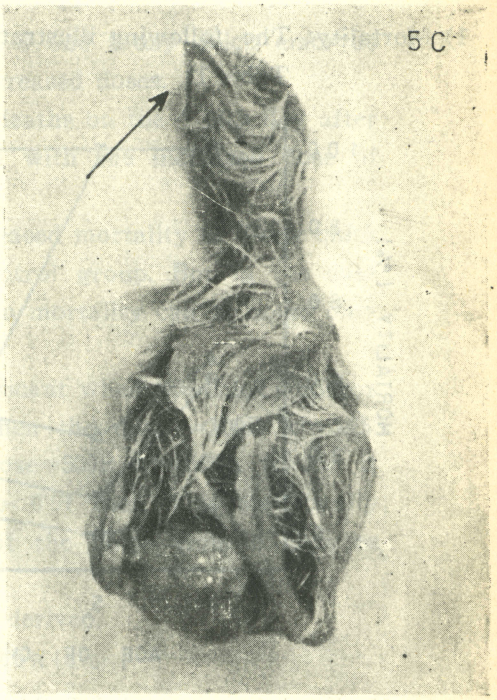
(a) Features of 5-day chick embryos, 24 hours after doses of irradiation.



(b) Features of chick embryos (group D) at different ages, which died within 24 hours after exposure to 1100R.

(c) Arrows show the deformity formed after exposure to 700R:

"Shortage on the lower jaw and deformity on the right foot" Age of chick embryo at irradiation: 5 days



"Shortage on the lower jaw" Age of chick embryo at irradiation: 5 days

"Shortage on left foot" Age of chick embryo at irradiation: 7 days

"Both feet were bent and unable to stand up" Age of chick embryo at irradiation: 11 days

1) Mortality: The following illustrations shown below were derived from Table 1:

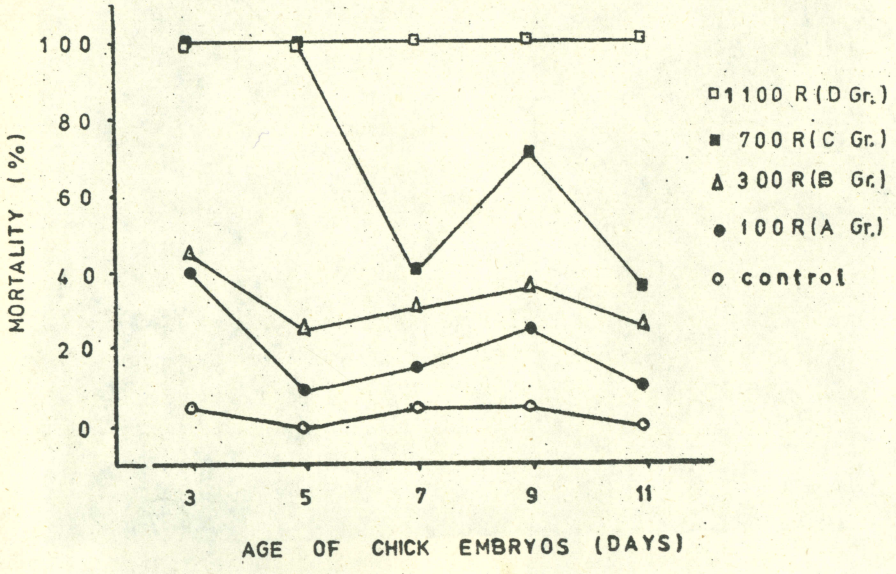


Fig. 2 % Mortality of groups of chick embryos at different ages after exposure to various doses.

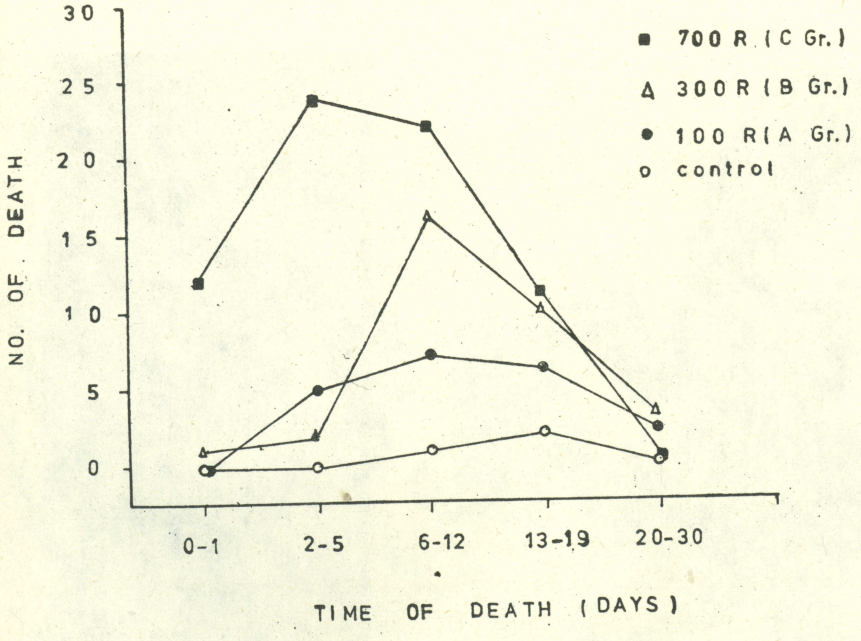


Fig. 3 Time of death and total no. of deaths of chick embryos after exposure to various doses.

As shown in Fig. 2, increased mortality occured in 3 and 9-day chick embryos after radiation and the mortality increased with the increased doses.

As shown in Fig. 3, there is increased total no. of deaths on the 6-12th day after exposures of 700R and less. The no. of deaths increased with the increased dose of radiation.

By means of the proportional method, there is increased mortality of 3-day chick embryos after exposure to 100R as compared to the control group. But in the case of 5, 7, 9, and 11-day chick embryos, obvious changes in mortality can be noted only when the doses are over 300R.

Chi-square test: The mortality change is not significant when the chick embryos at different ages are radiated with 100R or 300R. As when the dose =100R $x^2=8.25$. $p>0.05$ so it is statistically not significant. When the dose=300R $x^2=2.574$, $p>0.05$ it is not significant either. When the dose=700R $x^2=36$, $p<0.01$ then it is statistically significant. Thus the mortality changes when the age of the chick embryo changes are significant.

2) Deformity rate: According to Table 1, Fig. 4 was derived,

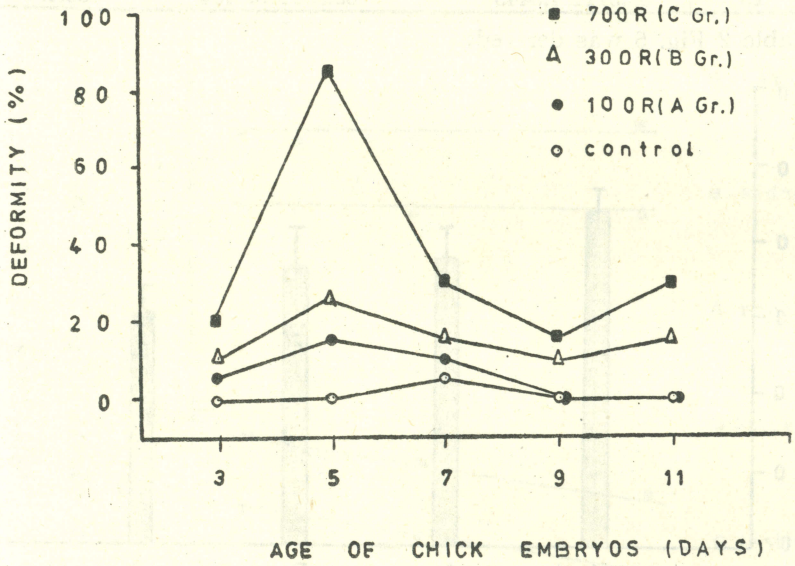


Fig. 4 The deformity rate of chick embryos at different ages after exposure to various doses.

As shown in Fig. 4 the deformity of all 5-day chick embryos after radiation increased while all 9-day chick embryos decreased. The deformity of the chick embryos at the same age increased with the increased doses of radiation. All chick embryos in group D died after exposure to 1100R within 24 hours, so no deformity rate was shown.

Chi-square test: Chick embryos at different ages after exposure to 100R have no obvious changes in deformity rate. As when the dose=100R $x^2=9.5$, $p=0.05$ it is statistically significant. When the chick embryos at different ages were radiated with

300R the deformity rate did not change because of the difference in age. As when the dose=300R $x^2=2.4$, $p>0.05$ it is statistically not significant. When the chick embryos were radiated with 700R the deformity rate changes greatly as the age changes. As when the dose=700R $x^2=36$, $p<0.01$ it is statistically significant.

3) Growth rate: Upon hatching, 15 chickens, at about the same weight, in each group were chosen. They were weighed and raised under the same conditions. Two weeks later they were weighed again and the weight gained was calculated. The mean and standard error are also calculated in Table 2 as below,

Table 2

Item / Mean & S.E. / No. of chickens / Group		W. of chickens, initial (gm)	B.W. of chickens, two weeks later (gm)	B.W. gained (gm)
Control (0R)	15	35.593±1.7246	79.416±3.0228	43.823±2.5909
A (100R)	15	35.349±1.4006	73.053±4.1698	37.746±4.0035
B (300R)	15	35.483±1.8795	71.037±6.2191	35.613±6.1805
C (700R)	15	32.311±1.5443	62.350±5.2464	30.039±4.1013

From Table 2 Fig. 5 was derived:

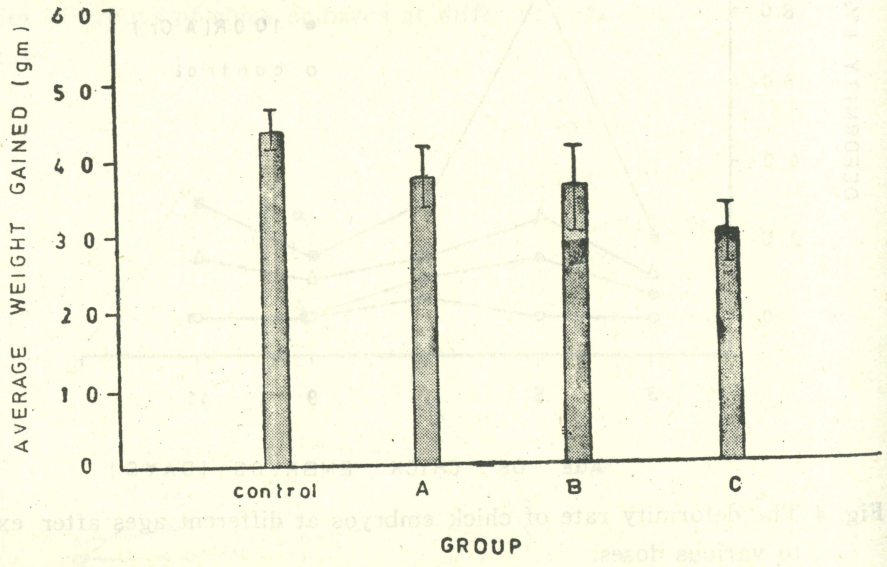


Fig. 5 Mean of weight gained (in two weeks) of chickens after exposure to different doses

As shown in Fig. 5, the more radiation the chickens were exposed to, the less weight the chickens gained in a two-week's period. From t-test, the values of P's when the chickens were radiated with 100R, 300R and 700R were all less than 0.01. It is statistically significant. Thus the growth rate of the chickens which have been exposed to 100R or higher in the embryo period at the age of less than 11 days are all smaller than those in the control group.

4) Proportion of organs to body weight: After the chick embryos which have been exposed to various doses of radiation hatched, 10 male chickens in each group were raised under the same conditions for two weeks. Then all chickens were weighed and dissected. The proportion of the visceral organs to the body weight was calculated.

Table 3

Item / Mean & S.E. / Chickens / Group		Liver (%)	Kidney (%)	Pancreas (%)	Spleen (%)	Testis (%)
Cont. (0R)	10each	3.51±.1536	1.32±.0658	.685±.0695	.0809±.0071	.0412±.0054
A (100R)	〃	3.33±.1776	1.26±.1200	.660±.0808	.0669±.0068	.0380±.0049
B (300R)	〃	3.38±.1642	1.29±.1019	.654±.0698	.0615±.0053	.0317±.0039
C (700R)	〃	3.48±.2264	1.33±.0700	.629±.0374	.0438±.0043	.0208±.0033

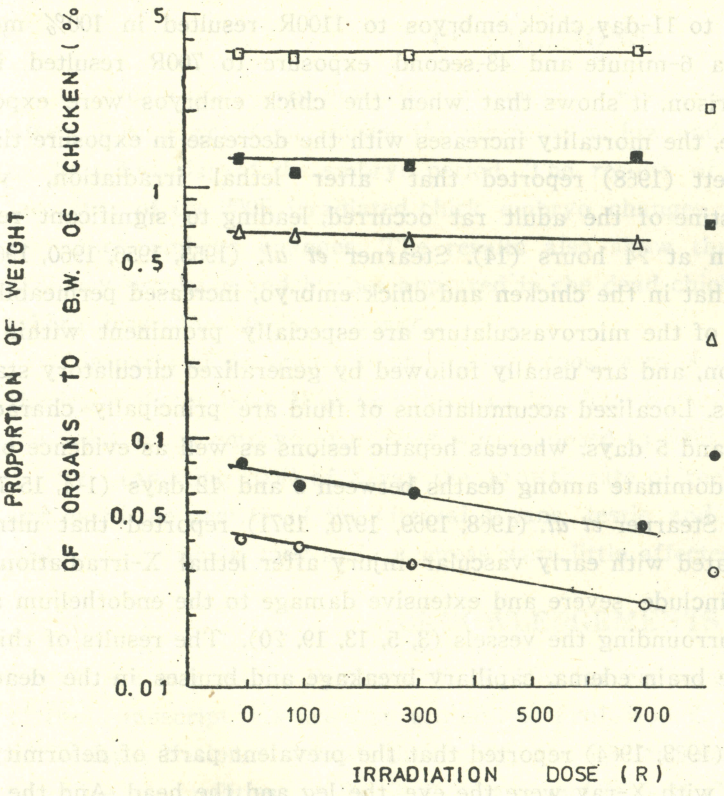


Fig. 6: (Derived from Table 3) Proportion of organs to body weight after exposure to various doses

As shown in Fig. 6, after the chick embryos which had been exposed to various doses of X-ray hatched and were raised for two weeks, the chicken's liver, kidney and pancreas were little affected by the radiation while spleen and testis were more

affected by the radiation.

By t-test, when the chick embryos were radiated with 300R, and 700R, the p of the testis and spleen were less than 0.01, it is statistically significant. The p's of liver, kidney and pancreas were all greater than 0.01, so it is statistically not significant.

DISCUSSION

The results of this experiment show that 50% of the 3 to 11-day chick embryos died within 6-12 days after exposure to 300R X-ray; 66.7% died within 2-12 days after exposure to 700R and all chick embryos died within 24 hours after xpeosure to 1100R. It reveals that early mortality of the chick embryos increased with X-ray doses. With lower X-ray doses, the mortality is low and the chick embryos died later in the 6-19th day of embryo period.

According to Stearner *et al* (1969) a 12-minute exposure of 3-day chick embryos to 1100R resulted in 95% mortality within 24 hours and when the dose was decreased to 700R the mortality reduced to 4% (13). In this experiment, a 10-minute and 41-second exposure of 3 to 11-day chick embryos to 1100R resulted in 100% mortality within 24 hours and a 6-minute and 48-second exposure to 700R resulted in 12% mortality. By comparison, it shows that when the chick embryos were exposed to the same X-ray dose, the mortality increases with the decrease in exposure time.

Eddy and Casarett (1968) reported that after lethal irradiation, vascular occlusion in the intestine of the adult rat occurred, leading to significant reduction of mucosal circulation at 24 hours (14). Stearner *et al.* (1955, 1956, 1960, 1965, 1966, 1967, 1968) reported that in the chicken and chick embryo, increased permeability and degenerative change of the microvasculature are especially prominent within a few hours after irradiation, and are usually followed by generalized circulatory stasis and death within 24 hours. Localized accumulations of fluid are principally characteristic of deaths between 2 and 5 days, whereas hepatic lesions as well as evidence of hematopoietic failure predominate among deaths between 6 and 12 days (1-4, 15-18). A microscope study by Stearner *et al.* (1968, 1969, 1970, 1971) reported that ultrastructural changes associated with early vascular injury after lethal X-irradiation in the 3-day chick embryo include severe and extensive damage to the endothelium and the mesenchymal cells surrounding the vessels (3, 5, 13, 19, 20). The results of this study show that there were brain edema, capillary breakage and bruises in the dead chick embryos.

Murakami *et al.* (1963, 1964) reported that the prevalent parts of deformity when mice were irradiated with X-ray were the eye, the leg and the head. And the highest deformity rate occurred when 11-day mice embryos were irradiated with 200-300R (6, 7). The parts of deformity formed after exposure to X-ray in this experiment were the leg 77%, the beak 18%, and the neck 5%. The highest deformity rate occurred in the 5-day chick embryos after they were exposed to 700R.

According to Pegelman *et al.* (1965), the X-irradiation will stimulate the growth

of chickens when the radiation is below 300R. A climax in the stimulating action was obtained at 20-50R. In this experiment when the chick embryo was exposed to X-ray below 300R, the growth rate of the hatched chicken was retarded rather than stimulated. When the dose reached to 700R, the growth rate decreased obviously. By irradiating the sublethal dose (600-1000R) the growth rate of the chick was retarded and the growth rate of the spleen, the thymus, and the genital organs were also retarded. The liver and the kidney were less affected (Pegelman 1965). In this study the same results were obtained when the chick embryo was radiated with 300-700R X-ray. The growth rate of the spleen and the testis were lowered, while the liver and the pancreas were little affected.

Stearner *et al.* (1969) reported that when the 3-day chick embryo was irradiated with X-ray for 12 minutes (i.e. dose rate 79R/min.) the $LD_{50}=948\pm9R$; and when it was for 240 minutes (i.e. dose rate 7.7R/min.) $LD_{50}=1849\pm41R$ (13). The LD_{50} changes with the different dose rates (22, 23). The dose rate employed in this study is higher (103R/min.) and the LD_{50} is lower. The relationship of age and species to LD_{50} should also be considered (24).

CONCLUSIONS

It reveals that early mortality of the chick embryos increased with high X-ray doses. With lower X-ray doses, the mortality is low and the chick embryos died later in the 6-19th day of the embryo period. The results of this study show that the mortality of the 700R irradiated chick embryo changes obviously when the age of the chick embryo changes. The results also show that there were brain edema, capillary breakage and bruises appeared in the dead chick embryos after exposure to a high dose (700-1100R) of X-ray.

The parts of deformity formed after exposure to X-ray in this experiment were the leg 77%, the beak 18% and the neck 5%. The highest deformity rate occurred in the 5-day chick embryo after they were exposed to 700R.

By irradiating 700R of X-ray the growth rate of the chick was retarded greatly, and as to the growth of the visceral organs, testis and spleen were retarded obviously while liver, kidney and pancreas were little affected.

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中 文 摘 要

X-射線對鷄胚發育之影響

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本研究選用600個秀拔（Shaver）288#之受精鷄蛋，置於鷄孵卵器中，並保持 38°-38.5°C之溫度及60%濕度。孵至第三天開始，每隔兩天處理一次，連續五次，每次選出100個發育良好之鷄胚，分成五組，每組20個，其中第一組爲對照組，其餘四組分別照射100R、300R、700R 以及 1100R之X-射線，然後觀察(1)死亡率與死亡時間之分佈，(2)畸形部位與畸形率，(3)孵出小鷄後之生長率，(4)各器官對體重百分比之變化等。

經用X-射處理鷄胚，照射劑量愈高者，其死亡率愈大而且多在發育早期死亡。照射劑量較低者其死亡率較小，其時間多在發育之中後期死亡。本研究發現照 700R 時，鷄胚之死亡率乃隨胎齡而有顯著的差別。鷄胚經X-射線處理所產生的畸形，其部位以脚部畸形佔77%，喙部畸形佔18%，其餘爲頸部。本研究並發現以700R照射 5 天胎齡所導致的畸形率爲最高。以700R 之 X-射線處理，對鷄胚的生長有顯著的抑制效果，並對睾丸及脾臟之生長也有顯著的抑制現象。但對肝、腎及胰等器官的影響不大。