The practical applications of findings such as these are limited. Since the protective value of castration is within the range of 30% or so, it might be recommended in human cases, only when the whole body exposure was believed to be close to, but not above, the LD/100/30 level, (or 600 r X- or gamma rays). In such cases a combined treatment with bone marrow (or spleen) and castration might well mean the difference between survival and death of the irradiated male.

Summary and conclusions. 1. Male mice of the ICR and CF1 strains were X-irradiated to the LD/50/30 range and some were castrated in order to determine whether the loss of testosterone might alter survival. 2. In both strains, post-irradiation castration (within 30 minutes) did indeed increase statistically the survival of the mice. 3. A delay in castration of 24 hours after irradiation (of the CF1 males) increased survival chances, and estradiol (at that time) further improved survival probability. However, estradiol benzoate given immediately after X-irradiation and castration had an unfavorable effect. 4. It is suggested that the mechanism of effect of castration may be in the encouragement of lymphocyte recovery, thus reducing the usual effects of lymphopenia attendant upon X-irradiation. 5. Since the survival improvement by post-irradiation castration is statistically significant, but amounts to only about 30%, its efficacy lies near the LD/100/30 range, and not higher. Possibly in combination with bone marrow the benefits would be additive, or even synergistic.

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Toxicity of High Levels of Calcium-45, Strontium-90 and Promethium-147 to Avian Embryos.* (29467)

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The increasing exposure of man to radioactive substances in his environment necessitates a knowledge of the biological effects of prolonged or continuous irradiation. The chick embryo is particularly suited for a study of irradiation effects as it is completely

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TABLE I. Embryonic Response to Varying Levels of Ca^{45} Injected into Eggs (6 Eggs per Treatment).

Ca^{45} inj, μc	Day of incubation 4th Hatched	4th Abnormal*	8th Hatched	8th Abnormal*	14th Hatched	14th Abnormal†
0	—	—	4	0	5	0
50	5	0	4	0	6	0
100	3	2	3	2	5	0
200	4	2	4	1	3	0
400	3	4	0	6	4	1

* Constricted area around center of long bone shafts. † Weak legs.

divorced from maternal considerations during its development. Karnofsky *et al*(1), who reviewed the literature on the toxicity of roentgen rays to the chick embryo prior to 1950, found them to exert both an acute and a delayed toxic effect depending upon age of embryo, total dose and dose rate. Stearner *et al*(2) exposed chick embryos to acute dosages of Co^{60} gamma rays on the 6th day of incubation and found that the mortality within 12 days falls into 3 time intervals which were closely correlated with the appearance of certain grossly visible pathologic conditions in decedents. Warren and Dixon(3) injected P^{32} in chick embryos to provide a source of continuous radiation. They found that the P^{32} concentrated largely in the bones and that the radiation caused an overall growth retardation, resulting in small but normally proportioned birds. Since various radionuclides differ in tissue specificity and type and strength of their emissions, it was considered essential to supplement this P^{32} data with that from other radionuclides. Ca^{45}, a bone seeker, has a weaker beta emission, 0.25 Mev., than P^{32}, 1.7 Mev. Sr^{90}, also a bone seeker, is chemically similar to calcium but has a 0.61 Mev. beta emission while its daughter Y^{90} has a 2.18 Mev. beta emission. Pm^{147} has a beta emission (0.22 Mev.) similar to Ca^{45} with its primary sites of deposition being the liver and the skeleton(4).

Methods. Single Comb White Leghorn eggs were candled and 6, except where noted, fertile eggs were aseptically injected with 0.2 ml of sterile distilled water or solutions containing Ca^{45}, Ca^{48}, Sr^{90} or Pm^{147} in the chloride form and then sealed with colloidin. On the 22nd day of incubation all unhatched embryos and chicks were examined for gross abnormalities.

In the first experiment, 4 levels of Ca^{45} (50, 100, 200 or 400 μc) were injected into eggs at 4, 8 or 14 days of incubation. In the second experiment, 400 μc of Ca^{45} were injected into eggs that had been incubated from 0 to 12 days. In the third experiment, eggs were injected with 60, 120, or 240 μg Ca^{48} on the 4th or 8th day of incubation to determine the effect of the size of the calcium atom. In the 4th experiment, eggs were injected with 50, 100, 200, 400, or 800 μc Sr^{90} at 4, 8 or 14 days of incubation. Y^{90} was in equilibrium with Sr^{90} at time of injection. In the 5th experiment, eggs were injected with 50, 100, 200, 400, 800 or 1600 μc of Pm^{147} at 4, 8 or 14 days of incubation. Some of the embryos were rendered transparent with potassium hydroxide and their skeletons stained with alizarin red-S which is specific for calcium, utilizing a procedure based on a process described by Davis and Gore(5).

Results and discussion. In the first experiment (Table I), the 50 μc level of Ca^{45} did not have any detrimental effects on embryonic development. Only 58% of the eggs that received 100 or 200 μc Ca^{45} at 4 or 8 days of incubation hatched, with 29% of the embryos from the total eggs injected having leg abnormalities. Only 25% of the 400 μc Ca^{45} injected group at these same periods hatched with 83% of those injected having leg abnormalities. Those embryos injected at the 14th day of incubation were not affected by the irradiation, although one of the chicks that hatched after receiving 400 μc of Ca^{45} had weak legs.

The characteristic leg abnormality ob-

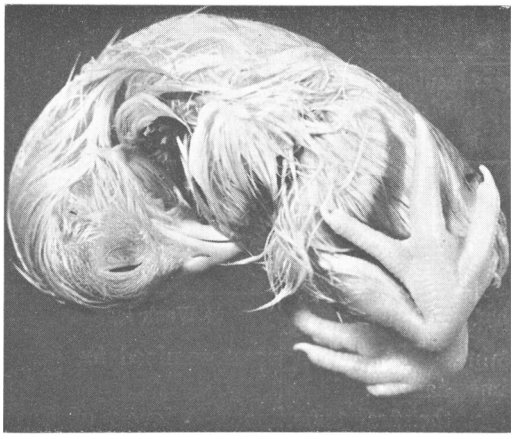

FIG. 1. A 22-day embryo injected with 400 μc Ca^{45} on the 8th day of incubation with broken tarsometatarsus.

served with high Ca^{45} injection is shown in Fig. 1. The legs were bent awkwardly and the feet were edematous, in most instances, and appeared to lack secure attachment to the tarsometatarsus. Similarly, the latter bones and femur were apparently weak or non-existent. The skeletal structures were examined after being stained with alizarin red-S (Fig. 2) and the abnormal legs and wings were found to be the result of broken or fractured shafts of the long bones. The leg bones were unusually thin at the center of the shafts, while the ends grossly appeared to be normal. In some embryos, a discontinuity of the clavicle separating the ramus and the hypocledium were observed. Hemorrhages were associated with the latter stages of embryo development suggesting that the breaking of the bones might be associated with greater activity of the embryo during the period.

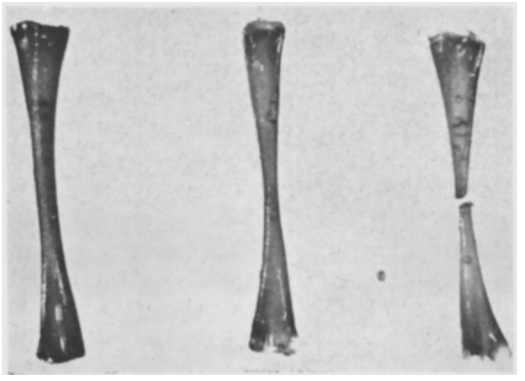

FIG. 2. Alizarin red-S stained tibia: left, from normal day-old chick; middle and right, from day-old chick injected with 200 μc Ca^{45} on the 4th day of incubation.

These abnormalities certainly differ extensively from those reported by Warren and Dixon(3) who used as high as 200 μc P^{32} at 8 days of incubation. They reported the bones to be dwarfed, but normal in proportion and external appearance. In their cortical bones, osteoblastic and osteoclastic activity, which normally is responsible for the definite modeling of the bone structure, was reduced. This retarded bone formation in the shaft, as evidenced by the thin cortical bone, was made up of a reduced number of bony lamellae which tended to be larger than normal. It may be the levels of P^{32} used were too low to produce fractures.

TABLE II. Embryonic Response to 400 μc Ca^{45} or Water Injected into Eggs on Different Days of Incubation.

Day inj	Fertile eggs inj	Mortality (wk) 1st	2nd	3rd	Hatched	Abnormal legs
			Ca^{45}-injected group			
0	5	4	0	0	1	1
1	5	2	2	0	1	0
2	6	6	0	0	0	0
3	6	2	1	3	0	3
4	6	1	1	2	2	4
5	6	1	1	3	1	4
6	6	3	2	1	0	1
7	6	1	4	1	0	1
8	6		4	2	0	2
9	6		2	3	1	3
10	6		1	4	1	3
11	5		1	3	1	2
12	5		1	3	1	1
			Water-injected group			
0	6	2	1	2	1	0
4	5	1	1	0	3	0
8	6		0	4	2	0

In the second experiment (Table II), mortality from 400 μc Ca^{45} was found to be high during the early incubation period for those embryos injected early, making it difficult to detect abnormalities in these embryos. No eggs hatched that were injected with 400 μc Ca^{45} at 2, 3, 6, 7 or 8 days of incubation. Leg abnormalities were the most severe in embryos injected at 9 or 10 days of incubation. Of 34 embryos that survived to the 3rd week from all groups, 74% were found to have abnormal legs and 24% abnormal

TABLE III. Embryonic Response to Varying Levels of Sr^{90}-Y^{90} Injected into Eggs (6 Eggs per Treatment).

Sr^{90} inj, μc	Day of incubation 4th		8th		14th	
	Hatched	Abnormal	Hatched	Abnormal	Hatched	Abnormal
0	3	0	5	0	3	0
50	3	1*	5	0	5	0
100	2	1*	5	1†	3	1*
200	0	0	1	1†	3	0
400	0	0	0	4†	2	2*
800	0	0	0	4†	0	5‡

* Deformed toes. † Constricted areas around center of long bone shafts. ‡ Heavy edema of head and neck.

wings upon external examination with no abnormalities being observed in the controls.

Since the 400 μc Ca^{45} dosage contained approximately 60 μg of calcium, it was believed possible that the abnormalities observed might be attributed to a spatial distortion of the hydroxyapatite crystal of bone caused by the Ca^{45} atom rather than to damage from its beta emission. In the 3rd experiment no specific abnormalities were observed, nor was hatchability affected, in embryos injected with up to 240 μg Ca^{48}. The long bone abnormalities, therefore, were due to the beta emissions from Ca^{45} which was deposited when the bones began to calcify after the 8th day of incubation and before the 14th day when the deposition of Ca^{45} would be diffused over a much larger bone surface. The first permanent staining of bones by alizarin red-S in potassium hydroxide cleared embryos occurred in the center of the tibiae of the 11-day embryos. From this period on, the calcifying portion of the bone covered a larger area, even though the center of the bone continued to be stained darker. At the 14th and 15th day of incubation most of the skeletal structure was permanently stained. Thus, the ionic unbound form of calcium injected prior to the 12th day of incubation would be deposited in a very concentrated area in the center of the bone. Here it would, because of its concentration, produce intensive radiation damage to the bone cells and result in weakening of the osseous structure and greater fragility of the shaft in this area.

In the 4th experiment (Table III), only the embryos injected at 8 days of incubation showed constricted areas around the center of the bones similar to Ca^{45} damage. The damage (Fig. 3) seemed more severe particularly to the tissues since the feet below these constricted areas were swollen, hemorrhagic and gangrenous. This damage was observed at the 100 μc Sr^{90} level and became more severe at the 400 and 800 μc level. Some of those injected at 4 or 14 days of incubation appeared to have spread or deformed toes. Some of those receiving 800 μc Sr^{90} at 14 days of age showed a heavy edema of head and neck with gelatinous material just under the skin of these areas. As the embryos matured, it required greater dosages of Sr^{90} to produce 100% mortality either because they were more resistant or because the total amount of beta irradiation received was less. It would appear that, though the beta emissions from Sr^{89}-Y^{90} were stronger than those from Ca^{45}, the specific leg abnormalities observed were more severe with Ca^{45}. This might be a reflection of more

FIG. 3. An 18-day embryo injected with 800 μc Sr^{89} on 8th day of incubation with gangrenous tissue below break in tarsometatarsus.

TABLE IV. Embryonic Response to Varying Levels of Pm^{147} Injected into Eggs.

Pm^{147} inj, μc	Eggs inj	Day of incubation 4th Hatched	4th Abnormal	8th Hatched	8th Abnormal	14th Hatched	14th Abnormal
0	12	8	0	9	0	6	0
50	6	3	0	4	1	4	1
100	12	9	0	11	1	12	0
200	12	5	1	5	4	10	1
400	12	3	2	3	2	7	1
800	6	0	0	5	0	4	0
1600	6	0	0	1	0	3	1

concentrated ionization by the beta emissions from Ca^{45} over a smaller area due to its weaker emission or of a difference in deposition of Sr^{90}. The Sr^{90} administered at 4 days could have been tied up elsewhere and therefore less readily available for bone deposition than calcium, while that administered at 8 days was given at a time when calcium deposition in the bone was starting and, therefore, was available for use. The gangrenous appearance of the tissues below the constrictions seemed to indicate that the ionization of tissues by the beta emissions of Sr^{90}-Y^{90} was occurring at a greater depth than that observed with Ca^{45}.

The higher levels of Pm^{147} produced higher mortality in the younger embryos (Table IV). The 8- and 14-day-old embryos were remarkably resistant to the high levels of activity, with 5 of the 6 embryos receiving 800 μc Pm^{147} at 8 days and 4 and 3 of those receiving 800 and 1600 μc of Pm^{147}, respectively, at 14 days hatching. The number of abnormalities were few, showed no pattern and consisted of developmental abnormalities (deformed toes, deformed beaks, shortened limbs or weak tendons) as experienced with external irradiation(1,2). The broken or fractured long bones observed with high Ca^{45} injection were not present even though the levels of Pm^{147} in the bone should theoretically have been equivalent to those found in embryos injected with half as much Ca^{45}(4).

It would appear that rather high levels of the soft beta emitters must be used to produce abnormalities in chick embryos. The type of damage depends upon the age of embryo at time of dosing, the level of activity and the sites of deposition in the embryo. The effect of the type of emission (alpha, beta or gamma), their energies and the half-lives of the radionuclide may also be factors influencing the damage.

Summary. As low as 100 μc Ca^{45} administered either at 4 or 8 days of incubation produced broken or fractured bones in chicken embryos. This same level of Sr^{90}-Y^{90} was less effective than Ca^{45} producing these fractures when administered at 8 days of incubation. The changes produced by Ca^{45} injected at 14 days of incubation, Sr^{90}-Y^{90} at 4 and 14 days and Pm^{147} injected at 4, 8 and 14 days were developmental abnormalities frequently produced with external irradiation.

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