Toxicity of Sulfur-35, Selenium, and Tellurium to Avian Embryos.* (29898)

P. L. WRIGHT AND F. R. MRAZ
Agricultural Research Laboratory, University of Tennessee, Oak Ridge†

Survival and morphologic development of chick embryos are adversely affected by acute X- or gamma-irradiation(1,2,3). Both immediate and delayed toxic effects are seen, their relative incidence depending on dose level and embryonic age(1). Reduced hatchability and increased morphological abnormality are related to age and dose level(2). The deformities produced by external irradiation are similar to those commonly observed among avian embryos.

Warren and Dixon(4) administered radioactive phosphorus (P^{32}) to chick embryos to provide an internal source of continuous irradiation. The only macroscopically visible abnormality was a reduction in growth rate and the production of well-proportioned midgets. Mraz *et al*(5) extended these studies using Ca^{45}, Sr^{90}, and Pm^{147}; specific abnormalities resulting from injection of Ca^{45} and Sr^{90} prior to the 14th day of incubation were described. The damage reported was due to the intense local irradiation resulting from the concentration of these radioactive substances in the osseous structures at the onset of calcification.

These results suggested that a further insight into developmental mechanisms of embryonic deformities, spontaneous or induced, might be achieved by using other radioactive substances utilized in specific metabolic sequences by avian embryos. Thus the effects of inorganic S^{35}, utilized by the chick embryo in cystine synthesis(6) and the formation of the cartilaginous skeleton precursor (7), were compared in this study with those resulting from toxicosis by 2 other chalcogens, selenium and tellurium.

Methods. Eggs from Single Comb White Leghorns were candled prior to aseptic injection of 0.2 ml of sterile saline or solutions containing graded levels of S^{35} (50 to 1600 μc) as carrier-free H_2SO_4 on the 4th or 8th day of incubation, selenium (2.5 to 40 μg) as Na_2SeO_3 or tellurium (40 to 640 μg) as Na_2TeO_3 on the 4th day of incubation; they were then sealed with collodion. On the 22nd day of incubation all chicks and unhatched eggs were examined for gross abnormalities. Abnormal embryos and chicks were rendered transparent with potassium hydroxide and the skeletons stained with alizarin red-S, utilizing a procedure described by Davis and Gore(8).

The normal appearing chicks from the S^{35} injected eggs were reared in conventional wire-floored batteries until 8 weeks of age. The females of this group were maintained and their egg production recorded until 10 months of age when they were sacrificed. Gross and histological examinations of reproductive tracts and ovaries were performed.

Results. There was no marked reduction in hatchability nor any increase in incidence of deformities in chicks hatched from eggs injected with up to 100 μc of S^{35} at either the 4th or 8th day of incubation (Table I). Higher levels of radiosulfur resulted in decreased embryo viability and increased the incidence and severity of deformities. Extent of damage was influenced by both amount of S^{35} injected and age of embryo at time of injection. Embryos receiving 200 or 400 μc S^{35} at 4 days of age or up to 800 μc at 8 days of age tended to assume the oval shape of the egg's interior (Fig. 1). This suggested that the irradiation from these levels of S^{35}, while insufficient to completely prevent the formation of the cartilaginous skeleton precursors or reduce growth, was sufficient to impair the spatial integrity of the cartilaginous skeleton and allowed the developing chick to conform to the shape of the egg's interior.

Embryos from eggs injected with 800 μc

* This manuscript is published with the permission of the Director of Univ. of Tennessee Agric. Exp. Station, Knoxville.

† Operated by Tennessee Agric. Exp. Station for U. S. Atomic Energy Commission under Contract AT-40-1-GEN-242.

on the 4th day (Fig. 2) or 1600 μc on the 8th day (Fig. 3) lived until approximately the 16th to 20th day of incubation but were deformed. They were characteristically dwarfed with extreme shortening of the leg and wing bones in relation to body size.

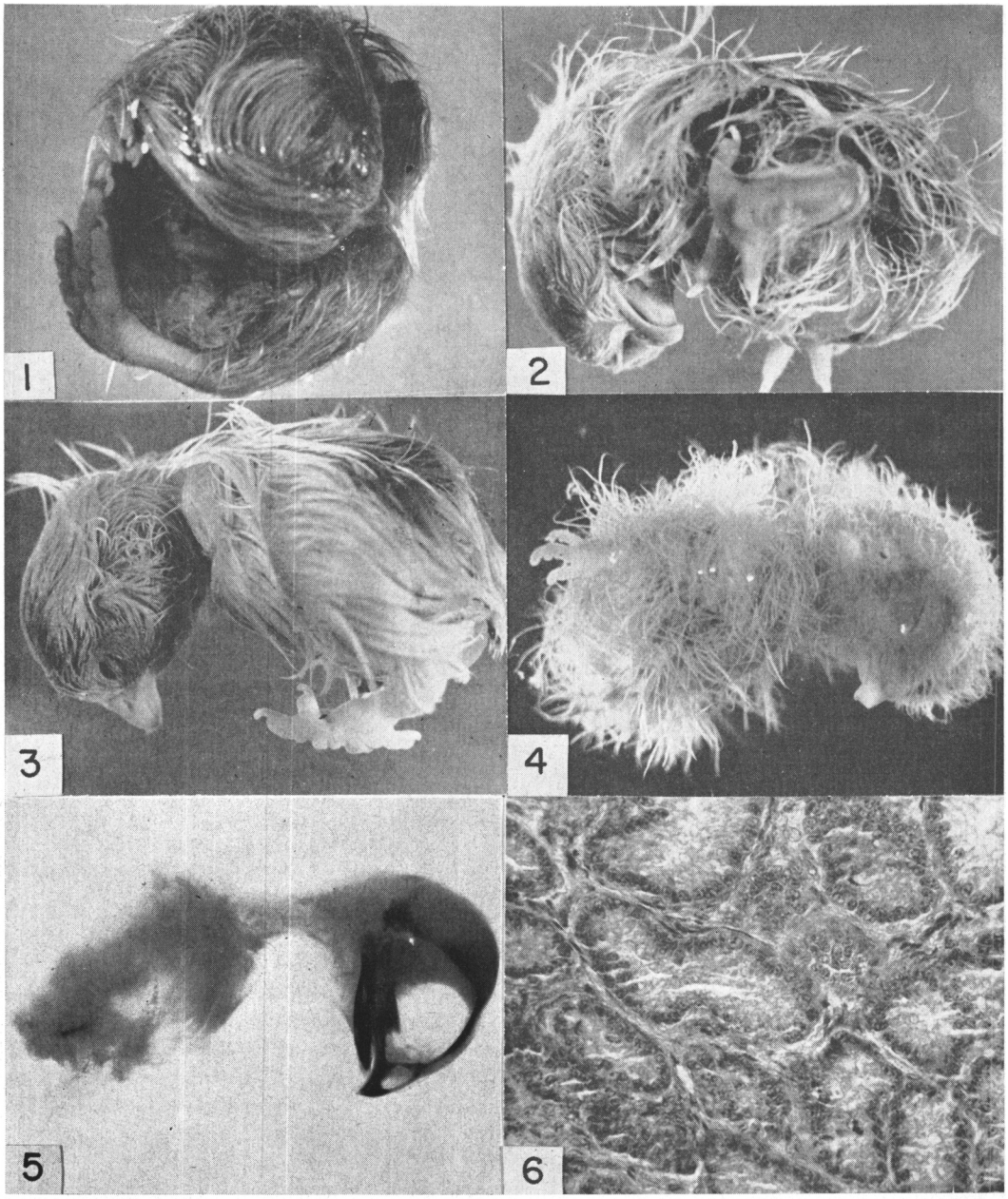

FIG. 1. Twenty-one-day embryo irradiated with 400 μc S^{35} from 4th day of incubation.
FIG. 2. Twenty-day embryo irradiated with 800 μc S^{35} from 4th day of incubation.
FIG. 3. Twenty-day embryo irradiated with 1600 μc S^{35} from 8th day of incubation.
FIG. 4. Fourteen-day embryo irradiated with 1600 μc S^{35} from 4th day of incubation.
FIG. 5. Alizarin-stained 14-day embryo irradiated with 1600 μc S^{35} from 4th day of incubation.
FIG. 6. Seminiferous-like tubules present in gonad of a 10-month-old hen injected with 800 μc of S^{35} as an 8-day embryo. $\times$ 144.

TABLE I. Embryonic Response to Sulfur-35 Injected into Eggs Containing 4- and 8-Day Chick Embryos.

Sulfur-35* (μc)	4-day-embryos			8-day embryos		
	Hatched (No.)	Developmental stage of dead embryos†	Abnormal (No.)	Hatched (No.)	Developmental stage of dead embryos†	Abnormal (No.)
0	4	19	0	6		0
50	5	18	0	4	21	0
100	4	10	0	6		1
200	3	21	1	2	20‡	1
400	1	21	1	5	10	2
800	0	16	6	6		3
1600	0	12	6	0	20	6

* 6 embryos per treatment.
† Estimated developmental age in days.
‡ 2 embryos died the day of injection.

TABLE II. Subsequent Growth of Chicks After Sulfur-35 Irradiation as Embryos.

		4-day embryos		8-day embryos				
μc sulfur-35	0	50	100	50	100	200	400	800
No. chicks	10	5	4	4	5	2	2	3
8 week wt*	868	808	840	795	786	744	695	707

* Average wt (g) adjusted for sex of birds.

These observations were further confirmed in the alizarin-stained skeleton preparations. The skeletons were complete but disproportioned. The femur, tibia, and tarsometatarsus were extremely short in relation to overall embryo length as were the humerus, ulna, and radius. Calcification and structural proportionality of the vertebral and visceral cranium and mandibula were normal.

Sixteen hundred μc S^{35} administered to 4-day embryos caused severe developmental damage (Fig. 4). The embryos died between the 10th and 14th days, when they were approximately one-half normal size. The limbs were little more than short undifferentiated appendages. A moderate degree of parrot beaking was observed and the feathers were wiry or hair-like. The skeleton was almost devoid of calcium (Fig. 5) except for the vertebral cranium, the visceral cranium, and the mandibula which were calcified as extensively as in control embryos of the same developmental stage.

The growth rate (Table II) of the normal-appearing chicks hatched from eggs injected with radiosulfur was slower than that of the controls. In some instances well-proportioned dwarfs approximately one-half normal size, similar to those described by Warren and Dixon(4), were observed. The egg production rate of the hens, irradiated with 50 and 100 μc S^{35}, during the first 100 days of individual lay were nearly equal to that of the controls, *i.e.*, 54% compared to 58%. No eggs were produced by hens which had received 200 μc or more as embryos.

Birds which had not produced any eggs by 10 months of age exhibited external changes similar to those observed in the sex reversals described by Hutt(9). Their combs and wattles were enlarged to a state approaching that found in males. Spur enlargement was also observed but the plumage was characteristically female. Upon autopsy the reproductive tracts of the 5 nonlaying hens hatched from eggs injected with 200 μc or more S^{35}, and of one hen which had received 50 μc on the 4th day of incubation, were found to be intact but juvenile in appearance. The left gonads grossly resembled the spontaneously arising or experimentally induced ovotestis previously described(9). No ova were outwardly visible on these gonads nor were any present upon histological examination. In each gonad from the nonlaying females seminiferous-like tubules (Fig. 6) were present, having the appearance of sterile nonfunctioning tubules found in irradiated males(4).

TABLE III. Embryonic Response to Graded Levels of Selenium and Tellurium Injected into Eggs Containing 4-Day Embryos.

Se* (μg)	Chicks hatched (No.)	Acute toxicity†	Delayed toxicity	Developmental stage of dead embryo‡	Te* (μg)	Chicks hatched (No.)	Acute toxicity†	Delayed toxicity	Developmental stage of dead embryo‡
0	9	1	2	20	0	9	1	2	19
2.5	6	4	2	14	40	6	4	2	19
5	4	7	1	11	80	10	2	0	
10	4	5	3	11	160	6	5	1	20
15	4	4	4	14	240	2	10	0	
20	0	10	2	17	320	3	8	1	17
25	0	8	4	17	400	0	12		
30	0	12			480	2	10		
35	0	12			560	0	12		
40	0	12			640	0	12		

* 12 embryos per treatment.
† Embryos died within 24 hr of injection.
‡ Estimated developmental age in days, excluding those embryos which died within 24 hr of injection.

The data from the injection of stable selenium and stable tellurium into eggs containing 4-day embryos are summarized in Table III. Approximately 30 to 50% of the embryos injected with up to 15 μg selenium hatched and were normal in appearance, confirming the report of Franke *et al*(10). However, in contrast to the earlier report(10), no chicks hatched from eggs injected with more than 20 μg of selenium; at levels greater than 30 μg all of the embryos were killed within 24 hours postinjection. Approximately 30% of the embryos that died 7 to 14 days after selenium injection were macroscopically abnormal. These deformities were similar to those previously described(10,11) for naturally occurring or experimentally induced selenium toxicosis of avian embryos. Included were dwarfness, shortened visceral cranium, or mandibula, shortened ulna, radius, and the more distal portion of the wing as well as a reduction in length of the tarsometatarsus and tibia. A fusion of the 3rd and 4th digits or the absence of one or more digits was also noted. The severity of deformities was not related to the amount of selenium injected.

Nearly 20 times more tellurium than selenium was required to kill all the embryos within 24 hours postinjection (Table III). No chicks that hatched from tellurium-injected eggs and no dead embryos were found to be grossly abnormal. The embryos dying within 24 hours after the tellurium injection contained a characteristic black precipitate. This precipitate, possibly elemental tellurium, was visible over the entire embryo and throughout the embryonic vascular system. This substance was not seen in those embryos dying at later developmental stages.

Discussion. The chemical similarities of the chalcogens sulfur, selenium, and tellurium have long been recognized. It has been postulated that the toxicity of selenium is mediated through its adverse effects upon sulfur metabolizing mechanisms(12). The present studies demonstrated that the abnormalities in avian embryos resulting from internal irradiation by S^{35} are very similar to those observed from selenium toxicosis. Such results as well as those of Mraz *et al*(5) suggest that damage from radioactive materials administered to avian embryos occurs most extensively in systems or organs where these materials are primarily incorporated. The more severe abnormalities resulting from internal S^{35} irradiation suggested that the embryo could tolerate the somatic irradiation until sufficient radiosulfur had been incorporated into specialized tissue components to cause irreparable damage. This sufficient S^{35} was incorporated into the cells of the rapidly developing limb buds of the 4-day embryos injected with 1600 μc to provide an irradiation level high enough to prevent differentiation and limb formation. Lower levels in the 4-day embryos and the highest level in the 8-day embryos did not prevent differentiation but did interfere with elongation of the long bones. An inherent problem in studies of this nature is the impossibility of determining the irradiation dose actually received at the cellular level in specific tissues.

The extent of embryonic damage was, however, directly related to the amount of radioactive material injected. On the other hand, the severity of the deformities resulting from selenium toxicosis was not related to the amount of selenium injected. At the higher levels of selenium where a sufficient interference with cellular differentiation might have been expected to produce more severe morphologic abnormalities, all the embryos succumbed to an acute selenium toxicosis and died before development had advanced to a degree at which macroscopic abnormalities were present.

The injection of tellurium was completely ineffective in producing embryonic deformities and it was apparent that the avian embryo was much less susceptible to acute toxicity of tellurium than of selenium. Franke and Moxon(13) reported that tellurium as sodium tellurite was more toxic, in rats, than selenium as sodium selenite. The black precipitate found in the chick embryos dying from acute tellurium toxicity indicates that a reduction of tellurite may take place in the embryonic tissues, possibly as a detoxification process. A similar deposition of black tellurium was found at the site of infusion following the accidental administration of sodium tellurite *via* a catheter into the ureter in two men(14).

Warren and Dixon(4) have demonstrated that the primitive ovum was the most radiosensitive element in the avian ovary. As little as 47.5 μc P^{32} injected during the first 8 days of incubation destroyed the ova. In the present study 50 μc S^{35} administered to 4-day embryos and 200 μc to 8-day embryos produced sterility in the females hatching from these eggs. This confirmed the suggestion(4) that ova age at time of internal irradiation was important in determining the degree of damage induced.

The sex reversals in hens hatched from eggs injected with S^{35} were similar to those arising from an overabundance of endogenous or exogenous androgen in the hen(9). No ova were found upon gross and histologic examinations of the ovaries from these birds. Histological examination demonstrated the presence of seminiferous-like tubules but no indication of active spermatogenesis. Essenberg and Zikmund(15) reported a similar degree of sex reversal in females exposed to X-irradiation. The interstitial cell mass in the ovaries of the S^{35} irradiated birds contained cells similar to the cells normally found in the testis. Such cells were presumed to have been responsible for the production of androgen-like substances which induced the outward symptoms of sex reversal.

Summary. Continuous internal irradiation of chick embryos with sulfur-35 administered on the 4th or 8th day of incubation produced abnormalities similar to those observed in selenium toxicosis of avian embryos. The highest level of sulfur-35 (1600 μc) injected into eggs containing 4-day embryos produced morphological abnormalities more severe than those produced by the same level of radiosulfur administered to 8-day embryos, or by any level of stable selenium tolerated. Selenium at levels greater than 30 μg killed all the embryos within 24 hours after injection. Nearly 20 times more tellurium than selenium was required to kill all the embryos within 24 hours; no abnormalities were observed in the tellurium-injected embryos.

1. Karnofsky, D. A., Patterson, P. A., Ridgway, L. P., Am. J. Roentgenol. Radium Ther., 1950, v64, 280.

2. Moreng, R. E., Hollister, K. W., Proc. Soc. Exp. Biol. and Med., 1963, v114, 99.

3. Stearner, S. P., Sanderson, M. H., Christian, E. J., Tyler, S. A., Radiation Res., 1960, v12, 286.

4. Warren, S., Dixon, F. J., Radiology, 1949, v52, 714.

5. Mraz, F. R., Wright, P. L., Ferguson, T. M., Proc. Soc. Exp. Biol. and Med., 1964, v116, 1114.

6. Suzuki, M., Nozaki, H., Bull. Nat. Inst. Agri. Sci. (Japan), 1959, Series G, No. 18, 179.

7. Romanoff, A. L., The Avian Embryo, 1960, p909, Macmillan Co., New York.

8. Davis, D. D., Gore, U. R., Clearing and Staining Skeletons of Small Vertebrates, 1947, Chicago Natural History Museum, 2nd Ed.

9. Hutt, F. B., Genetics of the Fowl, 1949, p465, McGraw-Hill Book Co., New York.

10. Franke, K. W., Moxon, A. L., Poley, W. E., Tully, W. C., 1936, Anat. Rec., v65, 15.

11. Moxon, A. L., 1937, S. Dakota Agri. Exp. Sta. Bull., 311, 22.

12. Shrift, A., Fed. Proc., 1961, v20, 695.
13. Franke, K. W., Moxon, A. L., J. Pharmacol. & Exp. Therap., 1936, v58, 454.
14. Keall, J. H. H., Martin, N. H., Tunbridge, R. E., British J. Indust. Med., 1946, v3, 175.
15. Essenberg, J. M., Zikmund, A., Radiology, 1938, v31, 94.

Received November 2, 1964. P.S.E.B.M., 1965, v118.

Effect of Time of Reduction of Renal Mass on Development of Adrenal-Regeneration Hypertension.* (29899)

F. R. SKELTON, L. L. BERNARDIS AND A. C. BROWNIE
Department of Pathology, State University of New York at Buffalo, Buffalo, N. Y.

Reduced renal mass often is associated with clinical hypertensive disease, and it has been demonstrated by Selye(1) that unilateral nephrectomy enhances the hypertensive potential of steroids in the rat. Skelton(2,3) has shown that uninephrectomy enhances the hypertension which develops during adrenal regeneration in the rat. Although the exact role which the reduction of renal mass plays in production of adrenal-regeneration and steroid hypertension is not understood, one hypothesis holds that it may be through reduced renal capacity to handle sodium.

The present paper deals with the investigation of the effect which time of nephrectomy might have on the development of the experimental syndrome of adrenal-regeneration hypertension.

Materials and methods. Weanling female Sprague-Dawley rats were used in these experiments. All rats were individually caged and fed Purina Lab Chow and given 1% sodium chloride solution to drink *ad libitum*.

Groups of rats were set up as shown in Table I. Saline intake was measured throughout the experiment and blood pressures of the rats were taken at weekly intervals using the microphonic method of Friedman and Freed (4). After 7 weeks the rats were sacrificed by decapitation and organs were dissected, examined for gross lesions, weighed and fixed in formalin. Sections were prepared and stained with hematoxylin and eosin for microscopic examination of the lesions. Severity of the gross and microscopic changes in the kidney, heart, brain and mesenteric vascular bed was graded on a zero to 3+ scale. Values so derived were summed for each rat and overall gross and microscopic lesion severity indices obtained.

Results. The blood pressure measurements from the various groups are depicted in Fig. 1 and 1a. Simultaneous unilateral nephrectomy, unilateral adrenalectomy and contralateral adrenal enucleation as performed in Group 2 resulted in the well-known adrenal-regeneration hypertension described by Skelton(2). The combination of operative steps used with Groups 3 and 6 also resulted in hypertension, kidney and heart enlargement, and high lesion severity indices. When the same surgical procedure was used as for Group 2 but in animals 2 weeks older (Group 5), there was some reduction in the hypertension. In Group 4, where the renal mass was reduced 2 weeks after the unilateral ad-

TABLE I. Arrangement of Experimental Groups.

Group No.	Operations at 'zero time'*	Operations at 2 wk
1	R. nephrectomy	—
2	R. "	—
	R. adrenalectomy	
	L. adrenal enucleation	
3	R. nephrectomy	R. adrenalectomy
		L. adrenal enucleation
4	R. adrenalectomy	R. nephrectomy
	L. adrenal enucleation	
5	—	R. nephrectomy
		R. adrenalectomy
		L. adrenal enucleation
6	R. nephrectomy	L. adrenal enucleation
	R. adrenalectomy	

* At "zero time" the rats were 24 days old.

* This investigation was supported by U.S.P.H.S. Grant HE 06975 from Nat. Heart Inst.