# Production of Beak and Skeletal Malformations of Chick Embryo by Semicarbazide. (22550)

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Aminoguanidine injected into the yolk of the embryonated egg produced a severe and characteristic inhibition of development of the chick embryo liver(1). The present paper reports the effect of the structurally related semicarbazide • HCl upon the development of the chick embryo. This compound produces a profound distortion of the beak and leg bones with only minor lesions of the liver.

*Methods.* The semicarbazide • HCl (SCH)† was dissoved in water and adjusted to pH 4.5-5.0. All solutions and water used for dilutions were sterilized using a Selas filter of 03 porosity. The embryonated White Leghorn eggs were incubated for 96 hours, and the solutions (0.4 ml) were injected into the yolk. After further incubation (usually for a total of 14 days), the embryos were removed for examination.

*Results.* The effect of SCH on mortality, skeletal structure and body weight of the embryo is recorded in Table I. Injections of SCH in amounts exceeding the 3 mg level resulted in 100% mortality. Most of the embryos died in the 3 mg treatment, but the percentage of survival increased sharply below this level. Irrespective of the levels tested, SCH had little effect on the body weight of the surviving embryos. Of particular interest was the skeletal malformation produced when SCH was administered at 2 and 3 mg/egg dosages. When injected at 4 days and harvested at 14 days, the lower beak of the embryo was shortened and distorted with an upward sharp bend, and the tibiotarsus was sharply bent (Fig. 1, 2). Other tissues appeared normal, although the liver occasionally revealed minute whitish areas at the borders of the lobes. Malformation occurred in milder form and with lowered incidence as the dosage levels were decreased.

The effect of different concentrations of SCH administered at embryonic ages from 0-16 days was then determined. Injections at incubation periods earlier than 4 days proved less effective, and administration of SCH at 7-10 days produced distortion and irregularities in that the legs frequently were twisted or flattened and beaks were crossed and/or twisted. The total effects of the 2-3 mg/egg dosage occurred to a decreasing degree as the time of injection approached 12 days. The 12-day embryos were practically normal. The most severe effect of SCH was obtained by injections at 6 days. At the 2.0-2.5 mg dosages, ¾ of the embryos had, in addition to the sharp bending of the tibiotarsus, another distinct bending of the tarsometatarsus. These effects are strikingly similar to that of hereditary chondrodystrophy in the fowl(2).

TABLE I. Effect of Semicarbazide • HCl on Mortality, Skeletal Structure and Body Weight of Chick Embryo.

| Quantity inj. (mg/egg) | No. of eggs | Embryos survived | Wt at 14 days (mean ± avg dev.) |
|---|---|---|---|
| 0 | 6 | 6* | 10.09 ± .75 |
| 1.0 | 4 | 4† | 9.70 ± 1.09 |
| 1.5 | 8 | 7‡ | 9.86 ± .24 |
| 2.0 | 8 | 7§ | 8.88 ± .83 |
| 2.5 | 8 | 6‖ | 8.46 ± .51 |
| 3.0 | 6 | 2¶ | 9.22 ± .98 |
| 4.0 | 4 | 0 | — |
| 5.0 | 4 | 0 | — |

* All normal.

† One embryo with normal beak but bend in tibiotarsus; other embryos apparently normal.

‡ One embryo with malformed beak but normal legs; other embryos with bent tibiotarsus and malformed lower beak.

§ All embryos with bent tibiotarsus, legs not folded back behind body; one embryo with normal beak, one embryo with slightly malformed beak.

‖ All embryos with acutely bent tibiotarsus, legs tend to fold behind back; beaks malformed.

¶ Legs of all embryos with acutely bent tibiotarsus and folded on back; beaks malformed.

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†Distillation Products Industries, Rochester, N. Y.

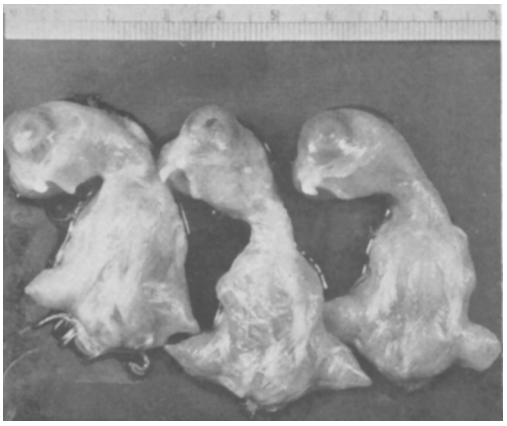

FIG. 1. Appearance of 14-day embryos treated with 2.5 mg semicarbazide • HCl at 4 days. Note tibiotarsus bent to such degree that remainder of leg is folded under embryo.

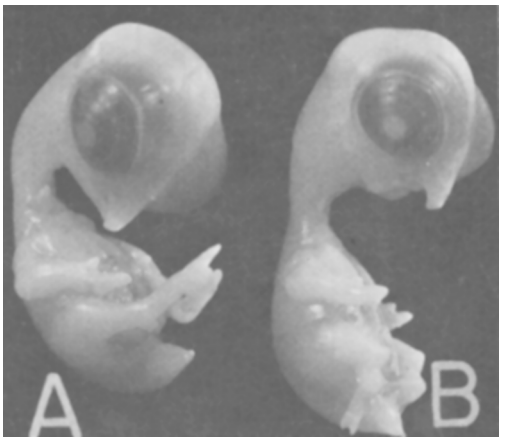


FIG. 3. Appearance of 8-day embryos. A—Control; B—Treated with 2 mg semicarbazide • HCl 48 hr previously.

Eggs were injected after 6 days incubation and embryos were examined at 24-hour intervals up to an age of 16 days. A gross malformation of the beak and legs was evident as early as 48 hours after injection (8-day-old embryo). This is shown in Fig. 3. The embryos were fixed and stained. In Fig. 4, sections of the bones from normal and treated 8-day-old embryos are compared and tibiotarsal and tarsometatarsal bends at increased magnification are shown. It is difficult to ascribe the bending to histological changes. A closely related compound, acetone semicarbazone, was toxic at the same level as SCH, and the typical SCH syndrome was produced at the 2.5 mg/egg level. If the semicarbazone dissociated in the egg, the results may have been due to SCH *per se*.

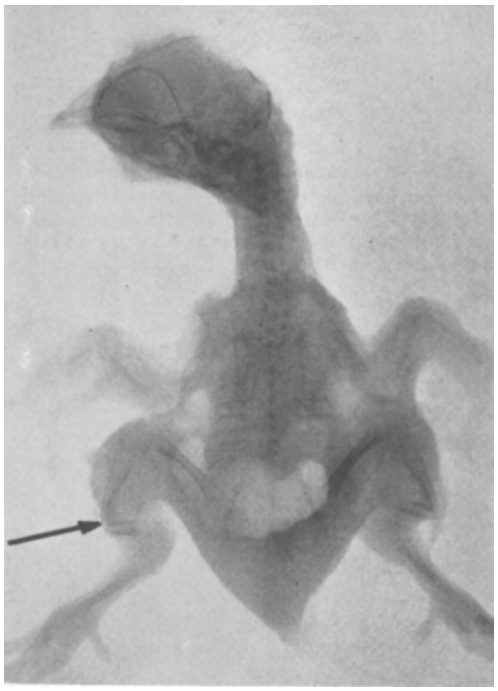

FIG. 2. X-ray photograph of 16-day embryo treated with 2.0 mg semicarbazide • HCl at 4 days.

When the carbonyl reacting portion of SCH was blocked as in 1-phenylsemicarbazide, the resulting structure was still capable of producing abnormalities. The 2.0-6.0 mg dose of 1-phenylsemicarbazide produced severe foreshortening of the legs, feet and wings; shortened beak; whitish areas of the borders of the liver; and moderate to extreme edema. The gizzards in half of the embryos examined were bloated with a clear viscous fluid. Thiosemicarbazide was much more toxic (L.D. 0.5 mg/egg) than SCH. At lower concentrations about 5% of the embryos displayed a bend in the tibiotarsus. p-Hydrazinobenzoic acid caused effects similar to those of SCH, but benzoic hydrazide did not produce abnormalities.

*Discussion.* SCH and hydroxylamine have been recognized as carbonyl reacting reagents. Zeller(3) used them to inhibit diamine oxidase and suggested that the oxidase contained ketone or aldehyde groups which reacted with diamines. Subsequently, SCH has been used

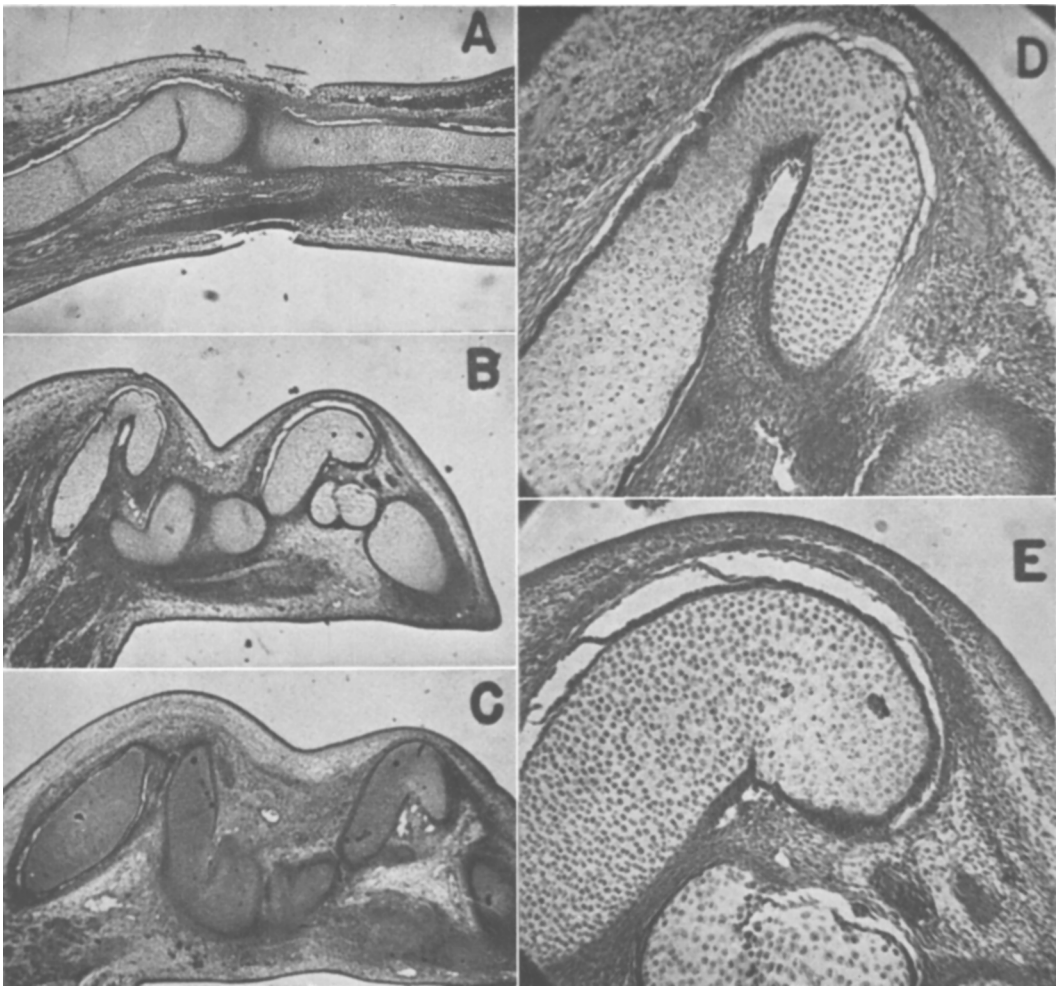


FIG. 4. Median sections of tibiotarsal and tarsometatarsal portions of leg bone of 8-day chick embryo when inj. at 6 days. A—Control, × 25. B and C—Typical sections from treated embryos (2 mg SCH/egg). Examination of serial sections revealed that tibiotarsus and tarsometatarsus were intact; × 25. D—Enlarged tibiotarsal section of B, × 100. E—Enlarged tarsometatarsal section of B, × 100.

to inhibit other enzymes such as, pancreatic lipase(4), the deaminating enzymes of histamine, isoamylamine and putracine(5), and spermine oxidase(6). In the spermine oxidase studies, Hirsch reported that SCH would specifically block the enzyme while certain amines and cyanides would bring about only partial inhibition. Clark *et al.*(7) reported the isolation of an enzyme from hog and guinea pig kidney which would specifically decarboxylate 5-hydroxytryptophan. Semicarbazide • HCl would almost completely inhibit this enzyme in concentrations of $10^{-3}$ M which suggested that the coenzyme involved may be pyridoxal phosphate. Addition of pyridoxal phosphate would not reverse the inhibition as it has been shown to do in the case of DOPA decarboxylase when inhibited by SCH. Shulman(8) reported that 0.26-0.34 M concentrations of SCH would reversibly inhibit the clotting of fibrinogen, indicating that the inhibition did not involve the destruction of the proteins involved. Sang and McDonald(9) showed that SCH would produce phenocopies in *Drosophila* larvae.

Micromelia has been produced by sulfanil-

amide and eserine sulfate(10), insulin(11), thallium(12), boric acid(13), and pilocarpine (14). Lyons and Insko reported chondrodystrophy in the chick embryo produced by manganese deficiency in the diet of the hen (15). Nicotinamide has been used to overcome the teratogenic effects of insulin(16), sulfanilamide(17), eserine sulfate(18), and pilocarpine(14). In the present studies, administration of 5 mg nicotinamide concurrently with 1-3 mg of SCH in no way modified the characteristic syndrome in the chick embryos.

Antagonistic effects of vit. $B_6$ for hydrazides in bacterial and tumor growth have been discussed(19). Yet pyridoxal • HCl and pyridoxine • HCl in 1 mg quantities administered with 2 mg SCH did not alter the results in the present work. A condition similar to the SCH effects resulted in embryos from hens on a biotin-deficient diet(20). Nevertheless, when administered to the embryo treated with 1-2 mg of SCH, biotin up to 2 mg/egg did not alter the production of abnormalities described in this paper. Similarly, riboflavin in quantities up to 1 mg/egg did not affect the action of SCH.

*Summary.* Semicarbazide • HCl when injected into the embryonated White Leghorn egg at 4-6 days produced shortened and malformed lower beak and bent tarsometatarsal and tibiotarsal bones. The embryos were most sensitive to this injection at 6 days of incubation. Gross and histological effects were quite marked 48 hours after injection. Nicotinamide, pyridoxal, pyridoxine, biotin, and riboflavin administered simultaneously with SCH did not overcome the effects. Different effects on bone and soft tissues were produced with 1-phenylsemicarbazide. p-Hydrazinobenzoic acid, but not benzoic hydrazide, produced abnormalities very similar to those following SCH treatment. Only a small incidence of skeletal defect resulted from administration of thiosemicarbazide.

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