Production by Semicarbazide of Gross Skeletal Changes in Rats Similar to Osteolathryism.*† (23661)

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Neuman, Maxwell and McCoy, in an attempt to produce liver lesions in chick embryos similar to those produced by the chemically related aminoguanidine, injected solutions of semicarbazide HCl into yolk sacs of incubating White Leghorn eggs(2). While semicarbazide had little or no effect on the developing chick liver, it produced skeletal changes which seemed to me to be strikingly similar to lesions which have been produced in the chick embryo by β-aminopropionitrile (BAPN)(3). Since BAPN produces osteolathyrism in the rat(4-6), it seemed likely that semicarbazide might have a similar effect.

Methods. Weanling, male rats of the Sprague-Dawley strain were used. The basal diet was Rockland Rat Stock Diet, finely milled by a commercial miller. Experimental diets were made up by drying solutions of semicarbazide HCl (SCH)‡ on suitable quantities of milled Rockland Diet. The diets were supplied *ad libitum*. All animals were autopsied at death or at the end of the experimental periods.

Results. In a preliminary experiment, rats weighing 60-62 g were divided into groups of 3 and fed diets containing 0.1%, 0.5%, and 1.0% SCH respectively. All rats receiving 0.5%, and 1.0% levels of SCH lost weight and died within 10 days. None of these exhibited any gross symptoms of lathyrism. The animals which received the 0.1% level of SCH developed skeletal exostoses, spinal curvatures and thoracic cage deformities similar to those seen when BAPN or *Lathyrus odoratus* (sweet pea) seeds are fed to weanling rats(4-8). One of these showed severe slipping of the right distal femoral epiphysis within 10 days. All 3 animals survived until sacrificed after 10 weeks of feeding.

In a second experiment rats weighing 42-51 g were divided into groups of 10 and fed diets containing 0.05%, 0.1% and 0.25% SCH respectively. Growth rate was depressed in proportion to the level of SCH in the diet as shown in Fig. 1. On the lowest level growth was good, approaching that of rats fed unsupplemented Rockland stock diet. All groups developed skeletal lesions typical of osteolathyrism.

Symptomatology. The gross skeletal lesions

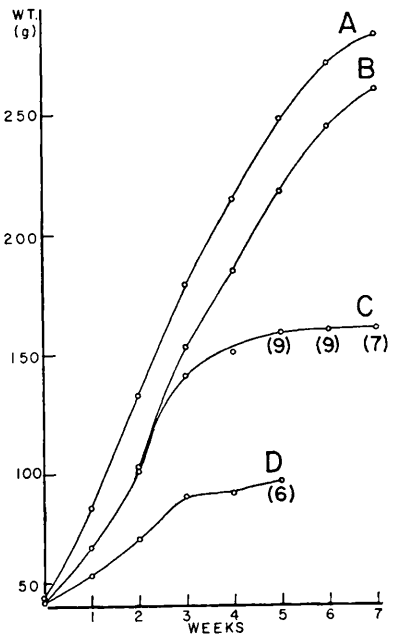

FIG. 1. Growth curves of rats receiving SCH. A—normal controls; B—0.05% SCH; C—0.1% SCH; D—0.25% SCH. All points represent average weight of 10 rats except as noted in parentheses.

* *Osteolathyrism* is a term introduced by Selye(1) differentiating the syndrome produced by β-aminopropionitrile, aminoacetonitrile, and certain species of *Lathyrus* (*e.g., odoratus*), in which marked skeletal lesions occur, from *neurolathyrism,* in which the symptoms are primarily neurologic and which results from the ingestion of certain other species of *Lathyrus* (*e.g., sativus*) or bis-(β-cyanoethyl)-amine.

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‡ Fisher Certified Reagent.

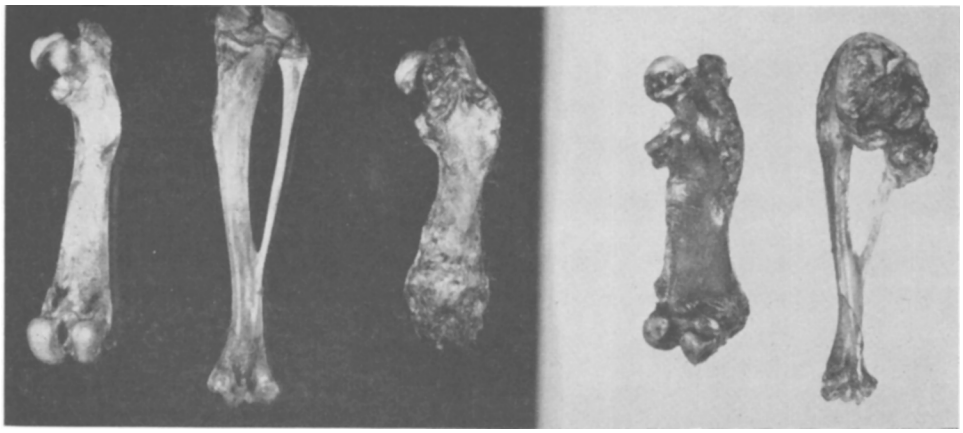

FIG. 2. Effect of SCH on femora and tibiae of weanling rats. Left: normal femur and tibia; center: 0.25% SCH for 28 days, distal articular surface damaged during removal; right: femur and tibia from rat fed 0.25% SCH for 56 days

were strikingly similar to those produced by BAPN(4-6). Exostoses on the ventral and lateral aspects of the mandibles could be palpated in rats on the 0.1% and 0.25% levels of SCH within 1-2 weeks after the feedings began. The long bones became thickened and prominent exostoses appeared at the sites of muscle attachment (Fig. 2). The bones of animals receiving the 0.25% level had the abnormal red color often seen in sweet pea lathyrism(9). Severe spinal curvatures and thoracic cage deformities occurred; even in rats receiving the lowest level of SCH (0.05%) marked spinal and thoracic cage deformities were present at the end of the experimental period (8 weeks). A marked S-curve at the base of the tail and more or less crooking or spiralling of the tail occurred in all rats, (Fig. 3). Crooking or spiralling of the tail has not been observed in rats receiving BAPN or aminoacetonitrile alone, but a marked curling of the tail has been reported by Selye in rats in which the effect of aminoacetonitrile was intensified by somatotrophic hormone(10). It should be stated, however, that the identification of the skeletal lesions with those produced by BAPN must await histological confirmation.

Neurologic symptoms occurred in all groups. These included paralysis of the rear legs, incontinence, paraphymosis, and atony of the colon. The incidence of neurologic manifestations increased with increasing levels of SCH. Thus, the number of rats exhibiting paraphymosis at the 0.05%, 0.1%, and 0.25% levels of SCH were 2, 4, and 6, respectively (10 rats in each group). All these neurologic effects have been commonly observed in this laboratory in rats fed BAPN. As has been explained elsewhere in connection with a discussion of BAPN toxicity, it is felt that the neurologic symptoms are not due to a direct neurotoxic effect of the supplement, but are secondary to the osseous changes(11).

Dissecting aneurysms of the aorta, commonly seen in rats and mice fed BAPN or sweet peas(8,12-14) were not observed in any of the animals. One animal on the 0.25% level died of a ruptured saccular aneurysm of the aortic arch (non-dissecting) and one ani-

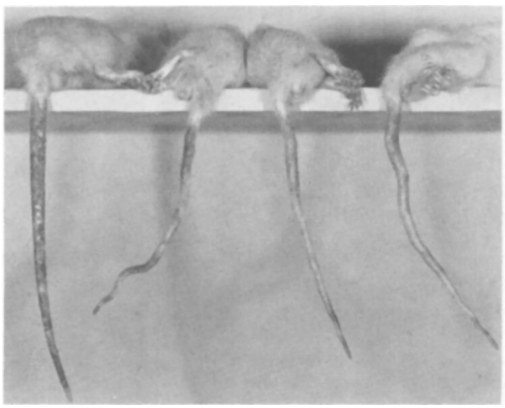

FIG. 3. Crooking of tails of rats fed SCH compared to normal at left. Rats photographed under nembutal anesthesia.

mal on the 0.1% level showed a grossly thickened and enlarged aortic arch (non-dissecting) when sacrificed at the termination of the experiment. The gross appearance of all other aortas was normal. Preliminary *microscopic* examination of the aortas, however, indicates that there was some damage to the aortic media in many of these animals.§

The incidence of deaths which occurred before the termination of the experiment (8 weeks) was as follows: 0.05% level, 0 of 10; 0.1% level, 3 of 10 (all from rupture of the urinary bladder due to severe urinary retention); 0.25% level, 7 of 8 (1 ruptured aorta, 1 ruptured urinary bladder, 5 cause unknown; 2 of original 10 rats were killed by mistake after 4 weeks by animal caretaker).

Discussion. Only relatively few chemicals have thus far been shown to be osteolathyrogenic. The most prominent of these are β-aminopropionitrile (BAPN)(4-6) and aminoacetonitrile(6). The γ-glutamyl derivative of BAPN is responsible for the toxic properties of sweet peas(15) and of Singletary peas(16). Methyleneaminoacetonitrile has also been reported to be strongly lathyrogenic(17). Bis-(β-cyanoethyl)-amine is mildly osteolathyrogenic(4,6) but is markedly neurolathyrogenic, *i.e.*, it produces central nervous system damage unrelated to the osseous lesions(4,18). Other substances closely related chemically to BAPN(4-6), even γ-aminobutyronitrile (6), the next higher homologue of BAPN, were found to be inactive. Only one non-nitrile has previously been reported to produce these skeletal changes. Commercially prepared β-mercaptoethylamine was found to cause similar skeletal lesions in our laboratory (5), but not in another laboratory(17).

The finding that semicarbazide produces lathyritic skeletal lesions is important because it demonstrates again that the presence of a nitrile group is not a *sine qua non* for osteolathyrogenic activity, since *in vivo* transformation of semicarbazide into nitriles is unlikely. In addition, this observation opens up an entirely new group of substances among which lathyrogenic action may be sought. It is still not clear whether osteolathyrism results from a stimulation of, or from an interference with, certain metabolic processes. It is hoped that the finding of new osteolathyrogenic agents will aid in locating the metabolic defect of osteolathyrism.

Summary. Semicarbazide HCl, when fed to weanling male rats, produced gross skeletal lesions similar to those of osteolathyrism. Aortic damage seemed to develop more slowly and to be less severe than in rats treated with BAPN or aminoacetonitrile and having skeletal lesions of comparable severity.

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