Prevention of Aminonitrile Lesions in Rats with L-Triiodothyronine.* (23382)

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We have described the striking lesions of the skeleton and of other mesodermal structures produced in vertebrates by aminonitriles (1,2,3,4). The histological similarities between these experimental lesions and certain human skeletal diseases were demonstrated (5,6). The metabolism of aminoacetonitrile (AAN) in the rat was studied and the possible protective effect of many compounds against the aminonitrile lesions was tested (7,8). Recently Selye and Bois(9,10) were able to influence the severity of these lesions in rats with somatotrophic hormone and with cortisol. Selye also indicated(11) that intensive overdosage with thyroxine can suppress these lesions. The effects of l-thyroxin (Tx) and 3,5,3′ l-triiodothyronine (TRI) in rats treated with AAN are here reported.

Methods. In the first experiment (Table I) weanling male Sprague-Dawley rats weighing 42 to 50 g were fed *ad lib.* ground Purina chow containing 0.075% AAN. The animals were housed in cages in groups of 4. Four groups of rats received Tx incorporated into the AAN diet in the following proportions: 0.25, 0.50, 1.25, and 10 mg per 100 g diet, respectively. Four groups of rats received TRI incorporated into the AAN diet in the following proportions: 0.02, 0.06, 0.10, and 0.25 mg/100 g diet, respectively. Groups of 3 rats served as controls: one group was fed Purina chow alone, another group received AAN diet alone, and other groups were fed Purina chow with the same percentages of Tx and TRI as in experimental groups. The animals were weighed every 4 days. The second experiment was similar to the first except that the AAN diet contained 0.06% AAN. The doses of Tx and TRI were slightly changed (Table II). Thyroid extract 250 mg/100 g

TABLE I. Effect of L-thyroxine* (Tx) and L-triiodothyronine† (TRI) in Prevention of Skeletal Lesions in Rats Given Purina Chow Containing 0.075% of Aminoacetonitrile (AAN).

Treatment, mg/100 g diet	Control diet‡ Avg Starting wt (g)	Control diet‡ Avg Final wt (g)	AAN diet§ Avg Starting wt (g)	AAN diet§ Avg Final wt (g)	Severity of lesions
Purina	44	137			0
Purina + AAN 75			46	98	5+
Tx .25	44	126	42	96	5+
Tx .50	49	121	44	98	5+
Tx 1.25	47	148	48	99	5+
Tx 10 ‖	—	—	50	98	3+
TRI .02	44	132	43	100	5+
TRI .06	44	129	46	88	2+
TRI .10	48	114	48	92	1+
TRI .25¶	48	91	49	74	1+

Four rats were used to test each dose in the experimental groups; 3 rats for each control group. Duration of experiment, 17 days.

* Nutritional Biochemicals.

† "Cytomel," courtesy of Smith, Kline & French.

‡ Finely ground Purina chow. The rats on this diet and Tx or TRI did not develop skeletal lesions.

§ Finely ground Purina chow + 0.075% AAN.

‖ One rat died on 14th day of experiment.

¶ Two rats of control group and 3 of AAN group died from 11th to 13th day of experiment.

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TABLE II. Effect of L-thyroxine (Tx) and L-triiodothyronine (TRI) in Prevention of Skeletal Lesions in Rats Given Purina Chow Containing 0.06% of Aminoacetonitrile (AAN).

Treatment, mg/100 g diet	AAN diet* Avg Starting wt (g)	Final wt (g)	Severity of lesions
Purina only	54	173	0
Purina + AAN 60	59	148	3+
Tx .40	60	152	3+
1.0	64	153	3+
5.0	53	140	2+
TRI .03	62	137	1+
.06	57	130	0
.09†	61	120	0
Thyroid extr. 250	57	140	2+

* Finely ground Purina chow + 0.06% AAN.
† One rat died on 6th day of study.

of diet was added to the diet of one group of rats. In Exp. 3 (Table III) the rats were fed Purina chow containing 0.075% AAN as in the first experiment. Several doses of Tx and TRI were injected subcutaneously daily in slightly alkaline solutions. In Exp. 4 (Table IV) the daily doses of Tx and TRI were injected simultaneously with 5 mg AAN. In Exp. 5 (Table V) 2 μg TRI was injected daily simultaneously with 6 mg AAN for the first 6 days and 9 mg AAN for the following 11 days. Duration of experiments was 17 days. At termination of each experiment the animals were sacrificed and roentgenograms were made. At post mortem examination no instance of dissecting aneurysm of the aorta was noted in either control or experimental

TABLE III. Effect of Injected L-thyroxine (Tx) and L-Triiodothyronine (TRI) in Prevention of Skeletal Lesions in Rats Given Purina Chow Containing 0.075% Aminoacetonitrile (AAN).

Treatment,* μg/day/rat	Avg Starting wt (g)	Final wt (g)	Severity of lesions
Control†	51	115	5+
Tx 10	48	116	5+
50	49	121	4+
100	52	116	3+
250	48	107	2+
TRI 2	49	119	2+
5	51	111	2+
10	46	95	1+

* Tx and TRI were administered in slightly alkaline solutions and inj. subcut.
† 0.1 ml normal saline.

TABLE IV. Effect of Injected L-thyroxine (Tx) and L-triiodothyronine (TRI) in Prevention of Skeletal Lesions in Rats Treated with 5 mg/Day of Aminoacetonitrile (AAN) Administered Subcutaneously.

Treatment,* μg/day/rat	Avg Starting wt (g)	Final wt (g)	Severity of lesions
Control†	48	107	3+
Tx 50	48	123	1+
100	51	130	0
TRI 2	51	145	0
5	49	118	0
10	48	116	0

* Tx and TRI were administered in slightly alkaline solutions. 5 mg AAN in 0.2 ml distilled water was inj. simultaneously.
† 5 mg AAN inj. subcut.

animals. The knees, including lower half of femur and upper half of tibia, were excised and decalcified in 6% aqueous solution of sulfosalicylic acid. Sagittal sections were stained with hematoxylin and eosin. Roentgenograms were evaluated for (a) width of epiphyseal plates adjoining the knees, (b) amount of periosteal new bone formation in femurs, (c) width of ilium, and (d) severity of osteoporosis. Approximate estimate of severity of lesions obtained from the roentgenograms was checked against the lesions observed in histological sections. Rats of the same experimental group showed only slight variations in severity of the lesions.

TABLE V. Effect of Injected L-triiodothyronine (TRI) in Prevention of Skeletal Lesions in Rats Treated with 6 mg/Day of Aminoacetonitrile (AAN) for 6 Days and 9 mg/Day AAN for Following 11 Days Administered Subcutaneously.

Treatment	Avg Starting wt (g)	Final wt (g)	Severity of lesions
Control *	52	165	0
TRI †	50	161	0
AAN ‡	51	144	4+
AAN + TRI§	51	142	0

Six rats were used in each group. Purina chow was fed *ad lib.*

* 0.2 ml normal saline inj. subcut. daily.
† TRI 2 μg administered daily in 0.2 ml slightly alkaline solution.
‡ AAN 6 mg/day in 0.2 ml distilled water for 6 days and AAN 9 mg/day in 0.3 ml distilled water for following 11 days inj. subcut.
§ TRI 2 μg was inj. daily simultaneously with AAN 6 mg/day the first 6 days and AAN 9 mg/day for following 11 days.

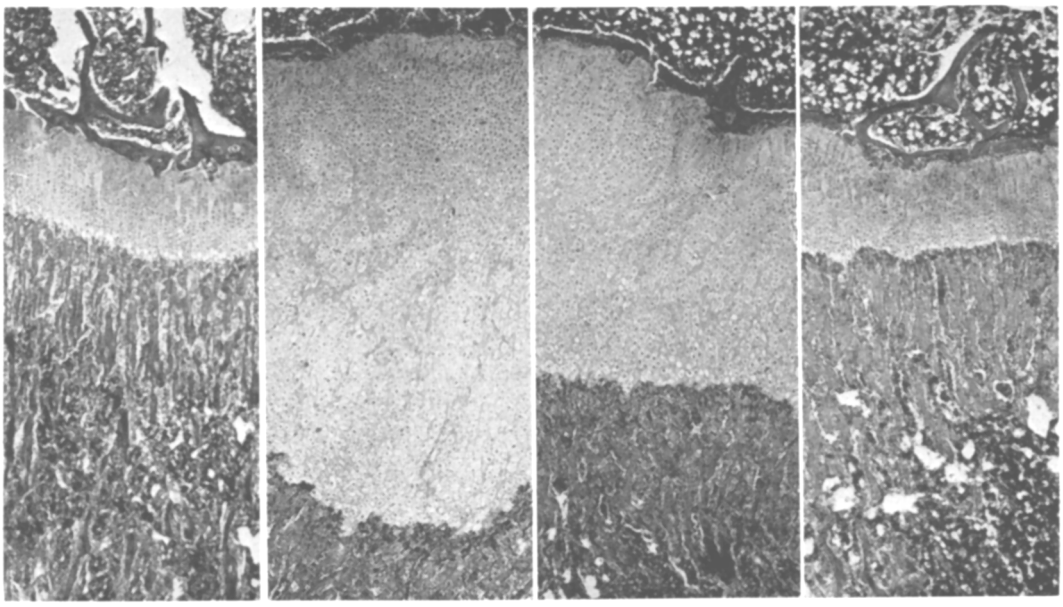

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FIG. 1. Proximal epiphyseal plates of tibia of 40-day-old rats (×20, hematoxylin and eosin stain) treated as follows for 17 days: (a) normal control, (b) AAN 0.075% diet, (c) AAN 0.075% diet + Tx 100 μg/day inj. subcut., (d) AAN 0.075% diet + TRI 10 μg/day inj. subcut.

Results. Neither Tx nor TRI completely prevented the lesions produced by a relatively high dose of AAN (0.075% of diet, Tables I and III). However, the lesions were greatly improved with high levels of TRI, whereas Tx had only a slightly suppressing effect even in doses 20 to 100 times higher than TRI (Fig. 1). Less severe lesions were produced with diet containing 0.06% AAN (Table II). TRI 0.09 and 0.06 mg/100 g diet prevented the lesions; these were not prevented with Tx in doses up to 5 mg/100 g diet. Moderately severe lesions were produced with AAN (5 mg/day) injected subcutaneously; TRI (2 μg/day) injected subcutaneously prevented the lesions; they were also prevented with parenteral administration 100 μg/day of Tx, but not with 50 μg/day of Tx (Table III). The experimental animals receiving very high doses of Tx and TRI grew less than the controls on AAN alone. The objection may be raised that the suppression of the AAN lesions by these compounds is related to the growth inhibition and is non-specific. In Exp. 5 (Table V) the growth inhibition produced by AAN was not incremented by administration of TRI 2 μg daily; however, this small dose checked completely the AAN lesions. Also the experimental animals of Exp. 4 (Table IV) weighed more than the controls, yet showed complete suppression of the AAN lesions. It appears evident from these experiments that Tx and TRI can suppress the lesions produced by moderate doses of AAN without interfering with growth of the rat.

No skeletal lesions occurred in the control groups of rats fed Purina chow with Tx and TRI or in the controls injected with Tx and TRI (not included in tables). However, animals on the highest doses of Tx (250 μg/day) and TRI (10 μg/day and 0.10 and 0.25 mg/100 g diet) had epiphyseal plates slightly narrower than the normal animals with fewer cartilage cells indicating some retardation of growth (Fig. 2). It is known that thyroxin produces precocious skeletal maturation in rats(12). Several investigators have estimated, on the bases of different criteria, the potency of TRI to be about 5 times that of Tx(13,14). Working on growth and development of embryonic chick limb-bones in tissue culture Fell and Mellanby(15) found

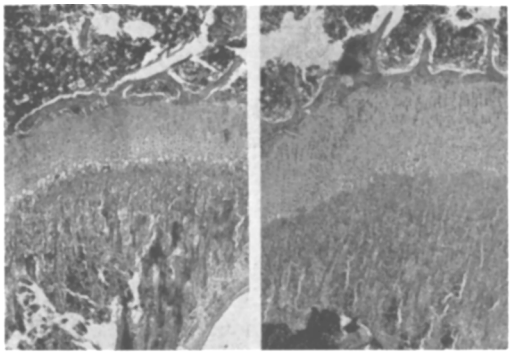

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FIG. 2. Proximal epiphyseal plates of tibia of 40-day-old rats (×20, hematoxylin and eosin stain) treated as follows for 17 days: (a) TRI 10 μg/day inj. subcut., (b) Tx 100 μg/day inj. subcut.

that the inhibitory effect of TRI on growth rates of femora and tibiae was about 4 times that of Tx. Our experiments with prevention of AAN lesions in rats showed TRI to be over 100 times more active than Tx when administered orally. It is known that Tx is less than one-half as active when administered orally as when administered subcutaneously, whereas TRI is almost equally effective by either route(13,14). The results of our third and fourth experiments indicate that when administered parenterally TRI was over 50 times more potent than Tx in the suppression of the AAN lesions.

No qualitative differences could be observed between effects of TRI and Tx in rats treated with AAN. Punched out areas filled with very cellular connective tisue were observed in the long bones of AAN-treated rats on the larger doses of TRI (10 μg/day, and 0.10 and 0.25 mg/100 g diet); similar lesions were observed in Tx 250 μg/day AAN-treated rats. More than half of the animals on TRI 0.25 mg/100 g diet died of unknown cause.

The suppression of the AAN epiphyseal plate lesions with Tx and TRI may be related to the skeletal maturation effects of these compounds. However, the much greater activity of TRI on these lesions cannot be explained. Experiments now under way indicate that the beneficial action of TRI on the AAN lesions is not limited to the skeleton but is a generalized effect on all mesodermal tissues. The possible interrelation between AAN action and thyroid secretion is suggested by our observation that aminonitriles become effective in developing vertebrates at a period corresponding closely with onset of activity of the thyroid gland(16), *i.e.* the 16th day of gestation for the rat embryo(17), the 13th day of hatching for the chick embryo(4) and stage 25 for the frog larva(4).

Summary. Low doses of l-triiodothyronine suppressed the mild forms of aminoacetonitrile poisoning in rats, whereas extremely high doses of thyroxine were necessary to accomplish the same result. On the basis of the prevention of aminonitrile lesions in rats, l-triiodothyronine was estimated to be over 100 times more active than l-thyroxine when administered orally and over 50 times when administered parenterally.

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