old. Dosages were calculated from the amount of feed consumed. Thiouracil was used as a standard, and effects of the drugs were determined by measuring changes in weight and iodine content of the thyroid glands compared with controls. The results of this experiment are summarized in Table 2, and statistically significant changes were noted. Thyroid weights were calculated as milligrams per 100 g body weight, and iodine $I_2$ as micrograms per 100 mg thyroid. Only thiouracil produced any significant increase in height of thyroid acinar cells; and, aside from the thyroid changes, no other discernible effects were produced by these drugs in the amounts given. The allyl and glyceryl derivatives were tested twice, since it seemed unlikely to us that the former compound would possess appreciable activity and the latter none. Nevertheless, the results were the same in both experiments.

The antithyroid activity of some of the compounds used here and the lack of such activity in others may be regarded as presumptive evidence that S-substituted thiouracils are not active *per se*. Moreover, although we found the S-carboxymethyl derivative to be active in the rat, this compound has been shown to have no effect on human hyperthyroidism (*5*). The same result was obtained here with the S-carboxyethyl derivative (*6*), although urine samples from three normal human beings showed a typical, but transient, blue-green color with Grote reagent (for the C=S group) following ingestion of 0.5 g of the compound. Hence, if the color of these samples was due to 6-methyl-2-thiouracil resulting from cleavage of the S-substituent, the rate of cleavage was too slow to affect the symptoms of human hyperthyroidism.

In further studies with rats, samples of the S-carboxyethyl compound were incubated with slices of liver and kidney, with the contents of the small intestine, and with cultures of bacteria normally found in the intestine. Since no evidence of cleavage was produced by these treatments, 10 rats, as previously described, were given daily subcutaneous injections of the drug at the same dosage as was received by those on oral treatment. After 15 days of injections, the thyroids of this group showed no changes whatever from those of control animals. When urine from the injected animals was examined by paper chromatography, only the unchanged S-substituted derivative could be found. When the drug was given orally, in addition to unchanged derivative, small amounts of 6-methyl-2-thiouracil could usually, but not always, be demonstrated in the urine. Hence, cleavage of the substituted drug was not quantitative, as is also indicated by the magnitude of the antithyroid effect shown in Table 2. Also, it would be expected that a large percentage of free drug released by cleavage would be destroyed in the tissues of the animal prior to excretion.

From the results of this work, we conclude that S-substituted thiouracils are not antithyroid compounds *per se* but must undergo prior cleavage to free the sulfur, and that the S-carboxyethyl derivative is partially cleaved in the intestinal wall of the rat during the process of absorption. Furthermore, in view of the chemical stability of this derivative, it is necessary to assume that cleavage of the S-substituent was brought about through enzymic attack, although the nature of this attack had not yet been demonstrated.

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## Epiphyseal Plate Lesions, Degenerative Arthritis, and Dissecting Aneurysm of the Aorta Produced by Aminonitriles


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β-Aminopropionitrile fed to weanling rats at a concentration of 0.1, 0.4, and 1.0 percent in their diet produced widening and extensive disruption of the epiphyseal plates, widespread periosteal new bone formations, loosening and detachment of the ligamentous and tendinous insertions, degenerative arthritis, and dissecting aneurysm of the aorta. Roentgenograms taken 2 wk after the beginning of the experiment showed that the epiphyseal plate lesions and periosteal new bone formations were extremely severe in the rats fed a 1-percent concentration; they were severe in rats fed 0.4 percent, and slight in rats fed 0.1 percent. Histologically, these lesions appeared to be identical to the lesions observed in rats fed diets containing 50-percent *Lathyrus odoratus* (sweet pea) seeds (*1*).

The possible relationship between these lesions and similar lesions commonly observed in human beings has been discussed in other papers (*2*). Working with C. Y. Chang and E. Witschi (*3*), we have found that β-aminopropionitrile at a concentration of 1:2,000,000 produces in *Xenopus* larvae dislocations of the joints of the hind legs and loosening of the septa of the metameres of the dorsal musculature. These lesions are produced within 1 wk if the compound is given about 10 days before metamorphosis.

Dupuy and Lee (*4*) reported the isolation from *L. pusillus* of a crystalline substance that produces the skeletal lesions characteristic of lathyrism. Schilling and Strong (*5*) identified a crystalline compound isolated from *L. odoratus* as β-(γ-L-glutamylamino)-propionitrile. Following the method of Dupuy and Lee, we also isolated this compound from germinating

*L. odoratus* seeds and found it to be active in producing the skeletal lesions when fed at the 1-percent level. In order to ascertain the active moiety of this compound, we have fed (*6*) to weanling rats the following diets: 1 percent β-alanine together with 1 percent L-glutamic acid, 1 and 2 percent propionitrile, 1 and 2 percent acetonitrile, 1 percent indoleacetonitrile, 0.4 and 1 percent bis(β-cyanoethyl)amine, 0.4 and 1 percent tris(β-cyanoethyl)amine, and 0.1, 0.4, and 1 percent β-aminopropionitrile.

The diets with the β-aminopropionitrile produced the skeletal lesions in all the rats within 2 wk. One of the three rats fed this compound at the 0.4-percent level died of dissecting aneurysm of the thoracic aorta after 34 days of feeding. The rats fed a diet containing 1 percent bis(β-cyanoethyl)amine died within 16 days of feeding and had no apparent skeletal lesions. However, the three rats fed this compound at the 0.4-percent level survived and developed skeletal lesions 50 days after the onset of feeding. No skeletal or aortic lesions were observed in the animals fed the other compounds listed (Table 1).

The possible way of action of the β-aminopropionitrile was further investigated. Since β-aminopropionitrile slowly decomposes into acrylonitrile and ammonia, we administered to weanling rats diets containing 0.1 and 0.4 percent acrylonitrile. No skeletal lesions were observed. However, since the toxicity of acrylonitrile has been ascribed to the slow liberation of cyanide ion in the animal body (*7*), we injected a number of rats with sublethal doses of potassium cyanide. It was found that a single dose of 0.4 mg was fatal and that a dose of 0.3 mg given three times a day was fatal to one of two animals. It was decided, then, to inject subcutaneously several groups of rats with doses of potassium cyanide varying from 0.21 to 0.057 mg three times daily. The potassium cyanide was dissolved in 0.5 ml of a 5-percent gum acacia solution. These animals gained weight but had not developed any skeletal lesions after 28 days of treatment.

The possible effect on the skeleton of other aminonitriles was investigated. It was found that aminoacetonitrile incorporated in the diet of weaning rats at the 0.2-percent level produced extremely severe skeletal lesions 2 wk after feeding. The same compound at the 1-percent level killed the rats within 6 days. On the other hand, α-aminopropionitrile at the levels of 1.0 and 0.2 percent did not produce any

Table 1. Intake of various compounds incorporated into the diet of weanling, male albino rats.

| Compound | Percentage of diet | Duration of experiment (days) | No. of animals | Average gain in weight (g) | Severity of skeletal lesions |
|---|---|---|---|---|---|
| Acetonitrile | 2.0 | 52 | 1 | 149 | 0 |
| | 1.0 | 52 | 1 | 190 | 0 |
| Aminoacetonitrile* | 1.0† | 6 | 3 | 3 | 0 |
| | 0.2† | 34 | 3 | 68 | ++++ |
| Propionitrile | 2.0 | 52 | 1 | 143 | 0 |
| | 1.0 | 52 | 1 | 137 | 0 |
| α-Aminopropionitrile‡ | 1.0† | 6 | 3 | −10 | 0 |
| | 0.2† | 34 | 3 | 3 | 0 |
| β-Aminopropionitrile | 1.0† | 30 | 4 | 10 | +++ |
| | 0.4† | 38 | 3 | 76 | ++ |
| | .1† | 38 | 3 | 112 | + |
| Bis(β-cyanoethyl)amine | 1.0 | 16 | 3 | −16 | 0 |
| | 0.4† | 98 | 2 | 68 | + |
| Tris(β-cyanoethyl)amine | 1.0 | 27 | 3 | 20 | 0 |
| | 0.4 | 10 | 2 | 10 | 0 |
| β-(γ-L-glutamylamino)propionitrile | 1.0 | 21 | 2 | 64 | ++ |
| Acrylonitrile | 0.1–0.4† | 38 | 3 | 127 | 0 |
| γ-Aminobutyronitrile‡ | 0.2 | 34 | 3 | 137 | 0 |
| Indoleacetonitrile | 1.0 | 38 | 1 | 70 | 0 |
| L-Glutamic acid + β-Alanine | 0.7 + .4 | 56 | 2 | 140 | 0 |
| Control§ | | 45 | 4 | 172 | 0 |
| Potassium cyanide | 0.00‖ | 28 | 2 | 126 | 0 |
| | .05‖ | 28 | 2 | 124 | 0 |
| | .11‖ | 28 | 2 | 109 | 0 |
| | .21‖ | 28 | 2 | 129 | 0 |
| | .30‖ | 1 and 20 | 2 | | 0 |
| | .40–1.0‖ | 0 | 6 | | 0 |

* Fed as a salt of $H_2SO_4$ with $NaHCO_3$.
† Diet made fresh daily and presented for 1½ hr.
‡ Fed as a salt of HCl with $NaHCO_3$.
§ The control diet consisted of casein (18 percent), yeast (10 percent), Crisco (5 percent), Wesson Oil (5 percent), sucrose (29.6 percent), starch (29.7 percent), salt mixture (2.5 percent), haliver oil (0.2 percent), and choline (0.04 percent). This was the base diet used in all the experiments reported in this paper.
‖ Milligrams per injection 3 times per day.

detectable skeletal lesions, although the rats were stunted. γ-Aminobutyronitrile at the 0.2-percent level did not produce any lesions, and the animals developed normally during the 34 days of feeding.

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## Purification of Angiotonin (Hypertensin)


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Several reports have appeared recently describing the purification of angiotonin (*1–3*). Recent observations in these laboratories have provided evidence that angiotonin may be purified to yield preparations of higher purity than those previously reported. However, these preparations are unstable under all the conditions we have studied.

The specific activity of the various preparations varies widely (Table 1). The data also show that specific activities based on comparison with tyramine are significantly different from those based on direct comparison with angiotonin. The difference is undoubtedly the result of the fact that each investigator has used a different type of animal preparation for assay. Further, tyramine is not a suitable reference standard for angiotonin assay (*1, 4*). For comparative purposes, a standard method of assay and a stable, standard preparation of angiotonin would be of great value.

Table 2. Summary of purification steps.

| Step | Yield | | Specific activity (AU/mg) |
|---|---|---|---|
| | Grams | Percentage of starting activity | |
| Crude | 1104 | 100 | 1.59 |
| Methanol and phenol extractions | 33.0 | 88.1 | 46.6 |
| Partition column | 2.02 | 17.4 | 151 |
| Countercurrent distribution | 0.8641 | 10.1 | 205 |
| Electrophoresis | .0585 | 5.7 | 1710 |
| Ether precipitation | .0042 | 16.4 | 68000 |

Crude angiotonin was prepared (*5*) by the method of Plentl and Page (*6*) from hog renin substrate and hog renin. The clear filtrate was concentrated in vacuum, autoclaved, filtered, and frozen-dried.

Purification of the material was effected by a series of steps involving (i) extraction into methanol, (ii) extraction from an aqueous solution into phenol, (iii) partition on a silica column using a solvent system composed of *n*-butanol, acetic acid, and water, (iv) counter-current distribution with a solvent system composed of acetate buffer *p*H 3.0, *n*-butanol, and methanol, (v) electrophoresis (*7*) in acetate buffer, *p*H 4.2, (vi) precipitation from methanol solution by addition of ether. As an illustration, data for one series of experiments are summarized in Table 2; 7090 lit of hog blood was treated with renin to obtain the 1104 g of crude angiotonin.

Angiotonin with high specific activity is unstable and loses pressor activity rapidly. The initial specific activity, 68,000 angiotonin units per milligram (AU/mg), was obtained when the sample (No. 221-194B-74-5) was assayed immediately the morning it was prepared. Assays that afternoon indicated an activity of 24,000 AU/mg, and the next morning, 424

Table 1. Specific activity of angiotonin preparations reported in the literature. The angiotonin unit, AU, is the pressor activity of 30 μg of a frozen-dried preparation of angiotonin (*11*). In the pithed rat preparation (*12*) it has a pressor activity equivalent to 0.155 Goldblatt unit, GU (*13*), to 0.863 of the unit used by Bumpus, BU (*13*), and to approximately 0.18 mg of tyramine hydrochloride. The nitrogen content of angiotonin is assumed to be 16 percent.

| Preparation | Tyramine phosphate (mg/mg) | Tyramine hydrochloride (mg/mg) | AU/mg based on tyramine | AU/mg based on angiotonin | GU/mg *N* | BU/mg *N* |
|---|---|---|---|---|---|---|
| Edman 1945 (*4*) | 39* | | 161 | | | |
| Helmer 1950 (*11*) | | | | 37* | | |
| Clark *et al.* 1954 (*1*) | | 5.8* | 32 | 245 | | |
| Skeggs *et al.* 1954 (*2*) | | | | 5150 | 5000* | |
| Bumpus *et al.* 1954 (*3*) | | | 402 | 2780 | | 15000* |
| Kuether and Haney | | | | 68000* | | |

* The original datum used in the comparison.