trol of apple scab, celery early blight, muskmelon leaf spot, onion-foliage diseases, leaf diseases of ornamentals, potato late blight, tomato anthracnose, dollar spot, and diseases of turf caused by Helminthosporium. The results of advanced evaluation studies will be reported elsewhere.

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## Antithyroid Activity of Some S-Substituted Thiouracils


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Since S-alkyl derivatives of thiouracil do not possess antithyroid properties (*1*), it is generally assumed that the activity of thiouracil and its derivatives depends upon the presence of unsubstituted sulfur in the thiourylene group. Available evidence supports this assumption insofar as the ultimate antithyroid mechanism is concerned, but the possibility remains that other S-substituted derivatives might be active either *per se* or through the removal *in vivo* of the S-substituent. Thus, Lawson and Searle (*2*) have recently described the antithyroid activity of thioimidazole derivatives of the —S—CO—OR type (R=alkyl or aryl) and have suggested that these compounds are cleaved *in vivo* so as to produce the free thioimidazole. Chemically however, this type of thio derivative is readily hydrolyzed to produce a free sulfhydryl group; hence, in animal work, some cleavage might be demonstrated regardless of any possible enzymic attack. On the other hand, thiouracil derivatives of the thioether type are generally quite stable toward hydrolysis at physiological *p*H, and if they are converted to free thiouracil *in vivo*, enzymic cleavage may be assumed. Therefore, it was decided to test several thiouracil derivatives containing a thioether linkage and, if antithyroid activity were found, to determine whether these derivatives were active with or without removal of the S-substituent (*3*).

Table 1. Data on new S-substituted thiouracils.

| Thiouracil derivative | Capillary mp (°C) | Kjeldahl nitrogen (%) | |
|---|---|---|---|
| | | Calc. | Found |
| 2-Allyl | 145–149 | 16.68 | 17.00 |
| 2-Malonyl | 158–159* | 12.17 | 12.05 |
| 2-Glyceryl | 230*† | 13.39 | 13.49 |
| 6-Methyl-2-carboxyethyl | 200*† | 14.00 | 13.77 |

* With decomposition. † Preheated bath.

Of the seven derivatives prepared and tested, four have not been previously reported and are shown in Table 1. The allyl and malonyl derivatives were prepared by reacting sodium-2-thiouracil in a 1:1 alcohol-water solution and were subsequently acidified with acetic acid. The glyceryl derivative was prepared by the same method using epichlorohydrin. This derivative is readily soluble in cold water even after acidification but is precipitated by addition of acetone. It has a markedly bitter taste. 6-Methyl-2-carboxyethyl-thiouracil was prepared by condensing b-isothioureidopropionic acid with acetoacetic ester (*4*). All the compounds used here proved stable toward hydrolysis at physiological *p*H except the allyl derivative, which produced small amounts of allyl sulfide when heated in water.

For the animal testing, the compounds were ground with Purina Lab Chow, and the mixture was given *ad libitum* to Sprague Dawley male rats 2 to 3 mo

Table 2. Effect of S-substituted thiouracils on rat thyroid.

| S-substituent | No. of animals | Dosage (μM/day) | Days treated | Thyroid changes in percentage | |
|---|---|---|---|---|---|
| | | | | (wt., mg %) | ($I_2$, μg %) |
| —H (thiouracil) | 20 | 50 | 25 | +45† | −71† |
| —$CH_3$ | 10 | 45 | 25 | 0 | − 7 |
| —$CH_2CH_3$ | 15 | 50 | 25 | − 6 | − 9 |
| —$CH_2CH{=}CH_2$ | 15 | 40 | 20 | + 8 | −30† |
| | 10 | 100 | 10 | +12 | −50† |
| —$CH_2COOH$ | 15 | 51 | 25 | +28* | −68† |
| —$CH_2CH_2COOH$ | 15 | 52 | 25 | +26* | −70† |
| —$CH(COOH)2$ | 15 | 47 | 25 | − 9 | −18 |
| | 15 | 96 | 25 | +10 | −24* |
| —$CH_2CHOHCH_2OH$ | 15 | 50 | 25 | +12 | +10 |
| | 10 | 102 | 10 | − 4 | + 7 |

* $P < 0.05$; † $P < 0.01$. Control thyroid values, 20 animals: wt. = 7.6 mg %, $I_2$ = 64 μg %.

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