

strain animals. On diet 31PMC, the minimum daily choline requirement was approximately 4 to 6 mg for F₃ generation LCR strain rats, and above 10 mg for HCR strain rats of the same generation. In both strains the incidence of kidney hemorrhage was less in females than in males on the same diet and level of choline. All female rats of the F₃ generation of the LCR strain lived for the duration of the experiment regardless of the level of choline on which they were placed. This confirms the results of Griffith² and of Engel and Salmon,³ in which it was reported that choline deficiencies appeared more slowly and were less severe in female than in male rats on the same diet.

² Griffith, W. H., *J. Nutrition*, 1940, **19**, 437.

³ Engel, R. W., and Salmon, W. D., *J. Nutrition*, 1941, **22**, 109.

The results of the experiment show the necessity for additions of choline to purified diets for experimental purposes. The fact that the rats may vary in their requirement for specific nutriment, such as choline, is of significance in relation to experimental procedures. The possibility of such variations should also be considered in relation to the practical nutrition of humans and the domestic animals.

Summary. Two strains of rats were selected which showed significant differences in their requirement of choline. Tests on the progeny of each strain proved that this difference continued through the F₃ generation. The minimum daily choline requirement of the strain LCR was approximately 4 to 6 mg per rat on diet 31PMC, and the minimum daily choline requirement of the strain HCR on the same diet was above 10 mg per rat.

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The Mechanism of Action of Thiouracil.

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According to the investigations of MacKenzie¹ the thyroid-inhibiting action of thiourea, sulfaguanidine, etc., results from direct action on the gland itself whereby the secretion is inhibited and the gland hypertrophies as the restraining influence of the thyroid hormone on the pituitary is eliminated; there is also lowered metabolic rate, interference with growth and development, in other words, all symptoms of hypothyroidism.

These writers also discuss the problem of whether the circulating hormone (thyroxin) may become inactivated by the drug. But they denied this possibility since in thyroidectomized rats thiourea—which does further depress the b.m.r.—is not antagonized by *injected* thyroxin. In other words: thyroxin raises the metabolism of thyroidectomized rats

even if thiourea had been administered. Similarly, Astwood, Sullivan, Bissell, and Tyslowitz² concluded that artificially administered thyroid hormone is equally calorogenic and toxic in the drug-treated animals as in the controls.

In contrast to these findings, we have found that thiourea (and thiouracil) *do* interfere with the action of *orally administered thyroid*, if the well known Reid Hunt³ method is made the basis of observations. In this test as little as 1 mg of whole thyroid gland can be detected since it imparts to the mice a distinct resistance against otherwise fatal doses of acetonitrile. This increased resistance is partially removed by thiourea or thiouracil as the following experiments show.

¹ MacKenzie, C. G., and MacKenzie, Julia B., *Endocrinology*, 1943, **32**, 185.

² Astwood, E. B., Sullivan, J., Bissell, Adel, and Tyslowitz, R., *Endocrinology*, 1943, **32**, 210.

³ Hunt, Reid, *Arch. Int. Med.*, 1925, **35**, 671.

Experimental Methods. White female mice were obtained from our usual commercial source and kept in cages in lots of 3 to a cage. The mice were fed a diet of Pratt's Rabbit Pellets which were ground to a fine powder in a drug mill, and moistened with water so as to form a cake. Each 100 g of dry food was fortified by the addition of 2 cc of cod liver oil USP. After an interval of one week on this diet of 5 g of food per day per mouse, the food, with the exception of the controls, had added to it varying amounts of thyroid, thiouracil and thiourea. Each set of mice was kept under identical conditions for a period of 2 weeks while receiving the diet. At the end of 2 weeks the mice were weighed and given intraperitoneal injection of acetonitrile, dilution 20 mg per cc. Variation of the toxicity of acetonitrile is to be expected according to the season of the year (Munch⁴). Death within 24 hours was taken as the end point.

On our first series of mice (12 to a set) the following results were obtained. All data were compiled following a Karber condensation curve as modified by Muench.⁵ LD₅₀ is in terms of acetonitrile.

TABLE I.

Diet	LD ₅₀ mg/kg	% in terms of controls
Controls	500	100
Thyroid 1 mg	800	160
" 2 "	900	180
Thiouracil 2 "	500	100
Thiourea 2 "	525	105

⁴ Munch, J. C., *Bioassays*, 1931, 672,

⁵ Reed, L. J., Muench, H., *Am. J. Hygiene*, 1938, 27, 493.

As is shown by the Table I the feeding of thiouracil and thiourea did not decrease the resistance of white mice to acetonitrile.

A second series of mice was run under like conditions. The diet had added to it 1 mg of thyroid and varying amounts of thiouracil and thiourea as indicated in Table II.

TABLE II.

Diet	LD ₅₀ mg/kg	%
Controls	450	100
Thyroid 1 mg	850	191
" 1 " Thiouracil 1 mg	650	144
" 1 " " 2 "	550	122
" 1 " Thiourea 2 "	600	133

Results. On the basis of the data obtained in Table I thiourea and thiouracil have no effect in relation to the toxicity of acetonitrile in mice.

On the data obtained in Table II both thiouracil and thiourea reduce the LD₅₀ per kilo of mouse. In terms of percentage, 1 mg of thyroid protects 91% more than the control mice. When 1 mg of thiouracil is added to the daily diet the protection ratio dropped 47%. When 2 mg of thiouracil was added daily the protection ratio dropped 69%. Thiourea 2 mg reduces the protection ratio 58%. On the basis of these results it would appear that about 3 to 4 mg of thiouracil will neutralize the effect of 1 mg of whole thyroid gland on white mice. Thiourea is not quite as efficient as thiouracil.

Conclusion. Thiouracil and thiourea in addition to preventing the synthesis of the thyroid hormone has some effect on thyroid *per se* when fed to white mice.