and 5. These stages change during parathyroid tetany. Not all of them were uniformly altered in 31 animals studied. Rosenblueth and Cannon[4] showed that repeated periods of 10 to 15 sec. indirect stimulation of the gastrocnemius-soleus at a frequency of 500 per sec., with pauses of 10 to 15 sec., made increasingly clear the series of stages, 1, 2, 3a, and 3b, while the tension developed increased. This phenomenon, confirmed many times in this department, is regularly changed in hypocalcemic animals. Stages 3b and 3c do not appear; the stages do not become more distinct, and the tension does not increase. On the contrary, the muscle shows a marked tendency to fatigue. This always occurred, even when the first period of stimulation produced a response with stages 1, 2, 3a, and 3b.

In order of importance, the other changes are as follows: loss of the characteristic responses at frequencies of stimulation of 500, 400, 300, 200, 100, and 60 per sec. Frequently stage 3c as normally seen with 300 or 150 per sec. does not appear. Marked neuromuscular fatigue often occurs even at a frequency of 60 per sec.

In the group of animals in which the blood calcium was increased to normal values by injecting $CaCl_2$ the synaptic behavior resembles very much that of the normal cats.

Comment. These changes cannot be attributed to the removal of the thyroid glands, since the operated animal which received thyroid extract showed the same changes.

The abnormal synaptic reaction of the parathyroidectomized animals is probably due to the low value of blood calcium during tetany as it is normalized by increasing the blood calcium to normal values.

It is accepted that the chemical transmitter of the neuromuscular synapse is acetylcholine. Harvey and MacIntosh[1] showed that the calcium ion is fundamental for cholinergic transmission, since without this ion, acetylcholine is not released.

The marked fatigability of the neuromuscular synapse in animals with parathyroid tetany may be explained by the work of these authors. The decrease of calcium ion would hinder the release of acetylcholine and thus lead to the failure of transmission.

Conclusions and Summary. The responses of the soleus muscle of the cat on stimulation of its nerve at varying frequencies were studied in animals rendered hypocalcemic by parathyroidectomy. The responses at certain frequencies were markedly altered. These alterations were normalized by calcium administration.

[2] Rosenblueth, A., and Morison, R. S., *Am. J. Physiol.*, 1937, **119**, 236.

[3] Rosenblueth, A., and Luco, J. B., *Am. J. Physiol.*, 1939, **126**, 39.

[4] Rosenblueth, A., and Cannon, W. B., *Am. J. Physiol.*, 1940, **130**, 205.

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Strain Differences in the Choline Requirement of Rats.*

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In earlier work on choline in this laboratory, 2 strains of rats were used. Rats from these 2 strains appeared to differ in their requirement for choline. In order to determine whether these differences were genetic in character, females from the 2 strains were selected and bred to their respective littermates. The progeny were designated as the F_2 generation. It was previously reported[1] that the F_2 generation of the 2 strains showed significant dif-

* Published with the approval of the Director of the Alabama Agricultural Experiment Station.

[1] Engel, R. W., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 281.

TABLE I.
Number, Sex, and Kidney Condition of Rats in the F_3 Generation Receiving Various Levels of Choline.

		LCR strain rats				HCR strain rats			
Mg choline per day		2	3	4	6	4	6	8	10
Total number of rats	♂	13	14	19	7	14	15	10	4
	♀	10	18	33	9	15	19	11	4
No. of deaths	♂	4	2	2	0	12	10	0	1
	♀	0	0	0	0	7	7	0	0
No. of survivors showing kidney hemorrhage	♂	4	2	1	0	0	2	2	2
	♀	2	2	0	0	0	1	1	0
No. of survivors showing swollen or frosted kidneys	♂	1	3	2	0	2	3	3	0
	♀	1	2	2	0	7	8	4	0
No. of survivors showing normal kidneys	♂	4	7	14	7	0	0	5	1
	♀	7	14	31	9	1	3	6	4

ferences in their choline requirements. The rats having the higher requirement, designated as strain HCR, were hooded rats selected from the Wisconsin strain; those having the lower choline requirement, designated as strain LCR, were albino rats selected from the Wistar strain. It was desirable to determine whether the observed differences would be transmitted to the F_3 generation, and to measure these differences quantitatively. Brother-sister matings of the F_2 generations in the 2 strains were made, and the progeny were used for quantitative measurements of their respective choline requirements.

Choline Requirement of the F_3 Generation. At the age of 23 days, rats of the F_3 generation of both strains were placed on experimental diet 31PMC,† which was supplemented with adequate amounts of thiamine, pyridoxine, riboflavin, calcium pantothenate, inositol, carotene, and calciferol. They received various dosages of choline in order to see if the minimum choline requirement could be established for each strain. HCR strain rats of the F_3 generation were fed 4, 6, 8, and 10 mg of choline daily, and LCR strain animals of the same generation were fed 2, 3, 4, and 6 mg of choline daily. The rats remained on the experimental diet for 14 days. At the end of this period, the surviving animals were killed and their kidneys were examined. Ninety-two rats of the strain HCR and 123 rats of the strain LCR were tested over a period of 6 months.

The results of the experiments are given in Table I. Nineteen of the 29 HCR strain rats on a daily level of 4 mg of choline had fatal kidney hemorrhage; 9 of the survivors had injured kidneys at necropsy and only 1 was normal. Of the 52 LCR rats on the 4 mg daily level of choline, only 2 died and of the survivors 45 were normal. All the LCR strain animals tested on a 6 mg daily level of choline lived and had normal kidneys at necropsy, but this level was far too low for the HCR strain animals. Seventeen of the 34 HCR strain animals placed on the daily level of 6 mg of choline had fatal kidney hemorrhage; 3 had kidney hemorrhage but survived, and only 3 were normal. Even on the extremely low level of 2 mg of choline a day, the incidence of fatal kidney hemorrhage in rats of the LCR strain was not nearly as great as that of HCR strain rats on 6 mg of choline a day. Moreover, only 50% of the HCR animals on a level of 8 mg of choline a day were normal.

It is evident that the choline requirement of the F_3 generation of the strain HCR was more than twice as great as that of the LCR

† The breeding rats of the F_1 and F_2 generations were fed stock diet 30. This diet consisted of ground wheat 64, meat scraps 10, casein 10, alfalfa leaf meal 2, bone meal 2, iodized salt 1, molasses 5, lard 5, and cod liver oil 1%. It contained 6.7 mg of choline per 10 g by analysis. Diet 31PMC consisted of alcohol extracted peanut meal 30, alcohol extracted casein 6, sucrose 54, lard 6, and salt mixture No. 186 4%.

strain animals. On diet 31PMC, the minimum daily choline requirement was approximately 4 to 6 mg for F_3 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 F_3 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 Griffith[2] 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 nutriments, 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 F_3 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 MacKenzie[1] 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 Tyslowitz[2] concluded that artificially administered thyroid hormone is equally calorigenic 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 Hunt[3] 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.