cluding group 6. Group 2 contained less than groups 1, 3, 4, and 5, while group 7 contained less than groups 1, 4, and 5.

It seems clear from these results that on a normal diet growth hormone increases the nitrogen content of skeletal muscle while at the same time increasing the water content. The effect of the other factors studied, vitamins B_1 and D and excess phosphorus, is less obvious and further studies are in progress on this phase of the problem.

Summary. 1. The total nitrogen content of the quadriceps muscle of the rat in various nutritional states has been studied. 2. It has been found that, on a normal diet, growth hormone increases both the total nitrogen and the water content of the muscle.

9040

Effects of Prolonged Administration of Moderate Doses of Creatine in Rats.

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Since there were no observations reported in the literature on the long continued administration of creatine to experimental animals, it seemed worth while to conduct a series of observations on young and old animals in which creatine was fed in doses comparable to those used in certain clinical therapeutic experiments in man. This seemed especially desirable since unfavorable effects of long-time administration might conceivably occur. The series of experiments was further used in order to determine the influence of moderately high ingestion rates of creatine on the amount of that substance stored in various tissues in the body, particularly in muscle. Rats were, therefore, fed on the following stock diet, half of the animals receiving this diet alone, and the other half receiving the same food mixture with 2 gm. of creatine hydrate added to each kg. of food. Assuming an average food intake of 14 gm. per day per rat of 350 gm. weight, the creatine intake was 75 mg. creatine hydrate per kg. per day.

The rats were from 2 sources. One group consisted of 25 animals* age 3 years or over at the beginning of the experiment,

*We are indebted to Professor Lloyd Arnold for providing these animals.

STOCK DIET

Sweet butter fat	6.5%
Whole wheat flour	68.2%
Whole milk powder	9.2%
Crude casein	13.8%
NaCl	0.9%
$CaCO_3$	1.4%
	100.0

In addition lettuce was fed to all rats twice weekly.

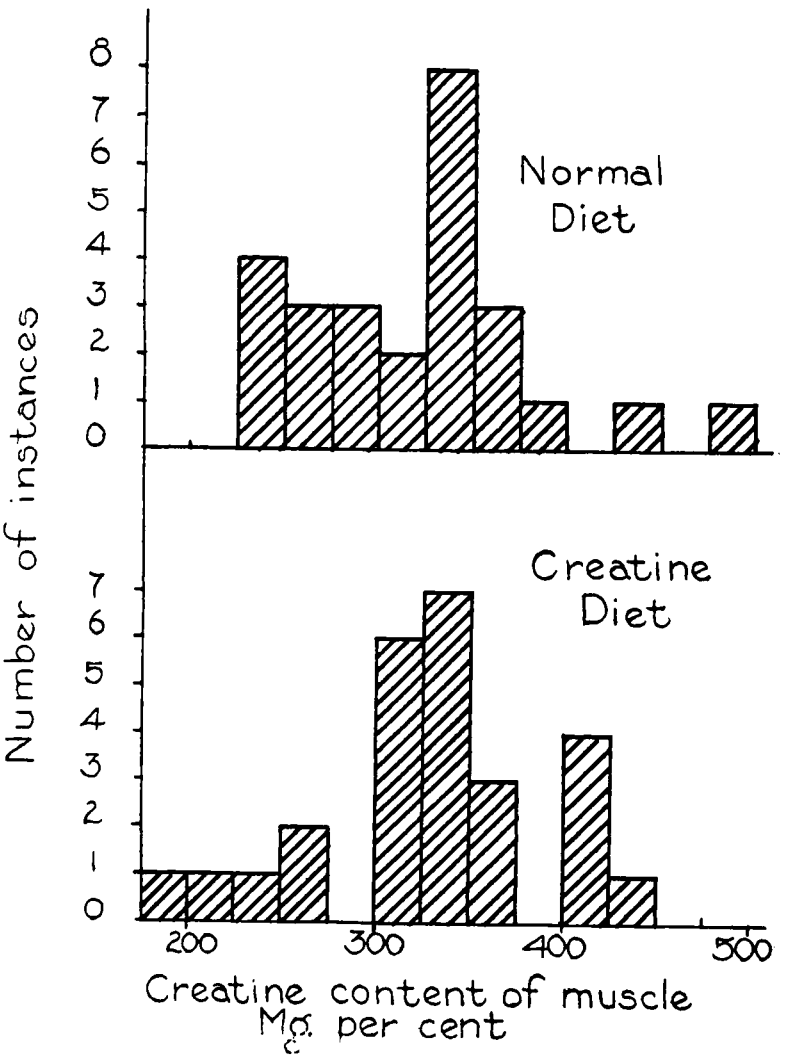

FIG. 1.

which had been obtained from the breeding colony of the Wistar Institute. The other group was of 50 Wistar strain animals 15 months of age at the beginning of the experiment. One-half of the animals in each group were placed on the control and the experimental diets for a period of from 4-6 months, at which time those still living were sacrificed and analyses made. The animals were anesthetized with amytal, 50 mg. per kilo, intraperitoneally. The tissues analyzed were the gastrocnemius muscle, the liver, and heart, which were excised in that order and plunged immediately into liquid air on excision. The tissues were weighed, ground with sand in a mortar containing trichloracetic acid after the method of Eggleton.[1] The creatine was estimated by the method of Folin-Benedict.[2]

TABLE I.
Creatine Content of Various Tissues of Rats.

	Normal diet			Creatine diet		
	Mean creatine content, mg./100g.	Std. dev.	No. of analyses	Mean creatine content, mg./100g.	Std. dev.	No. of analyses
Muscle	321.	58.	26	373.	69.	26
Liver	5.8	0.8	12	6.3	0.8	12
Heart	161.	56.	20	143.	43.	21

The distribution of creatine content in all of the analyses made on striated muscle is indicated in Fig. 1. It will be apparent that the most frequent concentration was the same in the animals on the stock diet as in those with creatine added. The mean values found in muscle, liver and heart, together with the statistically evaluated standard deviation for each, is shown in Table I. It is apparent that the means of the creatine content for the normal and the creatine series in no case differ by more than the standard deviation. Whatever differences exist are, therefore, probably those of random sampling. This seems the more likely because the direction of change is opposite for striated muscle and for cardiac muscle.

Particularly in the case of the rats 3 years of age and older a number of the animals died before the end of the experiment. Analyses were not made on those animals that died during the experimental period. Deaths in both the control and the experimental series of animals were due to a variety of causes, such as infections of the urinary tract, tumors, etc., which are common in animals of the age in question. There was no evidence that the animals on

1 Eggleton, G. P., and Eggleton, P., *J. Physiol.*, 1929, **68**, 193.
2 Benedict, S. R., *J. Biol. Chem.*, 1914, **18**, 191.

the creatine diet died as a result of specific poisoning. The analytical values for the young adult and the old animals showed no significant difference attributable to age. The figures have, therefore, been combined in the results presented.

It has previously been found (Chanutin[3]) that massive doses of creatine over shorter periods may increase the content of creatine in rats. Our results indicate that more prolonged feeding of lower doses, which in total exceed the quantities fed over the shorter periods, does not lead to an augmentation in creatine content of the tissues we have examined.

9041

Are Neutral Fat and Lecithin Present in Gall Bladder Bile?

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Since the time of Strecker[1] chemists who have worked with bile have appreciated the difficulty of separating lipoids from bile. The lipoid constituents as given by the text books at the present time are merely repetitions of the early figures given by Strecker, Hammarsten,[2] von Gorup-Besanez,[3] and others. These constituents are given as fatty acids, soaps, phosphatides, fat, and cholesterol. An examination of this early literature does not yield satisfactory evidence for the presence of either neutral fat or lecithin in bile.

Fresh bile may be extracted in 2 ways. One is to deproteinize by means of alcohol, then dry the protein-free bile and dissolve the residue in absolute alcohol, filtering off the inorganic salts, that are precipitated, and finally pouring the alcoholic solution into a large volume of anhydrous ethyl ether. This is the method used by Hammarsten.

The second method is to extract directly with ethyl and petroleum ether in the presence of some alcohol. In this manner a very persistent emulsion is formed that requires time to break. In either

3 Chanutin, A., and Beard, H. H., *J. Biol. Chem.*, 1928, **78**, 167; Chanutin, A., and Silvette, H., *J. Biol. Chem.*, 1928, **80**, 589; Chanutin, Alfred, *J. Biol. Chem.*, 1930, **89**, 765.

1 Strecker, *Compt. Rend.*, 1861, **52**, 1270.

2 Hammarsten, *Nova Acta Reg. Soc. Upsala*, 1893, June 15.

3 Von Gorup-Besanez, *Prager Vrtl. jahrshrift für prakt. Pharm.*, 1851, III, 86.