those of the equine strains. No immune serum was available for the Borna virus. It was noticed that while not all immunized guinea pigs responded by a marked antibody formation the serum of certain animals consistently gave positive results over varying periods of time with antigens made on different occasions. This was particularly true for serums of the Moscow No. 2 strain.

Two series of animals were immunized with live virus of the eastern and western American and the Moscow No. 2 strains, respectively, over a period of several months. Blood was removed from the heart and increasing doses of virus were administered at weekly intervals. One series was given intravenous and the other subcutaneous injections of virus, respectively. Neutralization and complement fixation tests were performed on serums collected weekly with results indicating a parallelism between the 2 reactions, although the neutralization test usually became positive at an earlier period than the complement fixation. As a rule the latter failed to take place with serums of the American equine strains until after 2 months of immunization and then was not strongly positive until massive doses of virus had been given. The animals inoculated with the Russian strain gave the strongest reaction in the shortest time.

From the results it seems apparent that the complement fixation test may be applied to the differentiation of the strains of equine encephalomyelitic virus and to that of the lymphocytic choriomeningitis virus of Armstrong and Lillie. So far the test seems specific for the homologous strains when strongly hyperimmunized serums are used together with a potent antigen. Further work is contemplated in this field with other viruses and a more comprehensive account of the results already obtained will be reported later.

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Total Nitrogen Content of Skeletal Muscle of the Rat in Various Nutritional States.

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It is generally conceded that one important function of the growth hormone of the anterior hypophysis is the promotion of protein anabolism. Evidence for this has been obtained from the results of numerous nitrogen balance studies which have been published in

recent years, all of which show a marked retention of nitrogen after single or multiple doses of the hormone. The same conclusion has been drawn from several studies of the nitrogen content of animals, in which it has been found that the tissue nitrogen is significantly higher after growth hormone has been administered. So far as we are aware, however, all of these analyses have been made on the entire carcass, the soft tissues, or on the liver. With the exception of liver, therefore, no information is available on the influence of growth hormone on the nitrogen content of individual organs or tissues. It seemed, then, to be of interest to include analyses of skeletal muscles in the course of a general study of the effect of various nutritional conditions on growth and development in rats.

The rats were of standard, pedigreed stock, and were placed on the special diets when 13 weeks old. Seven groups of 7 to 10 rats each were treated as follows:

Group 1. Control, on normal diet only.*

Group 2. High phosphorus diet: disodium phosphate was added to the normal diet in sufficient quantity to increase the phosphorus content to 1.25%-1.5%.

Group 3. Normal diet with addition of 22.5 units of vitamin B_1 per day in the form of Tiki-tiki extract† during the first 10 weeks, thereafter 45 units per day.

Group 4. High phosphorus diet with the same vitamin B_1 supplement as in group 3.

Group 5. Normal diet and growth hormone (Antuitrin Growth‡) given intraperitoneally.

Amount of hormone administered: First 2 weeks, 8 units per week in 4 equal doses. Next 6 weeks, 4 units per week in 4 equal doses. Remainder, 8 units per week in 4 equal doses.

Group 6. High phosphorus diet and growth hormone administered as in group 5.

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* The stock diet used was:

	%
Whole wheat flour	68.3
Crude casein	13.8
Whole milk powder	9.2
Sweet butter	6.4
Calcium carbonate	1.36
Sodium chloride	0.91

The calcium and phosphorus content of this diet varied between 0.5% and 0.6%. The protein content was approximately 23%, and the calorific value varied from 4.8 to 5.0 large calories per gram. These values were not significantly varied in the other diets.

† Supplied by Harris Vitamin Laboratories.

‡ Supplied by Parke Davis and Company.

TABLE I.
Results of Analyses on Quadriceps Muscle in Rats.

Group	Water				Nitrogen (Wet basis)			Nitrogen (Dry basis)			
	No. of analyses	Mean H_2O content, %	Std. dev.	Std. error	No. of analyses	Mean N_2 content, %	Std. dev.	Std. error	No. of analyses	Mean N_2 content, %	Std. dev.	Std. error
1	6	74.60	.27	.12	6	3.547	.066	.027	6	13.93	.334	.126
2	6	74.90	.46	.19	7	3.456	.069	.026	7	13.63	.198	.075
3	6	74.81	.14	.06	7	3.485	.053	.020	7	13.85	.166	.063
4	8	74.90	.48	.17	9	3.506	.063	.021	9	13.90	.269	.089
5	9	75.43	.53	.19	9	3.488	.059	.021	8	14.18	.142	.054
6	5	74.81	.31	.14	5	3.477	.060	.022	5	13.80	.145	.065
7	9	74.64	.63	.21	9	3.434	.044	.015	9	13.62	.322	.107

Group 7. Diet of group 4, with the addition of vitamin D§ in the form of viosterol of such a concentration that the volume of oil did not exceed 0.125 cc. daily per rat, for a total weekly supplement of 12.5 units of the vitamin.

The various diets were fed for 7 months without interruption; the animals being 10 months old when killed for analysis. All animals appeared to be healthy and vigorous when killed, with the exception of certain animals which had previously developed an infection of the labyrinths. The values found on analyses of the infected animals were the same as for the others, and are included in the results in Table I. Values are expressed in per cent. The effect of the growth hormone on the weight of the rats was not as marked as might have been the case if the administration had been started earlier. Rats receiving the hormone were, however, heavier than the controls. It was noted also that males in group 7 were heavier than the controls, while the females were lighter. The vitamin B_1 supplement in groups 3 and 4 apparently had no significant effect on growth or health of the animals.

The nitrogen determinations were made in duplicate by macro Kjeldahl technique on the quadriceps muscle. The animals were anesthetized with ether, the muscles excised and placed in solid carbon dioxide. A part was used for determination of water content, and the remainder for nitrogen determination. The results of the analyses are shown in Table I.

The probabilities of the results shown in Table I being random have been calculated by the statistical method of Fisher,[1] which takes into account the population of the series. From these it can be said that the water content of the muscles in group 5 was significantly higher than in any other group, with the possible exceptions of groups 2 and 6, and in group 6 the series is short. This is the more interesting when it is remembered that groups 5 and 6 received equal amounts of growth hormone, the only difference being the phosphorus content of the diet.

The nitrogen content of the moist muscles of group 7 was significantly less than all others except group 2. Group 2 contained less nitrogen than group 1. All the others were well within the experimental error.

When the nitrogen content is calculated as per cent of dry weight, the various groups show a more complex relationship. Group 5 contained significantly more nitrogen than all other groups, in-

§ Supplied by Mead Johnson and Company.

[1] Fisher, R. A., *Statistical Methods for Research Workers*, Second Edition, Oliver and Boyd, Edinburgh, 1928.

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.

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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.