was isolated as the crystalline brucin salt and had the properties of glucose-1-phosphoric acid; when added to frog muscle extract it was converted in a few minutes to the Embden ester.

8760 P

Effect of Excessive Dietary Sodium Chloride upon Liver and Muscle Glycogen in the Rat.

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Chaikelis,[1] in 1934, stated that the inverse ratio between blood chlorides and blood sugar was not a compensatory mechanism concerned with the maintenance of proper osmotic pressure relations. He suggested that the blood chloride change was associated with some phase of carbohydrate metabolism. Following his report an investigation was started in this laboratory to determine what effect variations in the amount of sodium chloride in the diet would have upon the deposition of glycogen in the liver and muscles of the white rat.

Methods. Series 1. Young male rats were divided into 18 groups consisting of three animals each, the majority of which did not vary in weight by more than 10 gm. One animal of each group was placed on a diet poor in chlorides, one was placed on a diet rich in chlorides, and the third received a control diet. The control diet consisted of casein 33 percent, agar agar 2 percent, Osborne and Mendel[2] salt mixture 4 percent, cod liver oil 2 percent, butter (unsalted) 7 percent, yeast 8 percent, and sucrose 44 percent. The chloride poor diet was of the same composition as the control diet except that the Osborne and Mendel "chloride free" salt mixture[2] was substituted for the complete salt mixture which was used in the control diet. The chloride rich diet consisted of a food mixture of the same composition as the chloride poor diet with the addition of NaCl to a level of 6.25 percent of the total diet. Distilled water was given *ad libitum.*

The animals were kept in individual cages over wire screens. The

1 Chaikelis, A. S., *J. Biol. Chem.*, 1934, **105**, 767.

2 Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1918, **34**, 131.

temperature of the room was controlled so that it did not vary from 75°F. by more than 4 degrees.

The diets described above were fed for 10 to 15 days, and the experimental period was terminated by a 24-hour fast, at the end of which time the animals were anesthetized with sodium amytal. The gastrocnemius muscle was exposed and frozen *in situ* by means of a mixture of solid CO_2 and ether, and the frozen muscle was removed and immersed in this mixture. The liver was then removed and frozen immediately in the same manner. The tissues were weighed in the frozen state and analyzed for their glycogen content by the method of Good, Kramer and Somogyi.[3] The glycogen hydrolysates were analyzed for their glucose content by the method of Shaffer and Somogyi.[4]

Series 2. Using the same technic, a second series of experiments was carried out upon 20 groups of three animals each. The liver glycogen only was determined in this series. The animals were maintained upon the experimental diets for 22 to 30 days. The diets were identical with those used in Series 1 with the exception that the chloride rich diet contained 9.09 percent NaCl.

TABLE I.
The Effect of Variations in Dietary NaCl upon Liver and Muscle Glycogen.

Diet	Series 1* Liver glycogen (gm./100 gm.)	Series 1* Muscle glycogen (gm./100 gm.)	Series 2† Liver glycogen (gm./100 gm.)
Control diet	0.18 ± .01	0.29 ± .005	0.30 ± .03
Chloride poor diet	0.19 ± .02	0.32 ± .007	0.37 ± .04
Chloride rich diet	0.31 ± .03	0.31 ± .010	0.75 ± .03

*Series 1. The chloride rich diet contained 6.25% NaCl.
†Series 2. The chloride rich diet contained 9.09% NaCl.

Results. The results of these experiments are given in Table I. In order to conserve space we are not reporting the glycogen values of individual animals at this time; therefore, only the mean values and the probable error of each mean are given. In both series of experiments the rats which received a diet rich in sodium chloride showed a large increase in liver glycogen. The chloride poor diet did not have any significant effect upon the liver glycogen stores. The results obtained from the muscle glycogen determinations showed that the mean values obtained from the groups on the chloride poor and chloride rich diets did not vary strikingly from the mean value obtained for those on the control diet. This, however, is being reinvestigated at the present time.

3 Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 485.
4 Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 695.

We have made no studies to ascertain the mechanism by which excessive amounts of sodium chloride in the diet cause an increase in liver glycogen stores, but we plan to do so in the future. A study is now in progress to determine whether the sodium ion, the chloride ion or a combination of the two is responsible for the results obtained.

These results are of considerable interest in connection with the observation of McQuarrie and coworkers[5, 6] that when large amounts of sodium chloride were added to the diet of diabetic children the fasting blood sugar was lowered and glycosuria was decreased.

8761 P

Volume Changes of Smooth Muscles of Phascolosoma During Activity.

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Meyerhof's method[1] of measuring the volume changes of amphibian muscles was applied to the smooth muscles of Phascolosoma, a marine worm, belonging to the Sipunculidea. Using the retractor muscle in the volume chamber, after single or tetanic stimulation, a temporary change in volume was observed, starting almost simultaneously with the isometric mechanogram. However, only in a few of the experiments was the course of the volume curves more or less parallel to the tension curves. Often the volume curves showed 2 phases; sometimes even 3 were present. The direction of the volume changes varied greatly from experiment to experiment; but in general there was a tendency towards volume increase. The maximum change occurring in the course of the volume curves was, on the average, of the order of magnitude of 0,000 005 of the total muscle volume. The dependence of direction of the volume change on the initial length of the muscles could not be demonstrated so clearly for the Phascolosoma retractor as for amphibian muscle

[5] Thompson, W. H., and McQuarrie, I., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 907.

[6] McQuarrie, I., Thompson, W. H., and Anderson, J. A., *J. Nutrition*, 1936, **11**, 77.

[1] Meyerhof, O., u. Hartmann, H., *Pflüg. Arch. f. d. ges. Physiol.*, 1934, **234**, 722; Meyerhof, O., u. Möhle, O., *Biochem. Ztschr.*, 1936, **284**, 1.