maxillary glands in some animals. The virus appears to be quite benign, because there were no accompanying infiltrative or degenerative changes in the kidneys, and, in the submaxillary glands, such alterations were more often absent than present. Measurements of the inclusions reveal considerable uniformity in size which is thought[11, 12] to indicate a static rather than an active process. Whether this virus in wild rats is capable of infecting humans is an open question although inapparent salivary gland viruses are remarkably species specific. It has been shown, however, that a virus ordinarily restricted to the salivary glands under certain conditions may be induced to extend widely through the body.[13]

8759 P

Mechanism of Formation of Hexosemonophosphate in Muscle and Isolation of a New Phosphate Ester.

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Experiments performed on intact frog muscle indicated that hexosemonophosphate, in contrast to hexosediphosphate, is formed by esterification with inorganic phosphate.[1] A further study of this problem was carried out on minced frog muscle which was almost completely inactivated (in regard to lactic acid formation) by 3 to 4 extractions with distilled water. When such muscle, which contains only 2 to 4 mg. % of organic, acid-soluble P, is incubated anaerobically for 3 hours in isotonic phosphate buffer, the organic P content rises to 8 to 13 mg. % due to the formation of hexosemonophosphate. Addition of small amounts of adenylpyrophosphoric or of adenylic acid greatly enhances the formation of hexosemonophosphate, as shown in Table I . The experiments indicate that hexosemonophosphate is formed from inorganic phosphate and that adenylic acid serves as the mediator of this reaction.

Observations after short periods of incubation showed that the first phosphorylation product is not hexose-6-phosphoric acid

11 Rector, E. J. and L. E., *Am. J. Path.*, 1934, **10**, 629.

12 Cowdry, E. V., and Scott, G. H., *Am. J. Path.*, 1935, **11**, 647.

13 McCordock, H. A., and Smith, M. G., *J. Exp. Med.*, 1936, **63**, 303.

1 Cori, G. T., and Cori, C. F., Summaries of Communications, XVth International Physiological Congress, p. 66, 1935.

TABLE I.

Effect of Coenzyme on Hexosemonophosphate Formation in Inactivated Muscle.

Minced muscle was extracted 3 times with 20 times its weight of distilled water and was incubated anaerobically in isotonic phosphate buffer (pH 7.2) at 20° for 3 hours.

Values in mg. per 100 gm. muscle.

Hexosemonophosphate				
hexose	P found	P calc.	Lactic acid	Additions (per 100 gm. muscle)
15		3	6	Incubated in isotonic KCl
68	10	12	8	
162	32	28	30	6 mg. adenylpyrophosphate P
75	11	13	10	
141	36	24	25	6 mg. adenylpyrophosphate P
68	13	12	10	
264	59	46	26	12 mg. adenylic acid P
48	8	8	10	
119	28	21	12	4 " " " "

(Embden ester), but a new ester which is slowly converted to the 6-phosphoric acid under the conditions of these experiments. (Table II.) After one and 2 hours of incubation much more organic P is present than can be accounted for on the basis of reducing power, assuming the latter to be due to hexose-6-monophosphate. Hydrolysis in N H_2SO_4 at 100° for 10 minutes revealed the presence of a compound which (unlike the 6-monophosphoric acid) is easily hydrolyzed in acid and which yields equivalent amounts of fermentable sugar and inorganic phosphate.

The new compound was isolated from 4 different preparations. About 100 gm. of minced and washed muscle, to which 120 mg. of

TABLE II.

Effect of Length of Time of Incubation on Phosphorylation.

Values in mg. per 100 gm. muscle.

Time hr.	Hexosemonophosphate fraction			Additions (per 100 gm. muscle)
	hexose	P found	P calc.	
0	5		0.9	
1	79	62	14	13 mg. adenylic acid P
2	131	69	23	" " " " "
3	252	67	43	" " " " "

	After 10 min. hydrolysis in N H_2SO_4.		
		Fermentable Sugar	
	Inorg. P	found	calc.
1	39	219	226
2	45	256	261
3	20	122	116

adenylic acid had been added, was incubated for one hour in phosphate buffer. After deproteinization with $HgCl_2$, the water-soluble barium salts were precipitated with alcohol. The soluble barium salts consisted of about 65% of the new compound, the remainder being the adenylic acid added and the small amount of hexose-6-phosphoric acid formed. Fractionation of the barium salts was not successful and they were therefore converted to the brucin salts. After removal of most of the adenylic acid by concentrating the aqueous solution of the brucin salts to a small volume, the remainder of the brucin salts was evaporated to dryness. The semi-crystalline residue was extracted with hot methyl alcohol containing 1% ethyl alcohol. On cooling the ester crystallized out in the form of fine needles which soon formed large aggregates. After recrystallization from methyl alcohol, the brucin salt was reconverted to the barium salt and purified further by repeated solutions in water and precipitations with alcohol. The final product contained 1.5% of an ester difficult to hydrolyze in acid as judged by the ratio of easily hydrolyzable to total organic P.

Elementary analyses for C, H, P, and Ba and determination of the sugar formed after hydrolysis (by means of hypoiodite) agreed with a compound of the composition of $C_6H_{11}O_5.PO_4Ba.3H_2O$. $[\alpha]_D^{25} = 75.5°$ (calc. for the anhydrous barium salt in water; conc. 1.26%). During hydrolysis in 0.01 N HCl at 70°, sugar and inorganic phosphate were liberated at the same rate, the percentages being 21.1, 38.3, 62.0 and 76.3 for the 30, 60, 120, and 180 minute periods, respectively. The percentages calculated for a velocity constant of 3.47×10^{-3} were 21.4, 38.1, 61.9, and 76.2, respectively.

The ester (when unhydrolyzed) does not reduce alkaline copper solution and does not react with hypoiodite, and it can be heated to 130° without discoloration, all factors which point to the absence of a free reducing group. We believe that the new ester is an aldose-1-phosphoric acid, the sugar being presumably glucose.

When the new ester is added to frog muscle extract, it is converted in a few minutes to an ester difficult to hydrolyze in acid and possessing reducing power, presumably hexose-6-phosphoric acid. The same change occurs in muscle extract inactivated by dialysis, so that the presence of the coenzyme system does not appear to be necessary for the wandering of the phosphate group.

Summary. In minced and washed frog muscle incubated in phosphate buffer, added adenylic acid transfers inorganic phosphate to carbohydrate resulting in the formation of hexosemonophosphate. The first phosphorylation product proved to be a new ester which

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.