The first very important work of SubbaRow with Fiske was to develop a rapid but accurate method for the determination of phosphorus as phosphate in biological samples. It paved the way for establishing the presence of several other biological phosphorus compounds. They discovered phosphocreatine followed quickly by ATP, glycerophosphate and others. Here we have reproduced the paper wherein they have described their work on muscle ATP, a significant contribution to DNA structure, and liver glycerophosphate.

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Phosphorus Compounds of Muscle and Liver

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I. MUSCLE

Embden and Schmidt[1] have recently made the highly interesting discovery that the adenosine phosphoric acid isolated about two years ago from voluntary muscle[2] is not identical with that obtained from yeast nucleic acid. Among the chemical properties by which the two may be distinguished the difference in resistance to hydrolysis by acid is particularly striking. The muscle nucleotide ("myoadenylic acid") is hydrolyzed (by 0.1 N sulphuric acid at 100°) only about one fifth as rapidly, as measured by the rate at which o-phosphoric acid is split off.[1]

That adenine (in nucleotide combination) is the source of the ammonia formed in muscle during contraction has been amply demonstrated by Embden and his collaborators. The physiological significance of this important work is presumably quite unaffected by the fact, which we now have to report, that myoadenylic acid is not a normal constituent of

1 G. Embden and G. Schmidt, *Z.physiol. Chem.,* 181: 130, 1929.

2 G. Embden and M. Zimmerman, *Z. physiol. Chem.,* 167: 137, 1927.

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muscle (except perhaps in traces), but a decomposition product. Our first intimation that this might be the case developed from observation that when a protein free muscle filtrate is treated with an alkaline solution of calcium chloride[3] a large part of the purine nitrogen comes down in the precipitate. The calcium salt of myoadenylic acid is soluble in water, and should consequently – if present – remain dissolved under these conditions.

The purine derivative precipitated by calcium has been isolated by (1) precipitation with mercuric acetate in the presence of 2 per cent. acetic acid, followed (after removal of the mercury) by (2) precipitation with calcium chloride and alcohol from hydrochloric acid solution, which yields an acid calcium salt. By repeating the entire process the acid calcium salt is finally obtained as a microcrystalline precipitate. The yield is not far from quantitative, and accounts not only for most of the purine nitrogen of muscle, but also for most of the acid soluble phosphorus not present as o-phosphoric acid, phosphocreatine or hexose monophosphate. In the case of cat muscle the yield of purified material may be the equivalent of nearly 50 mg of phosphorus per 100 gm of muscle.

The acid calcium salt is not well suited for analytical purposes, owing to the difficulty of removing all the water. It may, however, be converted to a silver salt – by precipitation with silver nitrate from nitric acid solution – and the composition of this product has been found to be $C_{10}H_{13}O_{13}N_5P_3Ag_3$. It contains, in addition to adenine and carbohydrate, three molecules of phosphoric acid, or two more than in adenylic acid. Two of the three molecules of phosphoric acid are readily removed by hydrolysis with acid, and this fact is doubtless sufficient to explain why Embden and Zimmerman[2] obtained a nucleotide (myoadenylic acid) which still retains the one resistant phosphoric acid group.

The new substance includes also the phosphorus which Lohmann[4] believes to be present in the muscle in the form of pyrophosphate, but whether or not it is an ester of pyrophosphoric acid remains to be determined.

II. LIVER

The greater part of the organic acid soluble phosphorus of liver may be precipitated from the protein free filtrate, after removing the inorganic phosphate with alkaline calcium

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[3] This is the frist step in the isolation of phosphocreatine (C. H. Fiske and Y. Subbarow, *J. Biol. Chem.,* 81: 629, 1929) where it serves the purpose of removing inorganic phosphate and other products.

[4] K. Lohmann, *Biochem. Z.,* 202: 466, 1928; 203: 164, 172, 1928.

chloride solution, by the addition of alcohol. Purification by dissolving in water and reprecipitating with alcohol finally yields a calcium salt, crystallizing in spherulites or aggregates of short needles, and having the composition $C_3H_7O_6PCa.1½H_2O$). It is the calcium salt of glycerophosphoric acid, which in spite of text-book statements has not – as far as we have been able to determine – been isolated from animal material before, at least under conditions which preclude its formation from lecithin and related substances.

Experiments are now under way which it is hoped will head to some procedure for the quantitative estimate of free glycerophosphate in tissue filtrates. From present indications this substance appears to account for at least one third of the total acid soluble phosphorus of the liver.

The properties of the above-mentioned calcium salt, together with the fact that it gives an intense greenish blue color on applying the Denigès codeine test after oxidation with bromine,[5] and forms no insoluble double salt with barium nitrate,[6] identify it as α-glycerophosphate. This is of particular interest since all preparations of lecithin so far examined by means of these tests have been found to contain mainly the β form of glycerophosphoric acid.[7]

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5 O. Bailly, *Ann. chim.*, 6: 96, 1916.

6 P. Karrer and H. Salomon, *Helv. chim. acta*, 9: 1, 1926.

7 O. Bailly, *Ann. chim.,* 6: 215, 1916; P. Karrer and P. Benz, *Helv. chim. acta,* 10: 87, 1927.