

ON THE OCCURRENCE OF A PHYTIN-SPLITTING ENZYME IN ANIMAL TISSUES.

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The salts of anhydro-oxy-methylene-diphosphoric acid, or phytin, have been shown by several investigators¹ to be widely distributed in plants. Hart and Andrews² have shown that inorganic phosphorus does not occur in vegetable feeding-stuffs in appreciable quantities. Wheat bran, which contains $1\frac{1}{2}$ per cent of phosphorus, was found to be essentially free from inorganic phosphates, the whole of the phosphorus being present as salts of phytic acid. This discovery led to a number of investigations by biological chemists in order to find what rôle this new organic phosphorus body plays in metabolism.

Gilbert³ and his collaborators and later Mendel and Underhill⁴ have shown that the salts of phytic acid are not toxic when administered *per os* and that very large quantities are necessary when administered intravenously, intraperitoneally or subcutaneously to produce disturbing symptoms. The latter observers found the free acid much more toxic. Scofone⁵ observed that the phytin ingested is excreted largely as inorganic phosphoric acid, and this result has been confirmed by Giascosa⁶ and by

¹ Posternak: *Compt. rend de la soc. de biol.*, lv, p. 1190, 1903; Patten and Hart: *Amer. Chem. Journ.*, xxxi, p. 564, 1904; Schulze and Costoro: *Zeitschr. f. physiol. Chem.*, xli, p. 476, 1904.

² Hart and Andrews: *Amer. Chem. Journ.*, xxx, p. 470, 1903.

³ Gilbert and Posternak: *La médication phosphorée*, Paris, 1903; Gilbert and Lippmann: *La presse médicale*, Septembre, 1904; G. Sécheret: thèse de Paris, 1904.

⁴ Mendel and Underhill: *Amer. Journ. of Physiol.*, xvii, p. 75, 1906.

⁵ Scofone: Abstract in *Biochem. Centralblatt*, iii, p. 606, 1905.

⁶ Giascosa: Abstract in *Biochem. Centralblatt*, iv, p. 572, 1905.

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Jordan, Hart and Patten,¹ who found no evidence of phytin in the urine of cows after the ingestion of very considerable amounts. Sécheret² stated that the enzymes of the digestive tract do not alter phytin and suggested that it is probably decomposed in the intestinal epithelium. According to Jordan, Hart and Patten³ pepsin and trypsin are without action on phytin. Further than this the fate of this substance in the body has not been investigated.

Recently Suzuki⁴ has reported the existence of a phytin-splitting enzyme in rice bran.

With a view to finding where phytin is decomposed in the body we have examined several tissues for the presence of a phytase. The method employed was to digest the sodium salt of phytic acid, which was prepared by extracting wheat bran according to the method of Patten and Hart,⁵ with an aqueous or glycerine extract of the tissue under examination at 38° to 40° C. Both toluol and chloroform were added to keep down bacterial growth. After one or two days the mixture was heated, after dilution when necessary, and the proteids removed by coagulation with the addition of the smallest possible quantity of acetic acid. The phosphoric acid was then determined in the filtrate by the method of Hart and Andrews⁶ by precipitating, in the presence of a small quantity of nitric acid, with neutral ammonium molybdate solution. In all cases blank determinations were made on the tissue extract alone and on a solution of sodium phytate which were digested in a similar manner.

EXPERIMENTAL.

Fresh calves liver was ground as finely as possible and treated with toluol water. After twenty-four hours it was filtered and the slightly bloody solution used for the digestion. The phytic acid was dissolved in water and neutralized by titrating with

¹ Jordan, Hart and Patten: *Amer. Journ. of Physiol.*, xvi, p. 268, 1906.

² Sécheret: *Loc. cit.*

³ Jordan, Hart and Patten: *Loc. cit.*

⁴ Suzuki: *Bull. of the Coll. of Agric., Tokyo Imper. Univ.* vii, p. 503, 1907.

⁵ Patten and Hart: *Loc. cit.*

⁶ Hart and Andrews: *Loc. cit.*

sodium hydroxide using phenolphthalein as indicator. This solution was added to the tissue extract and, after the addition of the antiseptic, it was incubated at 40° C. and examined as above described.

The following table gives the results obtained by digesting sodium phytate with water extract of liver:

TABLE I.

No. of Exp.	15 cc. of 1 per cent sodium phytate + 200 cc. liver ext.	200 cc. liver extract alone.	15 cc. of 1 per cent sodium phytate solution alone.	Difference.
	<i>gram P₂O₅.</i>	<i>gram P₂O₅.</i>	<i>gram P₂O₅.</i>	<i>gram P₂O₅.</i>
1.....	0.0980	0.0504	0.0070	+0.0406
2.....	0.1225	0.0644	0.0101	+0.0480
3.....	0.1365	0.0553	0.0123	+0.0197
4.....	0.0644	0.0336	0.0087	+0.0221

Table II gives the results obtained in two experiments in which sodium phytate solution was incubated with a glycerine extract of liver:

TABLE II.

No. of Exp.	15 cc. 1 per cent sodium phytate solution + 50 cc. glycerine ext. of liver.	50 cc. glycerine ext. of liver alone.	15 cc. 1 per cent sodium phytate alone.	Difference.
	<i>gram P₂O₅.</i>	<i>gram P₂O₅.</i>	<i>gram P₂O₅.</i>	<i>gram P₂O₅.</i>
1.....	0.0584	0.0367	0.0140	+0.0077
2.....	0.0672	0.0367	0.0140	+0.0165

Table III gives the results obtained in three sets of experiments in which calves' blood was incubated at 40° C. with a 1 per cent solution of sodium phytate.

Experiments showed that small amounts of phosphorus could be added to the tissue extracts and recovered by the method of Patten and Hart after removal of the proteids by heating in solution faintly acidified with acetic acid.

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TABLE III.

No. of Exp.	15 cc. 1 per cent Na phytate sol. + 50 cc. blood diluted to 200 cc.	50 cc. blood alone diluted to 200 cc.	15 cc. 1 per cent sodium phytate solution diluted to 200 cc.	Difference.
1.....	<i>gram P₂O₅</i> 0.0423	} 0.0150	<i>gram P₂O₅</i> 0.0087	{ +0.0186
	0.0364			
2.....	0.0364	} 0.0119	0.0087	{ +0.0158
	0.0322			
3.....	0.0315	} 0.098	0.0084	{ +0.0133
	0.0217			

The above results indicate, we believe, that the liver and blood have the property of cleaving the salts of phytic acid with the production of inorganic phosphoric acid. The wide distribution of inosite in the tissues renders it impossible for us to say from experiments yet made whether this is also produced in this cleavage. These results are in accord with those of Mendel and Underhill,¹ who showed that the intestine is not necessarily involved in the excretion of the metabolic products of phytin in certain animals, and also with the conclusions of Scofone, that the enzymes of the digestive tract do not alter phytin. We have examined ptyalin, pepsin and trypsin and have confirmed Scofone's results.

Experiments made with extracts of muscle and kidney did not give results which pointed toward the presence of a phytase in these tissues.

¹ Mendel and Underhill: *Loc. cit.*

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