80. *Purified Diastases.*

I. Purification.

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Through the work of Willstätter, Euler, Pringsheim, Northrop, Sumner and others, it has been concluded that diastase, like protease and other enzymes, is protein in nature and that its molecular weight lies between 20000–450000. In spite of the fact that these contentions are now generally accepted, the present author found that diastase must be regarded as a typical saccharide, not a protein, though the component agricone which contains nitrogen gives biuret reaction, the agricone being tripeptide of small molecular weight.

The author's conclusions, so fundamentally opposed to the current idea on the subject, are based on his extensive investigation on purified diastase, which he recently succeeded in obtaining by means of his newly devised method, and it is the purpose of this series of papers to give brief descriptions of the entire data.

Method of Purification.

The author succeeded in purifying diastase not only from mould diastase (Takadiastase) but also from malt diastase (Japanese Pharmacopoeia), bacterial diastase (Miyalase), etc. The method of purification, which applies to any of these starting materials, may be briefly described as follows:—

The clear water solution of diastase, previously filtered through paper was acidified with acetic acid up to 0.01–0.1%.

When lead acetate was added in excess to this solution, an yellowish white flocculent precipitate was formed, including a great deal of impurity, leaving diastase in solution.

The filtrate therefrom gave, when saturated with ammonium sulphate, a precipitate retaining chiefly diastase, which may be further purified by dissolving in water, removing lead sulphate and saturating again with ammonium sulphate.

This precipitate was now collected on a filter by suction and after washing with saturated solution of the same salt, it was dissolved in water and treated with barium hydroxide, adding a small quantity of ammonium carbonate so as to avoid the destruction by excess barium hydroxide.

On filtering the alkaline solution, the filtrate exhibited no diastatic power upon starch, but by precipitating the solution with acetone and alcohol, an active diastase was obtained.

The precipitate was now collected on a filter by suction, washed with the same solvents to remove the adhering ammonia, washed again with ether and dried in a vacuum desiccator.

The white powder thus obtained, was dissolved in water, the insoluble residue removed by filtration and precipitated again with the organic solvents.

The precipitate was further washed with ether and dried in a vacuum desiccator.

The purified diastase thus prepared, exhibited a strong amylolytic and saccharifying powers upon starch.

The method of purification of diastase above mentioned, can be schematically expressed as follows :

Crude diastase [Active]	
Dissolved in water, filtered through paper and acetic acid added	
Clear acidic solution	
Lead acetate added in excess and insoluble residue removed	
Filtrate [Active]	
Salted out by saturation with ammonium sulphate	
Precipitate [Active]	
Dissolved in water and lead sulphate removed	
Solution	
Precipitated again by ammonium sulphate and washed with the solution of the same salt	
Precipitate [Active]	
Water solution	
Barium hydroxide added and barium sulphate removed	
Filtrate [Inactive]	
Treated with acetone and alcohol	
Precipitate [Active]	
Washed with same solvents and ether.	
Dried in vacuum.	
Dissolved in water and the insoluble residue removed	
Filtrate	
Treated with acetone and alcohol	
Precipitate	
Dried in vacuum and extracted with 40% alcohol	
40% alcohol extract	
Treated again with the same organic solvents	
Precipitate	
Water solution	
Dialysed through animal membrane and treated with alcohol	
Precipitate	
Washed with ether and dried in a vacuum desiccator	
Purified diastase [Active]	

Chemical and Physical Properties of Purified Diastase.

While the purified mould diastase and the malt diastase were in the form of white amorphous powder, the bacterial diastase alone was brownishly coloured. But, all the three diastases were dissolved quickly in the cold water in the same manner. This water solution exhibited a strong diastatic power as above mentioned, but hardly the power of

other enzymes, i. e. pectase, pectinase, invertase, protease, lipase, etc. Moreover, the solution did not give the reaction of peroxidase, which was one of the hardest admixture to get rid of.

On adding inorganic or organic acids beyond the destruction point, diastase was destroyed and precipitated out of the solution. By heating beyond the destruction temperature, diastase was also destroyed and precipitated out with the aid of acids.

Diastase was also destroyed in alkaline solution such as sodium hydroxide, potassium hydroxide, calcium hydroxide, barium hydroxide, etc., except ammonia in which diastase was only inactivated within the limits of the destruction point. The destroyed diastase remained dissolved in the solution, but when the solution was neutralized and further acidified even weakly with organic or inorganic acids, it was precipitated out of the solution in a quite different manner before destruction.

Diastase was neither inactivated in nor precipitated from the water solution acidified with acetic acid, with lead acetate, copper sulphate, aluminium chloride, etc., though a great deal of impurities was precipitated. It was, however, completely precipitated with any of these salts when ammonia was added in excess. The insufficient acknowledgement of the inhibition of diastase with metallic salts and the recoverisation from the inhibition must be revised.

The purified diastases contained only a little inorganic impurities altogether and gave 0.5–0.7% ash when burned in crucibles. Nevertheless, no reactions of phosphorous and sulphur were given.

The pure preparation did not reduce Fehling's solution, the reduction which was always given intensively by crude samples. From this, it was obvious that the purified diastase had no free sugar group and the substances which gave the reduction above mentioned were all impurities. On boiling with 1–2% sulphuric acid or hydrochloric acid for 1–2 hours, the purified diastase gave strong reductions of Fehling's solution, showing the true saccharides nature.

Both mould diastase and malt diastase equally gave the typical pentose reactions, i. e., Bial's reaction, etc., and for these were proposed by the author the name of "Pentosic diastase".

Bacterial diastase and others, however, gave no pentose reaction and hence were given the name of "Hexosic diastase" by the author.

It is most important to note that the construction of diastase is not protein but saccharide in nature and that the pentose as well as hexose contents amounted to 33–38%.

While mould diastase and malt diastase gave typical biuret reaction, it was not clearly detected in the case of the bacterial diastase. All three gave the typical ninhydrin reaction and the similar murexide reaction, but not Nestler's reaction.