## CHEMICAL NATURE OF ENZYMES.

	Potas	sium antimonyl tartrate. Grams.	Potassium chloride. Grams.	Atomic weight of antimony.
I	• • • • • • • • • • • • •	1.19481	0.27539	120.345
2	• • • • • • • • • • • • •	1.57004	0.36186	120.359
3	••••	2.00912	0.46307	120.351
4	• • • • • • • • • • • •	2.04253	0.47073	120.379
5	••••	2.16646	0.49935	120.341
6	• • • • • • • • • • • •	2.25558	0.51982	1 20, 385
7	· · • · · · · • · · · •	2.61255	0.60215	120.350
8	•••••	2.95272	0.68064	120.311
		Mean = 120.353		
Minimum = 120.311				

Difference 0.074

The barium and silver antimonyl tartrates crystallize well and the hope was entertained that these salts might also be included in the circle of experimentation but thus far the results with them have not been satisfactory.

UNIVERSITY OF PENNSYLVANIA.

## THE CHEMICAL NATURE OF ENZYMES.

BY P. A. LEVENE. Received May 18, 1901.

There is comparatively little known about the chemical nature of the enzymes. In fact it is only in recent years that some attention has been given to these substances. Even the supposition that enzymes are of a proteid nature is not based on irrefutable evidence. Nevertheless, this is generally accepted. Very recently Hans Friedenthal claims that the enzymes possess the nature of nucleo-proteids.

It is chiefly in view of this last research that I have published some of the results of the investigation on the chemical nature of enzymes. The object of this work is to determine whether enzymes are actually of proteid nature. It was established during the last few years by the researches of Morochowetz, Lawrom, and Kutcher that proteids can be digested by means of trypsin to such an extent that the product no longer gives the biuret test, in other words the entire proteid material is decomposed. It was also demonstrated by Gulewitch that trypsin does not act on nitrogenous substances of non-proteid nature. Hence it seemed possible to test the proteid nature of enzymes by subjecting them to tryptic digestion.

The statement has, however, been made that some enzymes are destroyed even by a comparatively short digestion with trypsin, but this statement has also been contradicted. It therefore appeared advisable to repeat these experiments.

Popow has demonstrated that trypsin decomposes nucleoproteids, splitting off their phosphorus as phosphoric acid, and it seemed therefore possible to ascertain by means of tryptic digestion, whether enzymes were of the same nature as nucleoproteids.

I performed my first experiment in September, 1899. Several pounds of fresh pancreas glands were chopped, treated with 0.5 per cent. solution of sodium carbonate and a large quantity of chloroform. The mixture was allowed to stand over night. It was then strained through gauze and the liquid divided into several flasks, more chloroform added, and then placed in the thermostat at 40° C. The contents of the flasks were well shaken every day. After a couple of weeks of digestion the contents of the flasks were filtered, the filtrate transferred into acid bottles, a considerable quantity of chloroform added and placed in a very warm room. The bottles remained there until May, 1900.

The solution which was very dark in color, was then decolorized by means of animal charcoal, and tested for biuret and gave a negative result.

Another part of the same decolorized liquid was treated with a great excess of alcohol and the whole precipitate thus obtained tested for biuret without result.

From the above negative tests it was assumed that all the proteids of the original extract were decomposed. In order to test how far the decomposition of the nucleic acid went, a determination of the phosphorus in the form of organic and inorganic compounds was made.

In 25 cc. of the solution the phosphoric acid was precipitated by means of magnesia mixture. The precipitate slightly colored was redissolved with hydrochloric acid and reprecipitated with ammonia. The magnesium pyrophosphate weighed 0.257 gram.

Another 25 cc. of the same liquid was evaporated to dryness, and the residue fused with sodium carbonate and potassium nitrate. The phosphorus was estimated in the usual way. The magnesium pyrophosphate weighed 0.249 gram.

This experiment demonstrated that trypsin is able to decom-

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pose absolutely the nucleo-compounds of the pancreas, as well as the proteids. This solution of the self-digested pancreas extract, however, has no proteolytic activity.

It was then attempted to subject trypsin to self-digestion for a shorter period, so as either to break up all the nucleins and leave some proteid material intact or vice versa.

Grubler's trypsin was used for these experiments. About 3 grams of the substance were treated with 150 cc. of 0.5 per cent. solution of sodium carbonate and allowed to stand six weeks. At the end of that time the mixture was filtered. The filtrate gave a positive though very weak biuret test, and possessed tryptic activity. 50 cc. of the solution were used for the estimation of the total phosphorus, another 50 cc. for the estimation of phosphorus in the form of phosphoric acid. The total phosphorus weighed 0.00125 gram and the phosphorus as phosphate weighed 0.0012, thus showing the absence of nucleo compounds in the solution, and still the solution contained the proteolytic enzyme.

In experiment No. 2, 30 grams of trypsin (Fairchild) were treated with 50 cc. of 0.5 per cent. solution of sodium carbonate (a great excess of chloroform added as an antiseptic), and allowed to stand in an incubator.

After three weeks of self-digestion part of the mixture was filtered and tested for proteolytic activity. The result was positive. In 25 cc. of the filtrate, the total phosphorus was estimated, and it weighed 0.0162 gram. In another 25 cc. portion the phosphorus is estimated as phosphate; it weighed 0.0131 gram, thus showing the presence of traces of nucleo-compounds.

After four weeks of self-digestion another experiment was made similar to the former. The solution still possessed its proteolytic properties. 10 cc. of it contained 0.00715 gram phosphorus. Another 10 cc. of the same contained 0.00628 gram phosphorus as phosphate, again showing the presence of a slight amount of nucleo-compounds.

The experiment was again repeated after six weeks of selfdigestion. The solution was still active and gave the test for biuret. 10 cc. of the solution contained 0.00715 gram total phosphorus. 10 cc. of the same contained 0.00663 gram phosphorus as mineral phosphates, thus containing scarcely any nucleo-compounds and still possessing proteolytic activity. These experiments would scarcely justify the conclusions of Freslenthal that trypsin is a nucleo-compound.

The fact that only those solutions were active which gave a positive biuret test would seem to indicate that trypsin is of a proteid nature. However, in some cases the biuret test was scarcely perceptible, and yet the solution of the self-digested trypsin still contained the active ferment.

Experiments on other enzymes in the same direction are now in progress.

I wish to express my indebtedness to Doctor D. Sculley for the assistance received from him.

## CONTRIBUTIONS TO THE KNOWLEDGE OF REVERSIBLE REACTIONS.

BY W. N. STULL.

Received June 17, 1901.

THE object of the study outlined in the following pages was primarily to investigate the positions of the points of equilibrium when acid solutions of certain metals were treated with hydrogen sulphide, and to determine the influence of agitation and temperature upon these points. Incidentally to this main purpose, it was thought desirable to ascertain under what conditions the separation of certain metals, such as zinc and cadmium, could most accurately be effected by hydrogen sulphide. The author has been unable to find any account of accurate determinations upon this latter point, though the common method of separating zinc and cadmium by hydrogen sulphide is practiced in almost all laboratories.

At the beginning it was found that the attainment of equilibrium with acid solutions of zinc and cadmium presented serious difficulties, since the reactions are exceedingly slow, and, in fact, in no case was complete equilibrium reached even after the solution had been treated with a rapid stream of hydrogen sulphide for many hours. As the work advanced, therefore, the importance of the speeds of the reactions became more and more apparent, and as a result this factor became the chief object of study, and incidental thereto, the effects of temperature and agitation.

Since the author must discontinue the work for a time to devote himself to other duties, it is deemed best to publish the results, incomplete as they are, on account of their bearing upon practi-

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