phosphate which is regularly used in this laboratory in determining the activity of pancreatic amylase, and which gives favorable conditions for both its amyloclastic and saccharogenic action, seemed, in the case of takadiastase, to activate amyloclastic and at the same time to retard saccharogenic action.

It is evident that observations upon the iodine color reaction of digestion mixtures as an indication of the amyloclastic power require very different interpretations when dealing with different amylases.

We are greatly indebted to the Carnegie Institution of Washington for grants in aid of this investigation.

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[CONTRIBUTIONS FROM THE CHEMICAL LABORATORIES OF COLUMBIA UNIVERSITY, No. 226.]

STUDIES ON AMYLASES. VII. THE FORMS OF NITROGEN IN AMYLASE PREPARATIONS FROM THE PANCREAS AND FROM MALT, AS SHOWN BY THE VAN SLYKE METHOD.

BY H. C. SHERMAN AND A. O. GETTLER.

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Previous papers from this laboratory[1] have described the purification and some of the properties of amylase preparations from the pancreas and from barley malt, which preparations, while not chemically pure, are believed to represent high concentrations of the respective enzymes.

In the case of pancreatic amylase, the product obtained in many independent preparations is so uniform in diastatic power, as well as in other properties, as to justify the belief that we are here dealing with material which, while not absolutely free from impurities, is essentially a definit substance; and since the substance is able to digest at least one million times its weight of starch it seems not unreasonable to consider these preparations as essentially composed of pancreatic amylase.

The attempts to purify malt amylase have given products which are less uniform in composition and much less uniform in activity than the preparations from the pancreas, but which show considerably higher diastatic power than has previously been described for any amylase preparations, except the pancreatic amylase just mentioned.

Our amylase preparations show the nitrogen content and color reactions of protein substances, but the yields obtained are so small that it would be quite impracticable to prepare sufficient quantities for complete analysis of the hydrolytic products. We have therefore sought to gain a further insight into the chemical nature of these substances by determining the principal forms of nitrogen present according to the method of Van Slyke.

[1] THIS JOURNAL, 33, 1195; 34, 1104; 35, 1617.

This seemed likely to be of interest from more than one point of view. It would facilitate the comparison of our amylase preparations with a number of typical proteins, and the comparison of the amylases from different sources with each other, and it might throw light upon the nature of the chemical changes through which the enzyme is formed from antecedent substances.

Since the differences in power, sometimes encountered among preparations made in the same way, are probably due to deterioration during the processes of purification and drying as a result of some change not otherwise perceptible in the enzyme substance, we have included in this study both malt and pancreas preparations of different powers. It is interesting to note in the tables below that whatever change has taken place, to occasion the partial loss of diastatic power, has not resulted in any measurably different distribution of the nitrogen among the eight forms recognizable by the Van Slyke method.

In order that we might make such preliminary tests of our manipulation of this method, as would give us added confidence in comparing our results with the data obtained by Van Slyke in the analysis of typical proteins, Dr. Van Slyke very kindly furnished us a specimen of casein which he had examined, together with the results which he had obtained.

We would here express our great indebtedness to Dr. Van Slyke for his generous coöperation.

One gram of this casein was submitted to analysis by the Van Slyke method, the published descriptions[1] being closely followed in every respect, except that, in accordance with a suggestion kindly sent us by Dr. Van Slyke, the solution used to wash the phosphotungstate precipitate of the bases was cooled approximately to 0°.

The following statement shows the data furnished us by Dr. Van Slyke in the first column of figures, and the results obtained in this laboratory (by A. O. G.) in the second column.

NITROGEN FOUND IN DIFFERENT FORMS IN CASEIN, EXPRESSED AS PERCENTAGES THE TOTAL NITROGEN.

	Dr. Van Slyke. Per cent.	A. O. G. Per cent.
Ammonia nitrogen	10.27	10.0
Melanin nitrogen	1.28	1.8
Arginine nitrogen	7.41	7.3
Histidine nitrogen	6.21	6.2
Lysine nitrogen	10.30	10.1
Cystine nitrogen	0.20	0.3
Amino nitrogen of the filtrate	55.81	55.0
Non-amino nitrogen of the filtrate	7.13	7.2
Summation	98.61	97.9

[1] *J. Biol. Chem.*, 10, 15; 12, 275.

In subsequent work the details of the technique were always the same as in this test analysis with casein. It is therefore probably safe to infer that our results are not only comparable among themselves, but may accurately be compared with the data obtained by Van Slyke in his examination of various proteins. On account of the small amounts of material available, only about one gram was hydrolyzed in each case. While this is less than is desirable it has given good results in the test analysis of casein above.

Pancreatic Preparations.[1]

Sample A was a mixture of preparations made at intervals during the past two years, which, as tested separately, had shown powers of 3000 to 3600 (new scale), or, in other words, each preparation had shown a power of forming from 7500 to 9000 times its weight of maltose in thirty minutes at 40°. A duplicate sample under similar conditions formed over 200,000 times its weight of maltose in twenty-four hours and over 500,000 its weight in all.

Sample B was a mixture of preparations which had shown lower powers ranging from 1950 to 2450 (new scale), that is, they had formed from 5000 to 7000 times their weight of maltose in 30 minutes at 40°.

All this material had been kept in dry powder form, tightly stoppered and in a cool, dark place.

The results of analysis by the Van Slyke method were as follows:

RESULTS IN TERMS OF THE TOTAL NITROGEN PRESENT.

	Sample A. Per cent.	Sample B. Per cent.
Ammonia nitrogen	8.1	9.1
Melanin nitrogen	5.3	6.1
Arginine nitrogen	14.6	14.7
Histidine nitrogen	6.0	6.9
Lysine nitrogen	7.4	7.0
Cystine nitrogen	2.5	2.2
Amino nitrogen of the filtrate	50.4	49.1
Non-amino nitrogen of the filtrate	4.6	4.1
Summation	98.9	99.2

Malt Preparations.

Sample C was a mixture of the "50% precipitates" obtained in various experiments upon purification of malt amylase, *i. e.*, the precipitate thrown down when the solution containing the amylase is (after dialysis and filtration) mixed with an equal volume of absolute alcohol. This material is superficially much like the amylase preparation, except for this lesser solubility in alcohol; but it has little diastatic power. It was thought that some light might be thrown upon possible relationships between the malt amylase and the proteins which accompany it in the grain by ana-

[1] For more detailed descriptions see THIS JOURNAL, 33, 1195; 34, 1104.

lyzing this "50% precipitate" in comparison with the "65% precipitate," which contains the greater part of the amylase.

Sample D was a mixture of malt amylase preparations ("65% precipitates") which had been subjected to essentially the same process of purification which has yielded the products of maximum power, but which showed a power (for the composit sample) of only 350 (new scale).

Sample E was a mixture of preparations purified by the same method[1] and showing for the composit sample a power of 906 (new scale), corresponding to a power of about 1350 on Lintner's scale. Acting on 2% "soluble" starch at 40° it formed over 2200 times its weight of maltose in 30 minutes.

Analyses of these three preparations by the Van Slyke method resulted as follows:

RESULTS IN TERMS OF THE TOTAL NITROGEN PRESENT.

	Sample C. Per cent.	Sample D. Per cent.	Sample E. Per cent.
Ammonia nitrogen	7.8	7.3	7.9
Melanin nitrogen	4.1	3.9	5.6
Arginine nitrogen	14.5	13.1	14.2
Histidine nitrogen	4.6	6.5	5.4
Lysine nitrogen	7.5	6.7	5.5
Cystine nitrogen	4.0	4.0	4.9
Amino nitrogen of the filtrate	55.3	53.9	52.4
Non-amino nitrogen of the filtrate	3.5	4.3	4.5
Summation	101.3	99.7	100.4

Discussion.

These analyses by the Van Slyke method furnish additional, and it would seem sufficiently conclusive, evidence that our amylase preparations are essentially protein substances.

That the enzyme itself may be a substance of entirely different nature and the protein only a "carrier" has been suggested by some writers. The suggestion has never received much experimental support and has now been rendered highly improbable by work in which the attempts to purify the enzyme have been guided and tested by careful quantitative measurements of the activity of the products obtained.

The exact significance of the small differences in data, here obtained upon different amylase preparations, will doubtless become clearer as the corresponding data for other proteins accumulate. Just how far errors of determination enter into these small differences cannot be stated with certainty, since the amounts of material were too small to permit duplications of the analyses.

More striking, and doubtless more significant than the small differences observed, is the fact that all five of these preparations, including ma-

[1] Sherman and Schlesinger, THIS JOURNAL, **35**, 1617.

terials of maximum enzymic activity from the pancreas and from malt, yield all of the eight forms of nitrogen distinguishable by the Van Slyke method, in proportions within the range of variation shown by such typical protein substances as casein, edestin, hair, and hemoglobin.

We are greatly indebted to the Carnegie Institution of Washington for grants in aid of this investigation.

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A COMPARISON OF THE OBSERVED AND COMPUTED HEAT PRODUCTION OF CATTLE.[1]

BY HENRY PRENTISS ARMSBY.

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The first investigation by modern methods of the long-standing problem of the source of animal heat was that of Rubner.[2]

The quantities actually determined in Rubner's experiments were the total nitrogen of the excreta, the respiratory carbon dioxide and the heat produced. The carbon of the visible excreta was computed from their nitrogen, and the absence of carbon compounds, other than carbon dioxide, in the respiration was assumed. From the total excretion of carbon and nitrogen, the catabolism of protein and fat was computed, and from the latter the equivalent amount of heat, using Rubner's well-known factors; it being assumed that there was no material catabolism of carbohydrates. In the aggregate of experiments covering 45 days, the actual heat production, as determined by Rubner's form of animal calorimeter, differed from the amount computed from the body catabolism as follows:

	Cals.	
Computed heat production	17406.0	
Observed heat production	17349.7	
Difference	—56.3	= —0.32%

Laulanié[3] has reported similar comparisons on several different species, by what appear to have been somewhat less rigorous methods. These likewise show a close agreement, the observed differing from the computed heat production in the aggregate of all the experiments reported in full by +0.31%.

The most extensive investigations on this subject are those of Atwater and Benedict upon men. Their experiments are distinguished by the accuracy of their technic and by the fact that all the factors involved were,

[1] Investigations at the Institute of Animal Nutrition of The Pennsylvania State College, in coöperation with the Bureau of Animal Industry of the U. S. Department of Agriculture.

[2] *Z. Biol.*, 30, 73 (1894).

[3] *Arch Physiol.*, 1898, 748.