the presence or absence of barium sulfate, a portion of the original root ash was fused with a mixture of sodium and potassium carbonates, and total silica determined in the usual way. After being ignited and weighed, the resulting mass was heated with hydrofluoric and sulfuric acids until constant in weight.[1] The amount of sand and soluble silica determined by the modification of the official method already described was 52.87 per cent., while determination of total silica by fusion gave 49.87 per cent. Now, the presence of iron in the filtrate (containing soluble silica) from sand, previously noted, made it seem almost certain that the latter, also, contained iron. This view was confirmed by showing that the filtrate from total silica by fusion contained a larger amount of iron than did Solution A. The percentage of ferric oxide in the ash, as determined in Solution A, was 2.56 per cent.; that in the filtrate from total silica was 2.88 per cent. Undoubtedly a redetermination of other bases would have shown sufficient increases to account for the difference between the sum of sand and soluble silica in one analysis, and total silica in the other.

An assay of the leaf having shown that alkaloids were absent, attention was next directed to the alcoholic extract. It is worthy of note that in the preliminary consideration of this extract some of the matter (of which a large part seemed to be resin) removed was changed by oxidation or otherwise so rapidly that before the drug was exhausted the extract began to deposit a solid that could not afterward be dissolved in alcohol. In future work the amount separating at this stage will be determined as nearly quantitatively as possible, and other properties will be examined. This behavior, taken in connection with the statement already given regarding the poisonous character of the extracts prepared with various solvents, makes necessary a detailed study of the alcoholic extract. The work is now in progress.

LARAMIE, WYOMING,
May, 1911.

[CONTRIBUTION FROM THE HAVEMEYER LABORATORIES, COLUMBIA UNIVERSITY, No. 193.]

STUDIES ON AMYLASES: III. EXPERIMENTS UPON THE PREPARATION AND PROPERTIES OF PANCREATIC AMYLASE.[2]

BY H. C SHERMAN AND M. D. SCHLESINGER.

Investigations in this laboratory during the three years, 1907–1910, resulted in the development of a method for the determination of amylolytic power[3] and a somewhat extended quantitative study, by means of this method, of the action of pancreatic amylase.[4] On the basis of

[1] The slight non-volatile residue left contained iron, but spectroscopic examination failed to show even a trace of barium.

[2] Read before the New York Section, American Chemical Society, May, 1911.

[3] Sherman, Kendall and Clark, THIS JOURNAL, 32, 1073.

[4] Kendall and Sherman, *Ibid.*, 32, 1087.

this work we have been able to make use of activity as a criterion in extending our investigation to the preparation and study of a pancreatic amylase of much higher concentration and purity than had previously been available.

Of the numerous studies of amylases recorded in the literature the greater number deal only with extracts or secretions and when solid preparations have been described the amylolytic activity has not always been determined quantitatively.

Only a few previous investigations are therefore directly comparable with the experiments to be described in this paper, and these deal mainly with vegetable amylases.

Lintner[1] used a method which consisted essentially in extracting the enzyme from malt by means of water or very dilute alcohol, precipitating by strong alcohol, and purifying by repeated solution and reprecipitation. Of several preparations thus obtained the strongest had such diastatic power that 0.12 mg. acting upon 10 cc. of 2 per cent. soluble starch for one hour at room temperature formed enough sugar to reduce 5 cc. of Fehling's solution. One preparation of this strength was obtained from barley malt and one from wheat malt. Lintner proposed that these preparations be considered as having a strength of 100, and that the amylolytic powers of other precipitated diastases be expressed upon this scale. In one of his most active preparations Lintner found: Carbon, 44.33 per cent.; hydrogen, 6.98 per cent.; nitrogen, 8.92 per cent.; sulfur, 1.07 per cent.; oxygen, 32.91 per cent.; ash, 4.79 per cent. Lintner's diastases are described as giving the typical reactions of proteins.

Osborne[2] by a process of successive precipitation with ammonium sulfate and dialysis followed by a fractional precipitation by dialyzing against alcohol, obtained from an extract of barley malt a preparation having a diastatic power of 600 on Lintner's scale. This preparation showed 0.66 per cent. ash and the following composition calculated to the dry ash-free basis: Carbon, 52.50 per cent.; hydrogen, 6.72 per cent.; nitrogen, 16.10 per cent.; sulfur, 1.90 per cent.;[3] oxygen, 22.78 per cent. This substance gave "all the usual protein reactions" and yielded a flocculent coagulum when heated in solution. Osborne advanced the hypothesis that active diastase is a combination of albumin with some other substance, presumably a proteose, which combination breaks up on heating, yielding a coagulated albumin.

Other investigators, among whom may be mentioned Zulkowski,[4]

[1] *J. prakt. Chem.*, [2] 34, 378 (1886); 36, 481 (1887).

[2] THIS JOURNAL, 17, 593, 598, 601–3 (1895).

[3] Osborne suggests that the high figure for sulfur in this preparation may be due to the retention of a little of the sulfate employed in the process of its purification.

[4] *Wien Akad.*, 71, II. 453; 77, II, 647; *Jahr.-Thierchem.*, 5, 268; 8, 356.

Loew,[1] Szilagyi,[2] Jegorow,[3] Egroff,[4] Wroblewski,[5] Sykes and Hussey,[6] Frankel and Hamburg,[7] have also described preparations of malt or wheat diastase but without evidence of having secured any higher concentration of amylase than existed in Osborne's preparation.

Hüfner,[8] described in some detail an amylolytic preparation from the pancreas, but gave no quantitative data as to its activity.

Vernon[9] extracted pancreas glands with glycerol and precipitated with alcohol. Although many preparations were made under varying conditions the greater part of the diastatic power was always lost. Vernon concluded that the addition of alcohol to glycerol extracts of pancreas results in rapid destruction of its amylase.

Experimental.

Having in the course of previous work[10] been much hampered by deterioration of amylolytic power in pancreatin solutions, we preceded our attempts to purify pancreatic amylase by a number of experiments to test the influence of different solvents and precipitants upon the amylolytic activity of commercial pancreatin, the material which has served as a starting point for most of our preparations. The pancreatin showed marked loss of amylolytic power on standing 24–48 hours in water, brine, 5 per cent. salt solution, or in water containing the small amounts of salt and phosphate which had been found necessary to enable the enzyme to show its full activity.[10] For this reason the method used by Osborne in purifying malt diastase did not seem well adapted to pancreatic amylase.

Vernon's observation that extraction with glycerol followed by precipitation with alcohol involved a large destruction of amylase, or at least a large loss of amylolytic power, was also confirmed in our preliminary experiments.

On the other hand it was found that pancreatic amylase was fairly permanent in 50 per cent. alcohol and could be recovered in active form from this solvent by precipitation with strong alcohol or with a mixture of alcohol and ether. Using 7 volumes of a mixture of 20 per cent. alcohol and 80 per cent. ether, the precipitate was obtained as a yellowish, oily product, very readily soluble in water. This precipitate on account of its mechanical condition could not readily be transferred, but after the

[1] *Pflüger's Archiv. f. d. ges. Physiol.*, **27**, 203.
[2] *Chem.-Ztg.*, 1891, 349.
[3] *Jour. russ. chem. Ges.*, **25**, 80; *Chem. Centralbl.*, 1893, II, 212.
[4] *Mon. Sci.*, [4] **8**, II, 741.
[5] *Ber.*, 30, 2289; **31**, 1127.
[6] *Jour. Fed. Inst. Brew.*, **4**, 527; *J. Chem. Soc.*, **76**, I, 313 (1899).
[7] *Hofmeister's Beitrage*, **8**, 389.
[8] *J. prakt. Chem.*, [2] **5**, 372 (1872).
[9] *Jour. Physiol.*, **29**, 302 (1903).
[10] Sherman, Kendall and Clark, *Loc. cit.*

removal of the supernatant liquid was dissolved in water and poured into about 3 volumes of absolute alcohol, thus bringing it into the form of a flocculent precipitate which could be filtered, washed with ether, and dried over sulfuric acid.

Preparation 1, made as just described from a commercial pancreatin having a diastatic power of 101 (Lintner's scale), showed a diastatic power of 402 on the same scale.

Preparations 2 and 3 made in approximately the same way from the same material showed diastatic powers of 306 and 450 (Lintner scale) respectively.

Preparation 4.—About 0.5 gram of preparation 2 was mixed with about 30 cc. of 65 per cent. alcohol in which it did not entirely dissolve, transferred without filtering to a small collodion sack, and dialyzed for 6 hours against 200 cc. of 65 per cent. alcohol. The dialyzate showed no diastatic power. The liquid in the sack was then filtered, the filtrate poured into absolute (Kahlbaum's 99.8 per cent.) alcohol, ether added, and the precipitate filtered out, washed with alcohol and ether, and dried over sulfuric acid. This preparation showed a power of 531 (Lintner scale) and was therefore over 5 times as strong as the pancreatin from which it was prepared, and about 88 per cent. as strong as Osborne's strongest malt diastase.

In the next preparation the precipitation and dialysis were carried out somewhat differently.

Preparation 5.—Ten grams of commercial pancreatin were thoroughly stirred with 250 cc. of 50 per cent. alcohol, filtered, and 200 cc. of the filtrate poured into a mixture of 1120 cc. ether and 280 cc. alcohol. A yellow, oily precipitate was obtained. The supernatant liquid was decanted through a filter and the precipitate (still wet with alcohol and ether) was dissolved in as little water as possible, filtered, and poured into about 5 volumes of absolute alcohol. A white flocculent precipitate separated at once. A small amount of ether was then added as this seems to facilitate the subsequent handling of the precipitate. The precipitate was then separated by filtration, dissolved in as little 50 per cent. alcohol as possible, and dialyzed in a collodion sack for three days against 50 per cent. alcohol, which was renewed twice daily. The successive dialyzates showed biuret reaction but with constantly decreasing intensity indicating that this treatment was efficient in removing certain of the protein impurities from the enzyme solution. At the same time a precipitate separated from the enzyme solution within the sack. This precipitate, when removed by filtration and thoroughly washed with 50 per cent. alcohol, was found not to have diastatic power. It was insoluble in water, but soluble in salt solution.

The solution containing the enzyme, after having been thus dialyzed and filtered, was mixed with a large volume of ether and a small volume of absolute alcohol. A white flocculent precipitate formed readily and settled in about half an hour. The liquid was then decanted and the precipitate washed by decantation with alcohol-ether mixtures, the proportion of alcohol being decreased and of ether increased until the water was removed, then washed with absolute ether and dried over sulfuric acid. This preparation weighed 0.09 gram and showed a diastatic power of 790 on the new scale, corresponding to 1168 on Lintner's scale.

Preparations 6–15.—Here the method used for preparation 5 was modified in different ways, as by dialyzing for various lengths of time, and by the use of amyl alcohol or acetone as precipitant. All of these preparations were inferior to preparation 5 in diastatic power.

Preparation 16.—A sample of commercial pancreatin having a diastatic power of 150 (new scale)[1] was extracted with 50 per cent. alcohol, filtered, the filtrate pre-

[1] Sherman, Kendall and Clark, This Journal, **32**, 1082–4.

cipitated with alcohol-ether mixture, the precipitate thus obtained dissolved in water, reprecipitated by strong alcohol, and redissolved in 50 per cent. alcohol as in preparation 5. To this solution, which measured about 150 cc., we added 8 grams of maltose dissolved in a little water, thinking that this maltose might protect the amylase from deterioration, as Hudson has found it possible to protect invertase by sucrose under somewhat analogous circumstances. This solution was then dialyzed against 50 per cent. alcohol as in preparation 5. At the end of 19 hours the outer solution was replaced by fresh 50 per cent. alcohol; dialysis against the second portion of alcohol was continued 5 hours against the third 18 hours and against the fourth 49 hours —a total of 91 hours' dialysis. The solution in the collodion sack was then filtered, the filtrate shaken with 3–4 volumes of a mixture of equal parts of absolute alcohol and ether, the precipitate thus obtained dissolved in a small amount of water, reprecipitated by pouring into alcohol, filtered in dry air, and dried in partial vacuum over sulfuric acid.

This preparation showed a diastatic power of 1528 on the new scale[1] corresponding to 2270 on Lintner's scale.

A very dilute solution of this preparation in pure water yielded a white flocculent coagulum on heating. The solution was boiled down to about 10 cc. and filtered. The coagulum was submitted to the biuret test at the same time and in exactly the same manner as the filtrate. The filtrate gave the rose reaction characteristic of proteose or peptone, while the coagulum gave a blue-violet reaction such as is commonly observed with albumins. This result is in accordance with Osborne's suggestion, based on his study of malt diastase, that the enzyme may be a compound of a coagulable albumin with a proteose.

Preparation 17.—This was prepared in practically the same manner as the preceding, except that 10 grams of maltose (instead of 8 grams) were added to protect the enzyme from deterioration, and the time of dialysis was shortened, *viz.*, 17 hours for the first period, 23 hours for the second, and 28 hours for the third—a total of 68 hours. Although the dialysis in this case was not carried to completion, the last dialyzate containing maltose and giving a distinct biuret reaction, the product showed a diastatic power of 1910 on the new scale, corresponding to 2760 on Lintner's scale.

This preparation gave the same coagulation and biuret tests as preparation 16. This has also been found true of all subsequent preparations so far as tested, and will therefore not be repeated in the descriptions which follow.

Preparations 18 and 19, in which the method was varied, yielded products of lower diastatic power than the preceding.

Preparation 20.—Twenty grams of pancreatin 4,[2] carried through the same process as preparation 17, gave a product whose activity was 2000 on the new scale, corresponding to a Lintner figure of 2950.

Preparations 21–23 representing other experimental variations in the method showed no advance in diastatic power.

Preparation 24.—In this case parchment paper thimbles instead of collodion sacks were used for dialyzing. The final product appeared more powdery than in other

[1] Up to this point tests of diastatic power had been made by dissolving the enzyme in pure water and measuring portions of this solution into starch solutions containing the activating agents. This and the following preparations were dissolved at once in water containing the activating agents in order to avoid the deterioration which takes place in pure water.

[2] This sample of pancreatin which was used in preparations 19–28 was from the same source as that previously used, but showed an activity of 158 (new scale) corresponding to 228 on Lintner's scale.

cases, and showed a power of 2280 new scale, equivalent to a Lintner figure of 3360, but from the slowness with which the dialyzate acquired the yellow color of the enzyme solution, and from the results of tests for proteins in the dialyzate, it was judged that the removal of impurities went on less readily through the parchment than through the collodion membrane, and the latter was therefore used for subsequent preparations.

Preparation 25.—In this case the maltose for protection of the enzyme was added after 16 hours' dialysis and the removal of the first dialyzate. The dialysis was continued 64 hours in all, with three changes of dialyzate. The filtered solution was then mixed with an equal volume of a mixture of equal parts of alcohol and ether, the resulting precipitate dissolved in water, and reprecipitated by dropping into two volumes of absolute alcohol. The final product showed an activity of 2092 on the new scale, corresponding to 3090 on Lintner's scale.

Preparation 26 was made in practically the same manner as preparation 17, except that 5 grams of maltose (instead of 10 grams) were used to protect the enzyme during dialysis. This product showed an activity of 2280 (new scale) corresponding to a Lintner figure of 3360.

Preparations 27–30 showed no advance in diastatic power.

Preparation 31.—The starting material for this preparation and for all which follow, except preparation 37, was a new sample of commercial pancreatin (pancreatin 5) from the same source as the pancreatins previously used, but presumably made by an improved process since it showed an activity of 425 on the new scale of diastatic power.

Twenty grams of pancreatin 5 were submitted to practically the same process as in preparation 26, up to the point of beginning of dialysis. Here the work was interrupted and the solution of enzyme and maltose in 50 per cent. alcohol stood over night in a beaker. It was then placed in a collodion sack and dialyzed 29 hours against 2 liters of 50 per cent. alcohol, which was kept in circulation during the working hours of the day. The final product after washing with ether and standing over sulfuric acid in partial vacuum until air-dry weighed 0.7 gram and showed a power of 3120 (new scale) corresponding to a Lintner figure for the same temperature of 4520.

Preparation 32.—This was a duplication of preparation 31 except that the solution as soon as prepared for dialysis was placed in a collodion sack and dialyzed against 300 cc. of 50 per cent. alcohol at rest over night, then for 7 hours against a fresh portion of 2 liters kept in circulation, then for 16 hours against another portion of 2 liters at rest. The last dialyzate showed only a very slight biuret reaction. Total time of dialysis 40 hours; weight of air-dry product 0.65 gram, or 3.25 per cent. of the weight of original material; activity 3480 (new scale) corresponding to a Lintner figure for the same temperature (40°) of 5140.

Preparation 33.—This was prepared in the same manner as the preceding, except in dialysis. It was dialyzed about 40 hours in all against 4 portions of 50 per cent. alcohol, each 1200 cc. The last dialyzate was not entirely free from maltose and also showed a slight biuret reaction. The activity of the air-dry product was 3360 (new scale).

Preparation 34.—In this preparation the volume of each dialyzate was 2000 cc. instead of 1200 cc., otherwise it was prepared in the same manner as No. 33. Yield, 0.51 gram; activity, 3430 (new scale).

Preparation 35.—Prepared in the same manner as No. 34. Yield, 0.93 gram; activity, 3450 (new scale).

Preparation 36.—Prepared like Nos. 34 and 35, except in dialysis, which was continued only 19 hours, though with 5 changes of dialyzate. This was evidently insufficient to remove all of the added maltose, as the last dialyzate showed maltose

and the final product had a greater weight (1.08 gram) and a lower activity (2880) than the preceding preparations. Further evidence that this preparation was contaminated with maltose is found in the fact that the nitrogen content of the dry ash-free substance was only 13.65 per cent., while that of preparation 35 was 15.58 per cent.

Preparation 37.—Twenty grams of a commercial pancreas powder (H) from a different manufacturer than those previously used and which showed an amylolytic power of 405 (new scale) was subjected to the same process as in preparations 34 and 35 and yielded 0.87 gram of air-dry product, the activity of which was 3260 (new scale).

On the Probable Purity of these Preparations.

At present the principal criterion of purity or concentration of any enzyme preparation is its activity. Judged from this standpoint, it would seem that our later preparations must represent a high concentration of pancreatic amylase, since they exhibit about 8 times the amylolytic power of the strongest preparations which we have found described in the literature or which we have been able to obtain commercially. On the other hand, the nature of our products and the method of their preparation preclude the belief that they are chemically pure. Like other preparations of a protein nature they contain moisture and ash even after having been through an extended course of purification. It is possible that our preparations may contain traces of maltose, since the maltose used to protect the enzyme from deterioration is not always completely removed by dialysis and the minute amount sometimes remaining in the enzyme solution might not be completely excluded in the final precipitation. The presence of any considerable proportion of maltose or of other carbohydrate matter is, however, practically precluded by the analytical data given beyond, which show that our strongest preparations have essentially the composition and character of proteins. To what extent we have succeeded in separating amylase from other materials of a protein nature is a much more difficult question. In the extraction with 50 per cent. alcohol, which constitutes the first step in our process, much of the protein matter is left behind. The second step which consists in precipitating with a limited amount of alcohol and ether, evidently effects a further separation of the enzyme either from protein or other impurity, since the use of a larger amount of the alcohol-ether mixture yielded a heavier precipitate of lower diastatic power. When the solution of enzyme is dialyzed in 50 per cent. alcohol, material which responds to the biuret reaction passes into the dialyzate, and at the same time there occurs a gradual deposition within the collodion sack of protein matter[1] which is then removed by filtration before the final precipitation of the enzyme. Under these conditions, it would seem reasonable to expect a fair approach toward a separation of the

[1] This material is probably a globulin, soluble in 50 per cent. alcohol in the presence of salts, but insoluble when the salts are removed by dialysis. As noted above, it is insoluble in water but soluble in salt solutions.

enzyme from the unrelated proteins which accompany it in the pancreas. Whether those proteins which are closely related to the amylase are separated by the same process is still doubtful. If the amylase is formed from pre-existent protein, the difference between the enzyme and its precursor may or may not be sufficient to permit their separation by the process here used. Similarly, when a preparation deteriorates as this amylase does, for example, in water solution, there is doubtless some chemical change in the enzyme, but whether this is of such a nature as to permit the separation of the active and inactive forms by any process of dialysis and precipitation it is at present impossible to say. With the single exception of preparation 36, which was dialyzed only 19 hours, and evidently was not free from maltose, all of our later preparations show considerable similarity in amylolytic activity as is apparent from the following summary:

Preparation. No.	Activity-new scale.	
	Substance as tested.	Calculated to dry substance.
31	3120	3310
32	3480	3670
33	3360	3540
34	3430	3570
35	3450	3720
37	3260	3320
Mean of these six preparations........................		3520

Extreme deviations from the mean —6.0 to +5.7 per cent.

In view of the well-established fact that as enzymes are freed from the substances which ordinarily accompany them in nature, they become much more sensitive to external conditions and the quantitative measurement of their activity, always a sufficiently difficult problem, becomes much more difficult, these figures are significant for their agreement. That six independent preparations of this character should show such close agreement in diastatic power is a strong indication that this method of purification yields a fairly definit result.

Properties.

Some further properties of the preparations just described may be briefly summarized. The amount of material did not permit a detailed examination of each separate preparation, nor is this called for in view of the similarity of the six strong preparations. Each of the following statements of properties represents results obtained upon one or more of these six preparations.

The amylase as here prepared is obtained, depending upon the way in which the final precipitate is handled in washing and drying, either as a friable white powder, or as brittle, easily pulverized, slightly yellowish scales resembling dry egg albumin. It dissolves readily in water to a clear colorless solution.

It gives pronounced xanthoproteic and tryptophane reactions and also gives a typical protein reaction in the biuret test and with Millon's reagent.

Its water solution coagulates on heating, exhibiting the same appearance in this respect as an ordinary albumin. The coagulum gives a blue-violet reaction, while the filtrate gives a rose-pink reaction, to the biuret test. This filtrate if dropped into alcohol gives a white, flocculent precipitate.

In an experiment upon the temperature of coagulation a water solution containing 1 mg. per cubic centimeter showed opalescence at 60–65°, distinct turbidity at 70–75°, and a flocculent precipitate at 80°. Held at 80° until the precipitate had gathered into a few large flocks and then heated to boiling, no further precipitation occurred.

The air-dry preparations contained from 1.8–7.4 per cent. of moisture, and from 2.2–2.6 per cent. of ash. Ultimate analyses calculated to dry ash-free material showed:

Preparation. No.	Carbon. Per cent.	Hydrogen. Per cent.	Nitrogen. Per cent.	Oxygen and sulphur. Per cent.
31	52.8	6.7	15.5	25.0
35	53.1	6.6	15.6	24.7

These analyses, taken in connection with the properties already described, sufficiently demonstrate the protein nature of these preparations. Lack of material prevented more detailed study of the ultimate composition.

Regarding the activity of these preparations, the figures given above, 3120–3450 on the new scale of diastatic power, correspond to Lintner's figures of about 5000 for the same temperature (40°). Osborne gives the activity of his strongest malt diastase as 600 on Lintner's scale. It is difficult to decide whether it necessarily follows that our preparation of pancreatic amylase is actually 8 times as active as Osborne's amylase of malt, because of differences in experimental methods.

Wohlgemuth, in his work on saliva and other physiological fluids and tissues, has introduced a method of expressing diastatic power in terms of the number of parts of 1 per cent. solution of Kahlbaum soluble starch which are digested to products giving no reaction with iodine by one part of the substance under examination, usually during 30 minutes at 38°. Thus determined and expressed, the activity of our preparation 34 is:

$$D^{38}_{30} = 500,000.$$

The same preparation acting on 2 per cent. soluble starch formed 6,000 times its weight of maltose in 20 minutes at 40°. In 3 hours at the same temperature it digested 100,000 times its weight of starch, and produced 41,500 times its weight of maltose, together with dextrins which gave with iodine a purplish wine color. In 4 hours these dextrins had

been digested beyond the "erythrodextrin stage" and in 5 hours the 100,000 parts of starch had been completely digested to products giving no color reaction with iodine, and the enzyme had formed 55,500 times its weight of maltose. In 6 hours this preparation digested 200,000 times its weight of starch to the "erythrodextrin stage" with the production of 91,800 times its weight of maltose.[1] Mixed with 400,000 times its weight of starch it digested this in 10 hours to a stage which no longer gave with iodine the starch-iodide blue but instead a rich plum color. At this stage it had formed 118,000 times its weight of maltose. Within 22 hours it had digested the 400,000 parts of starch to the "erythrodextrin stage" and had formed 182,000 times its weight of maltose. In 30 hours it formed 211,000 times its weight of maltose. At the end of 48 hours the starch had been completely digested into products giving no color reaction with iodine.

We take pleasure in acknowledging our indebtedness to the Parke, Davis Company and to Messrs. Fairchild Brothers and Foster for the materials used in the later part of our work and from which we obtained our strongest preparations.

Summary.

By means of a process involving extraction of the dry pancreas powder with 50 per cent. alcohol, repeated precipitation, and purification by dialysis in 50 per cent. alcohol, preparations having diastatic powers up to 3480 on the new scale, corresponding to over 5000 on the Lintner scale or to $D^{38}_{30} = 500,000$ on Wohlgemuth's scale were obtained.

Six independent preparations showed in the dry substance activities of 3310, 3670, 3540, 3570, 3720, 3320. This agreement indicates that these preparations are substantially alike and that the process yields a fairly definit result.

This product shows the composition and characteristic reactions of a protein closely resembling Osborne's malt diastase, but differs from malt diastase as described by Osborne in its greater activity and in the conditions governing its action.

The pancreatic amylase as here prepared acting at 40° on soluble starch made by the Lintner method formed 6000 times its weight of maltose in 20 minutes and 211,000 times its weight in 30 hours. It digested 400,000 times its weight of starch to the "erythrodextrin stage" in less than 22 hours, and to products giving no reaction with iodine in 48 hours.

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[1] Between 10 and 22 hours this solution ceased to give any reaction with iodine whatever.