more easily cleaned, and the breakage of which would not be such an important matter.

The first flask designed to meet this requirement is that described by Wheeler and Hartwell.[1] In this apparatus the designers have used a straight-necked flask holding about 100 cc., fitted with a rubber cup channeled so as to receive the condenser. Some workers, however, have found this device somewhat unsatisfactory, owing to the short life of the rubber cups and some danger attending their use. The modification of this idea, shown in Fig. 1, was designed by Mr. F. W. Robison, of the Michigan Dairy and Food Department. As is indicated in Fig. 2, the seal consists of a maple cup made to fit over an ordinary rubber stopper through which the neck of the flask is passed. The seal is made by mercury in a manner similar to the device employed by Wheeler and Hartwell. This form of apparatus is now used in one of the laboratories of the Department of Agriculture and is considered a great improvement over the Knorr flask.

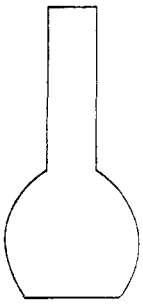

Fig. 1.

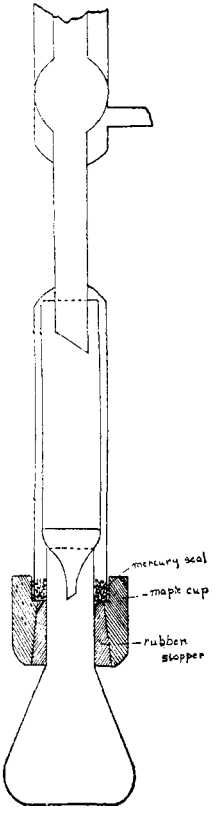

Fig. 2.

The flask designed by the writer and shown with connections, in Fig. 2, is a modification of the above. Being of the Erlenmeyer type, cleaning is more easily accomplished, while at the same time all the good features of the above-described flasks are retained. The one used in this laboratory holds about 100 cc. and weighs approximately 30 grams, this tare affording considerable strength while not affecting the accuracy in weighing. In practical use the flask is proving about all that could be desired in regard to safety and ease of manipulation, facility in cleaning and small expense for replacement.

BUFFALO LABORATORY.

A PROPOSED METHOD FOR THE ROUTINE VALUATION OF DIASTASE PREPARATIONS.

BY WILLIAM A. JOHNSON.

Received February 21, 1908.

In view of the rapidly increasing number of starch-digesting products on the market, and the exaggerated claims which are made for some of

[1] THIS JOURNAL, 23, 338.

them in the literature, through which they are advertised to physicians and others interested, the necessity for a simple and approximately accurate method of valuation becomes every day more apparent. This necessity would not be so urgent if the manufacturers of these amylolytic ferments had agreed among themselves on a uniform method by which the enzymic activity may be determined, but this they have not done and apparently are not likely to do.

In the last two years committees of the Council on Pharmacy and Chemistry of the American Medical Association have undertaken to pass on the validity of the claims of the manufacturers of medicinal substances for the strength and purity of their products, among which products the diastatic mixtures occupy an important place. In the course of some investigations on the subject, which I have carried out under the direction of one of these committees, I have made a number of observations which have a bearing on the question of the value of the methods and these I now wish to put on record.

It may be said at the outset that nothing fundamentally new will be offered here. Indeed this is not necessary in view of the classic researches of Brown and Morris, Brown and Heron, O'Sullivan, Roberts, Kjeldahl, Lintner, Effront and others, to say nothing of the older studies of Dubrunfaut, Payen and Musculus.[1]

But most of the methods of measuring the ferment activity as brought out in these long studies had for their main object the valuation of malt employed in the brewing industries, and because of this fact they are not usually available for the work we have in hand. In general, such valuation may be made by noting the amount of the diastase preparation required to completely discharge the color of the iodine reaction in a given weight of starch paste of definite strength, or by noting the amount of sugar formed from an excess of starch, by the enzymes in some definite time at a proper temperature. From a theoretical standpoint, methods based on the latter determination would seem to have the advantage, as sugar formation rather than starch disappearance is the end practically required. But in some classes of preparations the enzyme is mixed with so large a quantity of glucose or maltose that the determination of more sugar formed would be found difficult in practice, especially where the ferment activity is low. This objection does not obtain in the observation on the disappearance of the starch-iodine reaction.

It is true that this starch-iodine method has been frequently condemned

[1] The general literature on the subject of the digestion of starch is of course voluminous, and no attempt will be made to quote all of it. But reference may be made to the convenient résumés in the following works: v. Lippmann, "Chemie der Zuckerarten," III Edition. Oppenheim, "Die Fermente und ihre Wirkungen." Effront, "Die Diastasen."

because the end point indicated by the disappearance of the iodine reaction is a point measuring the formation of a mixture, possibly, of somewhat complex dextrins with sugar, rather than of maltose itself. But if it may be shown that the disappearance of the iodine reaction follows always when a rather constant amount of maltose is formed, the method may be made of value for practical purposes. The correspondence between sugar formation and starch disappearance obtains, however, only under definite conditions, the redetermination of which was the first object of my experiments.

Roberts was the first, apparently,[1] to work out a method for the comparison of diastatic activities through the aid of the iodine-starch reaction. This general method was first used by F. C. Junck[2] and later by J. M. Francis,[3] who supplied many working details, which contribute much to the general accuracy and convenience of the process. In the accurate comparison of diastases in this way the essential points to be observed are these: 1. A pure standard starch must be made and this must be used in the form of a thin paste of constant value. 2. The experiments must be so conducted as to show a sharp end reaction between the iodine and vanishing starch. 3. A standard time limit must be adopted and rigorously adhered to. 4. The reaction must be carried out at a constant temperature.

In my experiments, in agreement with many others who have investigated the subject, I have found potato starch the best material to use as a standard. Most of the corn starch on the market seems to be wholly unfit for the purpose; in fact, different samples tested have given often final results varying by 50 per cent. or more from each other. But potato starch comes from the market in nearly pure form and by a simple treatment may be made suitable for use. For my experiments I washed 500 grams repeatedly with distilled water, by decantation, then sucked as dry as possible on a Buchner funnel. The mass was then spread on glass plates and dried 3 hours in an air current at a temperature of 50°. This made it dry enough to rub in a mortar, after which it was dried at 80° through four hours, which brought the moisture content down to 9.5 per cent., when the product was rubbed up again and bottled. It is not advisable to try to dry beyond 90 per cent. of pure starch, as the anhydrous starch absorbs moisture so quickly as to introduce inaccuracies in weighing. Prepared in this way, the microscopic examination shows clean granules, free from fracture and free from foreign substances.

It is hardly necessary to insist that a colorless end point is much more accurately and easily observed than is the point where the blue starch-

[1] *Proc. Roy. Soc.*, 32, 145. Cit. Maly's "Jahresber." for 1881, p. 290.

[2] *Am. Jour. Pharm.*, June, 1883.

[3] *Bulletin of Pharmacy*, February, 1898.

iodine reaction gives place to the reddish starch-dextrin reaction. I have worked then, in every case, to the colorless end point. With a little practice this may be uniformly noted by different observers working with the same materials, yet many persons have failed to realize the importance of this, as will be shown below.

On the question of the time limit in the digestions, there is greater room for difference of opinion, and various intervals from five minutes to one hour have been suggested by different workers. As the rapidity of starch conversion is very accurately proportional to the amount of enzyme present, it is in any case possible to reduce the time required to secure the desired end reaction by starting with a larger weight of the ferment, and the time finally selected as the standard or limit must depend, therefore, on a practical acquaintance with a wide range of substances in which such tests are made. In carrying out a number of tests in parallel, as is always the custom in such work, five minutes is an inconveniently short time to complete the various manipulations necessary; on the other hand, an hour, or even half an hour, is a relatively long time, which must be considered in part wasted if the same degree of accuracy can be secured in a shorter period. Now, it has been found, as a matter of fact, that no one of the digestive ferments on the market is so strong as to convert over 300 times its weight of starch into reducing sugar in ten minutes, while many of them have between one-tenth and one one-hundredth of this activity. Suppose, therefore, that we start with 1 g. of starch, the weights of enzyme preparation necessary to effect conversion in this time would run from 3.33 up to 33.3 or 333 mgs., quantities which are reached by convenient dilutions. Ten minutes seem to afford ample time to make the final color tests and I have therefore adopted this period for all this work.

In making such digestions, a temperature of 60° is often used, but as we are dealing with products which in practice are to be used at the temperature of the body, generally, it is preferable to take a temperature of 40° as the standard, and this I have done.

Practical Details.

In working this method we need first a standard starch paste. This is made by weighing out enough of the starch, prepared as above, to correspond to 20 grams of pure anhydrous starch. Of 90 per cent. starch we take, therefore, 22.22 grams. This is stirred up to make a uniform cream with 100 cc. of water, and the mixture is then poured into 800 cc. of boiling distilled water, in a flask. The boiling is continued ten minutes and then more water is added to make 1000 grams by weight. The mixture is heated and shaken to distribute the starch uniformly. The contents of the flask should be practically clear and free from all lumps.

For each test quantities of 50 grams each are weighed into a series of 250 cc. flasks, clamped in a large water-bath kept at 40°.

The iodine test solution is made by dissolving 2 grams of iodine and 4 grams of potassium iodide in 250 cc. of water. Two cc. of this solution are then diluted with distilled water to make 1000 cc.

In making up the diastase solutions, the operator must be guided by the results of a few preliminary experiments in each case. For liquid malt extracts, for example, 10 cc. diluted to 100 cc. will be generally a proper strength, while in the examination of the dry preparations on the market, 200 to 500 milligrams dissolved or suspended in 100 cc. of distilled water will usually answer. These solutions are used in this way. Small definite volumes of the dilution are added to the flasks containing the starch paste in the thermostat, and with the least possible loss of time. The mixture is well shaken. The volumes added may be as follows, but all diluted to that of the largest volume before mixing: 1 cc., 2 cc., 3 cc., 4 cc., 5 cc., 6 cc. In about eight minutes tests are begun by removing volumes of 5 drops of each digesting mixture by a pipette and adding this to 5 cc. of the diluted iodine solution in a clear white test tube standing over white paper. It is best to have a row of these tubes mounted to receive the liquids to be tested. If at the end of ten minutes drops from one of the flasks fail to give the iodine reaction, we are ready for a second and more accurate test. Weigh out now 100 grams of the paste into each of 6 bottles and, assuming that the end point was found in the first test to be between 4 and 5 cc., add accurately to the different flasks these volumes of the diastase solution: 8 cc., 8.4 cc., 8.8 cc., 9.2 cc., 9.6 cc., 10 cc. These volumes should stand ready and all diluted to 10 cc. so that they may be poured into and shaken up with the starch without delay. The tests with the iodine solution are repeated as in the first trial and new limits are found between which the real value must lie. For example, at the expiration of ten minutes the paste to which 8.8 cc. of the diastase solution are added may show a faint yellowish dextrin color, while that with the 9.2 cc. is colorless. For all of our practical purposes it is not necessary to go beyond this. In fact, we cannot carry our readings to a much greater degree of accuracy because of the difficulty in distinguishing between the final shade from the disappearing erythrodextrin and the achroodextrin, using these terms in a general sense, rather than in the sense of assuming the actual existence of these forms.

Much of the uncertainty in the determination of diastatic values, as found in the literature, doubtless comes from the failure to recognize the importance of working to a colorless end point whenever this is possible. Roberts[1] pointed this out clearly in his work, but his suggestions

[1] *Loc. cit.*

have been generally overlooked, because perhaps they were made in the course of physiological, rather than in the usual technical, investigations. Most results for starch-converting power which we find advertised are evidently obtained by working to a rose-red end point, as the Pharmacopoeia allows in the case of the pancreatin test. For this reason many of the strong products which I have examined appear to be somewhat weaker than claimed. This is shown by the results of the following table, in which the statements of digesting power are given in both ways. The diastase products tested are among the best known in the market and are widely advertised. In giving the starch-converting power of such preparations anhydrous starch does not appear to be taken as the standard in any case. Average commercial starch contains about 15 per cent. of water, which should be allowed for in making fair comparisons.

TABLE OF STARCH-CONVERTING POWERS.

No.	Value in anhydrous starch, to colorless end point.	Value in anhydrous starch, to loss of blue color.	Value in commercial starch, to loss of blue color.	Value as claimed.
1	100.0	143.0	168.0	150.0
2	16.0	22.5	26.0	150.0
3(liq.)	0.0	0.0	0.0	7+
4	113.	170.0	203.0	200.0
5(liq.)	3.6	5.2	6.1	8.0
6(liq.)	6.0	8.6	10.1	.....
7(liq.)	6.0	8.6	10.1	.....

The values given in the last column are those claimed by the respective manufacturers, and are based apparently on digestion to the loss of the blue color only. From the advertising literature it is seldom possible to discover the exact basis of the valuation. The values in the second column, of the table, expressing the digestion carried to the achromic point, are much more accurate than those in the following column, where the disappearance of the blue color is recorded. It is not possible to judge of this as closely as desirable for a quantitative test.

Sugar Formation.

It is interesting now to note the amounts of sugar formed by these preparations in equal times, and such determinations were made in this manner. Having found the relative values of the preparations, amounts of each sufficient to convert 1 gram of starch to the achromic point in ten minutes were taken and mixed with 50 grams of starch paste. For each substance five tests were made, the flasks being kept in the thermostat through periods of 10, 30, 60, 120 and 180 minutes, respectively. At the end of the proper time a flask was removed, quickly brought to the boiling-point to check further action, and the sugar then determined as maltose by the Fehling titration. The results of these determina-

tions are shown in the following table, for some of the products described above:

MILLIGRAMS OF REDUCING SUGAR CALCULATED AS MALTOSE, FROM 1 GRAM OF ANHYDROUS STARCH.

	10 minutes.	30 minutes.	60 minutes.	120 minutes.	180 minutes.
1	613	788	866	866	866
2	611	822	933	1042	1094
3(liq.)	Inert.	Too weak to measure.			
4*a*	622	783	850	855	855
4*b*	630	788	845	858	858
6*a*	633	777	863	872	872
6*b*	635	777	866	866	866

In this table, as in the other, numbers 2 and 3 are preparations from a fungus. Numbers 1, 4 and 6 are pancreas preparations from three different firms, 4*a* and 4*b* are products of one firm purchased some months apart, while 6*a* and 6*b* are products of another firm bought at different times. It is interesting to note that the different products resemble each other very closely in their behavior, and that in all cases the amount of actual sugar formed in ten minutes, that is when the achromic point is reached in the iodine test, is about 60 per cent. of the theoretically possible complete amount, assuming that 1 gram of starch should yield 1.055 grams of maltose. At the end of one hour the amounts have gone up to over 80 per cent. of the possible, and beyond this there is practically no change.

The behavior of the preparation from the fungus is interesting. While its converting power for a short interval is like that of the others, the conversion becomes relatively stronger with time, and evidently proceeds beyond the production of maltose. The results of the calculations from the titrations must be interpreted in this way. It must be remembered, however, that the weight of the preparation required to convert starch with rapidity is much greater than with the other ferments used.

The above results are entirely in accord with those which have been found from time to time for the diastase from malt. A complete conversion is not practically possible, although by precipitating out the dextrins and adding fresh ferment it may be carried somewhat farther than is here done. The values here obtained are easily comparable with those of the iodine method.

The diastase preparations used in the above tests were all practically free from sugar to begin with. But we have as commercial articles a class of products made from malt by various extraction processes in which there is always a large amount of sugar, from the malt, and frequently added glucose. The examination of these mixtures, which are usually in the form of thick sirups, presents greater difficulties. Some

of these articles I have now in hand and expect to report on them at a later time.

NORTHWESTERN UNIVERSITY MEDICAL SCHOOL,
CHICAGO, ILL.

THE SEPARATION OF CLAY IN THE ESTIMATION OF HUMUS.

BY C. A. MOOERS AND H. H. HAMPTON.

Received March 6, 1908.

That a serious error may be introduced into the estimation of humus by the official method has been pointed out by a number of investigators. The chief cause of this error has been the weighing of clay with the humus extract and the consequent reckoning of the combined water in the clay as humus. To avoid this difficulty, Cameron and Breazeale[1] filtered the extract through a Pasteur-Chamberland filter and determined the humus in the clear filtrate; Peter and Averitt[2] have suggested the use of a factor with which to make a correction for the loss in the clayey residue; and a third, or evaporation method has been used by the authors.[3] This paper presents a comparison of the results obtained by the three methods.

In the filtration method the ammoniacal humus extract is filtered through a Pasteur-Chamberland filter, which retains all the clay, and the humus is estimated as usual, by evaporation, etc., of the clear filtrate. In getting the results here reported, a silver-plated, containing tube was used on account of the ready solubility in ammonia of the copper of the usual brass tube.

By the factor method Peter and Averitt make a deduction from the total loss in weight of 10 per cent. of the residue left after the humus has been burnt off. In Table I are given the results obtained by making both a 10 per cent. and a 14 per cent. deduction.

In the evaporation method the ammoniacal humus extract containing clay in suspension is evaporated to dryness over a steam bath, by which means the clay is flocculated so that after extraction with 4 per cent. ammonia it can be retained on a common filter paper. Two evaporations and filtrations are necessary as a rule in order to get a clear filtrate, in which the humus is determined as usual.

The percentages in the first column, obtained with clay present, are not only liable to be irregular, as is shown under 636, but are undoubtedly too high even when the clay is allowed to settle out for several weeks before taking out the aliquot portion for evaporation, as was done for Nos. 602 and 666.

[1] THIS JOURNAL, **26**, 29–45.
[2] *Ky. Sta. Bull.*, No. **126**, pp. 63–126.
[3] *Tenn. Sta. Bull.*, Vol. **19**, No. 4, p. 50.