THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Paul Francis Sharp final oral examination for the degree of Master of Science . We recommend that the degree of Master of Science be conferred upon the candidate.

Minneapolis, Minnesota

June 3 1920

C H Bailey
Chairman

F. H. Macdougall

J. B. McClendon

C. A. Morrow.

R. W. Thatcher

THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Paul Francis Sharp for the degree of Master of Science. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science.

R A Gortner
Chairman

P H MacDougall

R. W. Thatcher

C. A. Bailey

June 3 1920

SOME PHYSICO-CHEMICAL PROPERTIES OF THE GLUTENS FROM STRONG AND WEAK FLOURS.

By Paul Francis Sharp.

A THESIS

Submitted to the Graduate School of the University of Minnesota in partial fulfillment of the requirements

For the Degree

of

Master of Science.

St. Paul, Minnesota.

May 1920

TABLE OF CONTENTS

I INTRODUCTION

It is a well established fact, especially among millers and bakers, that the wheats grown in the north central portion of the United States produce flours of superior baking qualities to those grown in the southern and western sections. Those flours of good baking quality are called "strong", those of poor baking quality are called "weak". Two definitions of "strength" which are commonly accepted and are essentially the same are, first, that of Humphries and Biffen (1907).

"A strong wheat is one which yields flour capable of making large, well-piled loaves; the latter qualification thus excludes those wheats producing large loaves which do not rise satisfactorily."

The second defination is that given by Jago and Jago (1911).

"strength . . . . . is defined as the measure of the capacity of the flour for producing a bold, large-volumed, well-risen loaf."

Those flours which do not meet the above requirements are called "weak."

We thus see that the value of wheat for bread making largely depends on the strength of the flour that can be milled from it.

II HISTORICAL

A great number of investigators have tried to find the factors influencing flour strength, investigating almost every known factor that might possibly have anything to do with the problem. As soon as one investigator thought he had the problem solved, another worker would promptly show that his limiting factor did not hold in all cases.

Blish (1916) gives an excellent review of the different factors investigated in the effort to find some factor which influenced flour strength.

The strength of flour was early attributed to the gluten, and thus the laboratory test of determining the crude gluten was developed. This test consists essentially of washing the starch from 15 grams of flour in a stream of water, the doughy mass that remains being the "crude gluten". The crude gluten is weighed in both the wet and dry state. This test is of little value as ordinarily employed in view of the fact that both the strong and weak flours give practically the same amount of crude gluten and in view of the fact that Snyder (1901) was able to add up to 30% starch to the flour without decreasing its baking quality. Snyder also states that it is the "quality" rather than the "quantity" of gluten that is the determining factor.

This difference in quality of the crude gluten has been noted by a number of investigators. Gortner and Doherty (1918) mention that the strong flour gave a firm coherent gluten as did a medium flour. The three weak flours investigated "gave a more friable, sticky, and less coherent gluten which was much more

difficult to obtain than was the gluten from the other two flours".

The ratio of gliadin to glutenin was investigated and thought to be a limiting factor by Fleurent (1896) who claimed that his experiments showed the optimum to be 75% gliadin and 25% glutenin or 3:1. Snyder (1899) thought the ratio was 65-35.

Blish (1916) concludes from a careful series of experiments that "the ratio of gliadin to glutenin is much more nearly constant in flours of different baking qualities than has heretofore been supposed". He also concludes that, "the individual proteins of strong and weak flours are identical in their chemical constitution as shown by Van Slyke's method for the analysis of proteins".

The enzyme content, the soluble protein content and the hydrogen ion concentration in relation to strength have also been studied by various investigators without throwing any positive light on the subject.

With the comparatively recent development of colloid chemistry particularly the study of emulsoids, the attention of investigators on the subject of flour strength has been turned to the study of the colloidal factors that might influence the baking quality of the flour. Their attention naturally turned towards the gluten because it had been shown that the glutens from the strong and weak flours differed in physical appearance. Wood (1907) and Wood and Hardy (1908,1909) were the first to apply the methods used in the study of emulsoid colloids to the gluten from wheat flour. Hofmeister (1890) had previously studied the effects of acids, bases and salts on the imbibition of

of emulsoids.

Wood (1907) extended these studies to the gluten from wheat flour. He was the first to attribute the differences between strong and weak flours to the physical properties of the proteins rather than to their chemical properties. This conclusion was reached only after a careful investigation of the chemical properties such as, total nitrogen, total gliadin nitrogen, amide nitrogen in the gliadin, ratio of gliadin to total protein, total ash, total soluble ash, acid, and carbon dioxide produced in several different flours. He thought that the size of the loaf was influenced by the amount of fermentable sugars present which would regulate the amount of carbon dioxide evolved.

In their study of the effect of acids, bases and salts on the gluten, Wood (1907) and Wood and Hardy (1908,1909) suspended strings of gluten across V shaped glass rods and immersed these strings of gluten in beakers containing the solutions to be investigated. They found that if the gluten was suspended in distilled water it retained its coherence almost indefinitely but if suspended in dilute hydrochloric acid N/1000 it immediately began to disperse, the rate of dispersion increased as the concentration of acid increased up to about N/30, as the concentration is increased from this point the dispersion rate decreases until it finally became even more coherent in N/12 hydrochloric acid that it was originally. It was noticed that with sulphuric, phosphoric, and oxalic acids, dispersion took place even in high concentrations. Wood (1907) also noticed that if salts were added to the acid solutions the effect of the acid was in a large measure counteracted. He concludes from his experiments that -

"the variations in coherence, elasticity, and water content, observed in gluten extracted from different flours, are due rather to varying concentrations of acid and soluble salts in the natural surroundings of the gluten than to any intrinsic difference in the composition of glutens themselves".

Olson (1912) studied the effect of modifying the gluten surrounding of the flour. The method he used was to dough up 10 grams of the flour with 6 cubic centimeters of the solution being studied and after the dough stood one hour the gluten was washed out with distilled water and the results tabulated under the heads of percent of gluten, weight of nitrogen in the gluten, percent of nitrogen in the gluten, percent of soluble nitrogen, and percent of total nitrogen calculated from the nitrogen in the gluten from the original flour as 100.

These determinations were carried out with the original flour, with flour that had been made up into a dough and then dried and remilled, with flour that had been made up into a dough and then dialyzed for about three days with frequent changes of water and then dried and remilled, and finally with flour that had been stirred up with a large volume of water and allowed to settle and the supernatant liquid decanted. This process being repeated several times, the material was then dried and remilled. He found that by mixing the untreated flour with N/10 solutions of acids, salts, and alkalies the yield of gluten was decreased, the effect being in the following order, sodium phosphate having least effect: sodium phosphate, sodium chloride, magnesium sulphate, potassium phosphate,

calcium phosphate, aluminum sulphate, sodium hydroxide, potassium hydroxide, sulphuric acid, phosphoric acid, and hydrochloric acid. It was found that the mere doughing up and drying process changed the character of the gluten to a small extent. The glutens which had been dialyzed showed marked differences from the original flour, the gluten yield being smaller; in the case of the decanted flour it was almost impossible to collect the gluten at all and values were only obtained in the case of sodium and potassium hydroxide, and sodium sulphate and magnesium sulphate. The gluten could not even be collected when treated only with water. Olsen states that acids have a greater prejudicial effect than alkalies and of the alkalies, calcium hydroxide has a greater prejudicial effect than sodium or potassium. He concludes, "there is as yet some unknown substance which is important in causing a transformation of the physical properties of the gluten".

Gröh and Friedl (1914) made a careful study of the physical properties of the gliadins separated from the strong and weak flours. They studied the following physical-chemical properties of gliadin solutions of the different flours in potassium hydroxide: viscosity, surface tension, specific rotation, gold number, refraction, and the time it took a gliadin solution when shaken with a platinum sol to decompose half of a known amount of hydrogen peroxide. They separated the alcohol soluble protein of flour into four fractions and found them all to be the same within experimental error but the first which was shown to contain impurities. They show that the physical constants of the gliadins separated from four flours of different strengths are the same.

They conclude that the differences in strength are colloid chemical and that these differences are changed either by the extraction with alcohol or by the treatment with potassium hydroxide. They obtained from rye an alcohol soluble fraction which agrees with wheat gliadin in the above mentioned physico-chemical properties.

Upson and Calvin (1915,1916) studied the imbibition of gluten as influenced by acids, and acids in the presence of salts. A more exact method was used than that of Wood and Hardy. The gluten was washed from the flour and pressed into sheets from which discs were cut with a large cork borer. The discs were then weighed and placed in the various solutions for a given period of time when the discs were removed and again weighed. The results were calculated to increase in grams per gram of moist gluten. They confirmed the findings of Wood and Hardy, finding the greatest imbibition in the case of hydrochloric acid at moderate concentrations N/100 while with high concentrations N/5 to N/2 the solution actually took water from the gluten. They found that in the case of lactic and acetic acid this effect was not so marked at the higher concentrations, in the case of the lactic acid the swelling being almost as great in N/2 as in N/200 solution. They also showed that in the presence of acid and salts the swelling will decrease almost in proportion to the amount of added salt. They also demonstrated the reversibility of the action. Discs that had more than doubled in weight after remaining in acetic acid for two hours, when placed in N/10 dipotassium phosphate for one hour not only resumed their original weight but their original appearance and physical properties of toughness and elasticity as

well. Upson and Calvin (1916) also studied the effect of the use of various solutions of salts in the wash water in the crude gluten test and concluded that the best yield was given with carbon dioxide free water. Wood and Hardy (1909) had pointed out that there was enough carbon dioxide in the ordinary distilled water to affect the gluten. Upson and Calvin (1916) conclude, "that the bread making qualities of dough made from wheat flour are dependant on the quantity and quality of the contained gluten. Quality of gluten is regulated by the kind and concentration of the acids and salts present in the dough. If the kind and amount of acids and salts are such as to favor water absorption, the quality of the gluten will be poor, whereas the presence of acids and salts in such amounts as to tend to inhibit water absorption makes for an improved gluten."

Fisher (1915) did a tremendous amount of work on the swelling of emulsoids working with animal proteins, the largest series of his experiments being conducted with fibrin. He studied the effect on the imbibition in both acids and alkalies caused by the addition of salts and non-electrolytes. He found that the addition of non-electrolytes had but little effect, on the swelling of the colloids studied either in acids or alkalies on the other hand, the addition of salts markedly effected the imbibition causing a marked decrease.

An investigation which throws more light of a positive nature on the whole subject of flour strength than any other, was that carried out by Gortner and Doherty (1918). These investigators applied the method used by Upson and Calvin to flours of different strengths. The other workers in this field had worked

only with one type of gluten and had drawn conclusions for the other types. Gortner and Doherty found that the glutens from the strong flours had a greater rate of imbibition than the glutens from weak flours. This difference was noted in solutions of the following acids, hydrochloric, acetic, lactic, ortho phosphoric, and oxalic. They tried the effect on the swelling rate of five different glutens in various concentrations of the above named acids alone and, in the case of three of the glutens, the effect of the hydrochloric and lactic acids in the presence of M/200 solutions of potassium chloride, potassium phosphate, potassium tartrate, calcium chloride, mercuric chloride, aluminum sulphate, and magnesium sulphate, and in addition the effect of the last three named salts with oxalic and acetic acids. While the effect of the addition of salts to the various acids cut down the imbibition rate, the ratio of the effect on the strong and weak glutens remained about the same, that is, the strong flour glutens still had a higher rate of imbibition than the weak glutens. This is the first instance where a marked difference between the glutens from strong and weak flours in any chemical or physical property has been shown, except, of course, the appearance of the gluten. The results of Gortner and Doherty definitely refute the conclusion of Upson and Calvin regarding flour strength in that the crude glutens investigated by both Gortner and Doherty and by Upson and Calvin contained almost exactly the same amount of moisture. This would not be the case if the conclusion of Upson and Calvin were true. The differences in the imbibition shown by Gortner and Doherty are so great that they could not

possibly be correlated with the original moisture content of the wet gluten. These authors show that in addition to there being a marked difference in the rate of imbibition, there is a marked difference in the maximum hydration capacity, the weak flour gluten going to pieces much sooner and with a much lower water content than the strong flour gluten. These results also refute the contention of Wood and Hardy (1909) that the difference between them is due to the strength of an electric double layer. If this were true the glutens would all have the same imbibition rate but it would be found in different concentrations.

Gortner and Doherty conclude that the glutens are different even at the isoelectric point and that, "the difference between a strong and weak gluten is apparently that between a nearly perfect colloidal gel with highly pronounced physico-chemical properties, such as pertain to emulsoids, and that of a colloidal gel in which the properties are much less marked. It is suggested that such differences may be due to the size of the gluten particles and that at last a part of the particles comprising the weak gluten may lie nearer the boundary between the colloidal and crystalloidal states of matter than is the case with the stronger glutens."

III EXPERIMENTAL

The Problem

The object of this investigation was to continue the work on flour strength along the line begun by Gortner and Doherty to see if it were possible to correlate any other physico-chemical property with the glutens from flours of different strengths.

The following physico-chemical properties of the glutens were studied.

1. Imbibition in the presence of different strengths of lactic and hydrochloric acids.

2. Imbibition in the presence of different strengths of the following alkalies: potassium hydroxide, sodium hydroxide, calcium hydroxide, barium hydroxide, and ammonium hydroxide.

3. The effect on the imbibition rate of potassium and calcium hydroxide containing M/200 sodium sulphate.

4. The gold number of solutions of the different glutens in .005 N potassium hydroxide.

5. The specific conductance of the gluten ions.

6. The combining capacity in equivalents per gram of gluten.

7. The viscosity of the different glutens dispersed in both N/100 potassium hydroxide and N/100 lactic acid.

8. The isoelectric point of the glutens.

MATERIAL USED.

The strong flour used B-780 was milled from northern spring wheat and was an especially high grade patent flour prepared for a select trade. B-781 is a clear flour made from northern spring wheat. The weak flours used were B-782 a patent milled from a soft western wheat grown around Waitsburg, Washington, and B-783 a patent milled from soft club wheat grown at Genessee, Idaho. They showed the values for ash, total protein N x 5.7 and dry matter in the wet gluten given in Table I.

Table I.

Sample	ash	total protein	% dry matter in wet gluten average of five
B-780	.435	11.69	32.51
B-781	.90	13.74	37.55
B-782	.49	9.46	36.63
B-783	.53	10.15	35.25

The gluten was easily obtained from sample B-780 and was a firm elastic mass that could be separated from the starch very rapidly. More difficulty was evidenced in collecting the gluten from B-781, it was rather intermediate between B-780, and B-782 and B-783 the glutens from these last two flours could be collected only with extreme difficulty. They were whiter, it was harder to separate them from the starch, and they were not firm or elastic.

THE DRIED GLUTENS.

It is easily seen that if the gluten could be washed from a large quantity of flour and dried, that this dried material would be much more convenient to work with especially in carrying out some of the above named determinations, therefore the attempt was made to dry quantities of the glutens washed from the four

flours.

It is well known that emulsoid colloids will not withstand the effect of high temperatures so the drying must necessarily be carried out at a temperature that would not be at all unfavorable to bacterial and enzyme action. The first attempts to dry the gluten were made in a vacuum oven at a temperature of about 30° but the wet gluten retains the water rather tenaciously and the drying could not be accomplished at this temperature before the gluten began to decompose as evidenced by a bad odor. It was finally found necessary to dry at a temperature of 45-50°. The method finally adopted for preparing the dried gluten was as follows:

About 3 kilos of flour were made into a stiff dough with distilled water and allowed to stand under distilled water for at least an hour. The material was then placed in a dough mixing machine which was run by an electric motor. Distilled water under considerable pressure was run continuously into the mixer at rather a rapid rate. At the end of about 7 minutes all of the starch appeared to be removed, the liquid from then on remaining only slightly turbid, the treatment was continued for 13 minutes longer, the water by this time had begun to froth slightly. This material was then pressed out between glass plates into sheets 3 mms. thick and was cut into small squares weighing less than 0.5 grams. Glass plates were used for shelves in the vacuum oven and these little squares were placed on the plates at least 1 centimeter apart. The material was then placed in the vacuum oven and dried at a temperature of 45-50°, the gage on the oven reading around 27 inches.

It was possible to dry the material to a crisp in about 18 hours by this method, the drying was continued for a total of 48 hours, however. This material showed no evidence of decomposition that could be detected by odor. The dried material was then placed in a ball mill and ground to a fine powder. The powder so obtained was rather hydroscopic and showed a tendency to cling to the mill. The dried material was preserved in tightly stoppered glass bottles. Moisture determinations were made on the dried material and the four samples gave the results in Table II in terms of percent of moisture contained in the original. The results are the average of two determinations.

The percentage of protein, nitrogen x 5.70, was determined for the four dried glutens. The results are given in Table II.

Table II.

Sample	Percent Moisture	Percent of protein in dried gluten.
B-780	6.286	79.42
B-781	5.889	75.28
B-782	7.304	56.27
B-783	7.129	65.86

1. Imbibition in the presence of different strengths of hydrochloric and lactic acids.

(a) The gluten before drying.

In order that the method of carrying out the imbibition determinations may be clearly understood, the procedure used in the case of the glutens which were not dried is described in detail.

The following concentrations of acids were employed N/2, N/5, N/10, N/25, N/50, N/100, N/200, N/500, 200 cubic centimeters of each being placed in beakers of 800 cubic centi-

meter capacity. The beakers were kept covered when not in use.

150 grams of flour were taken and mixed with about 90 cubic centimeters of distilled water or enough to make a dough. The dough was then allowed to stand under distilled water for about one hour. The starch was washed out in a stream of distilled water at room temperature, the washing took about 15 minutes. The gluten was then pressed out into a thin sheet about 3 millimeters thick between glass plates. After standing about 5 minutes, it was placed on a wet board and cut into discs with a large cork borer. These discs weighed about 0.8 grams. Enough discs for the entire determination (45) were cut at one time and were placed in distilled water and allowed to stand for about 20 minutes. This permitted them to come to equilibrium with the distilled water after cutting and allowed them to take up any water that might have been pressed out by the cutting and pressing process. It was found that discs so prepared did not change weight in distilled water for several hours. Five discs were then taken out of the water and placed on an inclined perforated porcelain plate and allowed to drain for exactly seven minutes, they were then weighed to the nearest milligram and placed in the acid. The discs were allowed to remain in the acid for exactly 30 minutes (this was the length of time found by Gortner and Doherty to give a maximum swelling in the weak flours without the gluten going to pieces), the discs were then taken out and allowed to drain 7 minutes as before and were then weighed. The results were calculated on the basis of increase in weight in grams per gram of moist gluten, or relative increase in weight on the basis of the wet gluten. In all of

the work 5 determinations were made and the average taken.

An example of the determination and the percentage gain in weight of the individual discs is given in Table III. The gluten used was B-780. The gluten after washing had stood for 18 hours under distilled water at a temperature of 20 degrees which was the temperature at which all of the swelling experiments were carried out. The room was one that held this temperature very well plus or minus 2 degrees.

Table III

B-780 in hydrochloric acid before drying. Stood 18 hours before determination. Imbibition period 50 minutes.

N/2	N/5	N/10	N/25	N/50	N/200	N/500
-.0065	+.030	+.183	+.602	+.810	+.830	+.685
-.0115	+.062	+.172	+.678	+.945	+.677	+.574
-.0024	+.038	+.170	+.782	+.870	+.848	+.580
-.0336	+.020	+.192	+.660	+.975	+.835	+.627
-.0034	+.027	+.214	+.702	+.842	+.723	+.755
-.0115	+.0354	+.190	+.685	+.890	+.783	+.645

It is seen that while some of the individual determinations vary, as a whole the agreement is good.

Determinations of the swelling capacity of the four glutens were made for hydrochloric acid before drying. See Table IV and Figure I.

Table IV

Glutens in hydrochloric acid before drying. Imbibition period 50 minutes. (average of five).

Strength of acid	Stood 18 hrs before testing B-780	Determinations made soon after washing out gluten.			
		B-780	B-781	B-782	B-783
N/2	-.0115	-.0347	-.024	+.028	-.035
N/5	+.0345	+.054	+.061	+.061	+.033
N/10	+.190	+.225	+.150	+.155	+.151
N/25	+.685	+.686	+.400	+.374	+.367
N/50	+.890	+.923	+.553	+.514	+.453
N/100		+.938	+.633	+.422	+.477
N/200	+.783	+.773	+.517	+.459	+.478
N/500	+.645	+.727	+.296	+.428	+.412

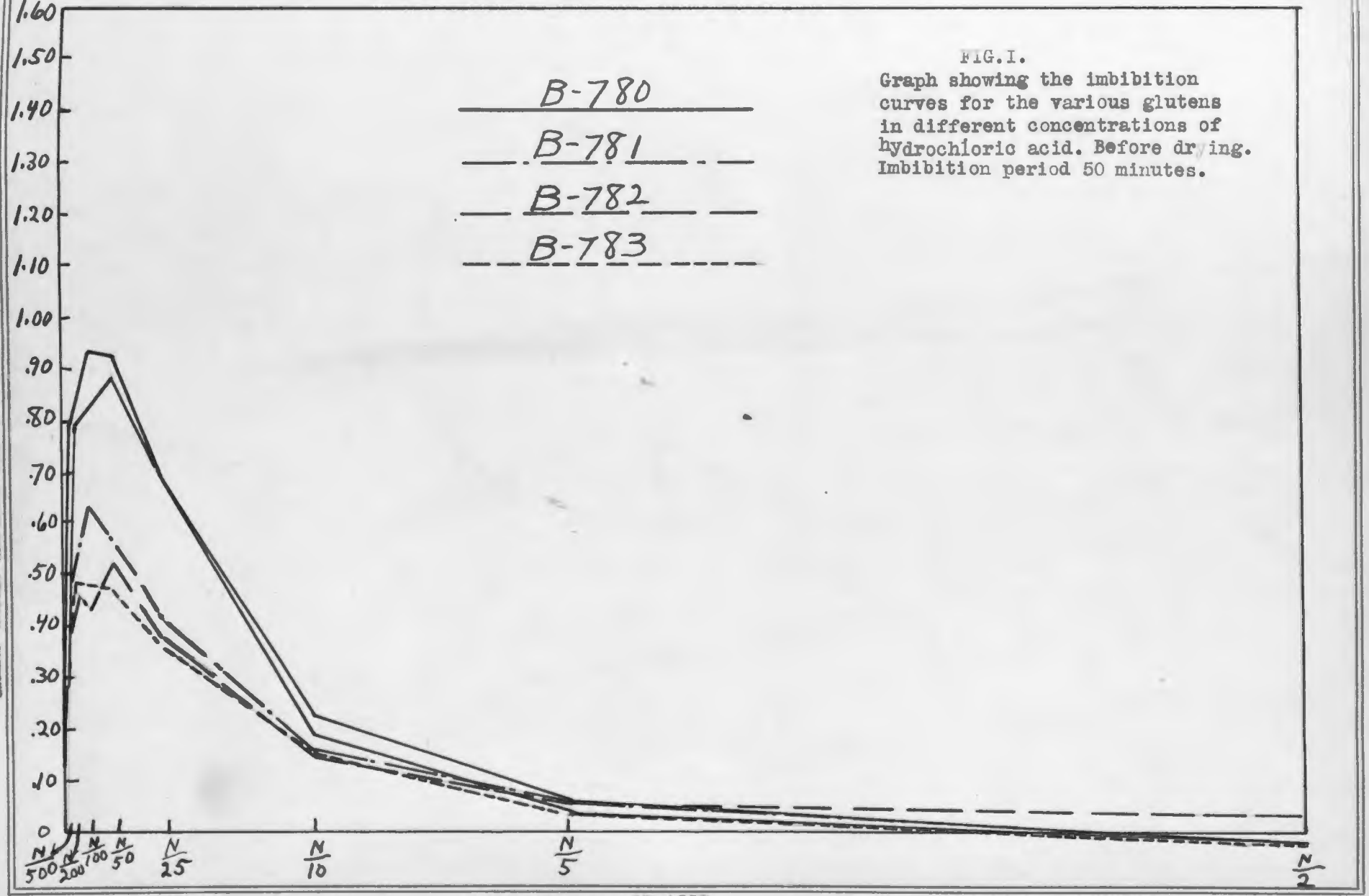


FIG.I.
Graph showing the imbibition curves for the various glutens in different concentrations of hydrochloric acid. Before drying. Imbibition period 50 minutes.

While the differences in the swelling capacity here is not very great yet there is a distinct difference between the gluten B-780 and the other three.

The swelling capacity of the various glutens was determined in lactic acid before drying. See Table V and Figure II.

Table V

Lactic acid before drying. Imbibition period 50 minute. (Average of five)

Strength	B-780	B-781	B-782	B-783
N/2	+1.328	+.669	+.546	+.406
N/5	+1.395	+.701	+.687	+.476
N/10	+1.518	+.816	+.627	+.528
N/25	+1.552	+.603	+.580	+.572
N/50	+1.417	+.642	+.611	+.598
N/100	+1.080	+.367	+.487	+.521
N/200	+1.231	+.421	+.457	+.460
N/500	+.787	+.232	+.467	+.497

It is noticed that there is a wide difference in the imbibition capacity of the B-780 and B-781 and there is still a great difference between B-781 and B-782 and B-783. The difference between these glutens is more marked than that found by Gortner and Doherty. The strong flour B-780 being stronger than the one worked with by them and the weak flours were weaker.

(b) The gluten after drying.

The method used in this case of getting the dried gluten back to the wet gluten state was to take about 20 grams of the dried material and mix it with just enough water to make a dough. This dough was allowed to stand under distilled water for one hour. The gluten so obtained was much more elastic than the original gluten before drying, in order to obtain sheets it was necessary to press it out between the glass plates as thin as possible and weights had to be placed on it to keep it from coming back together after remaining for some time this way, additional pressure was

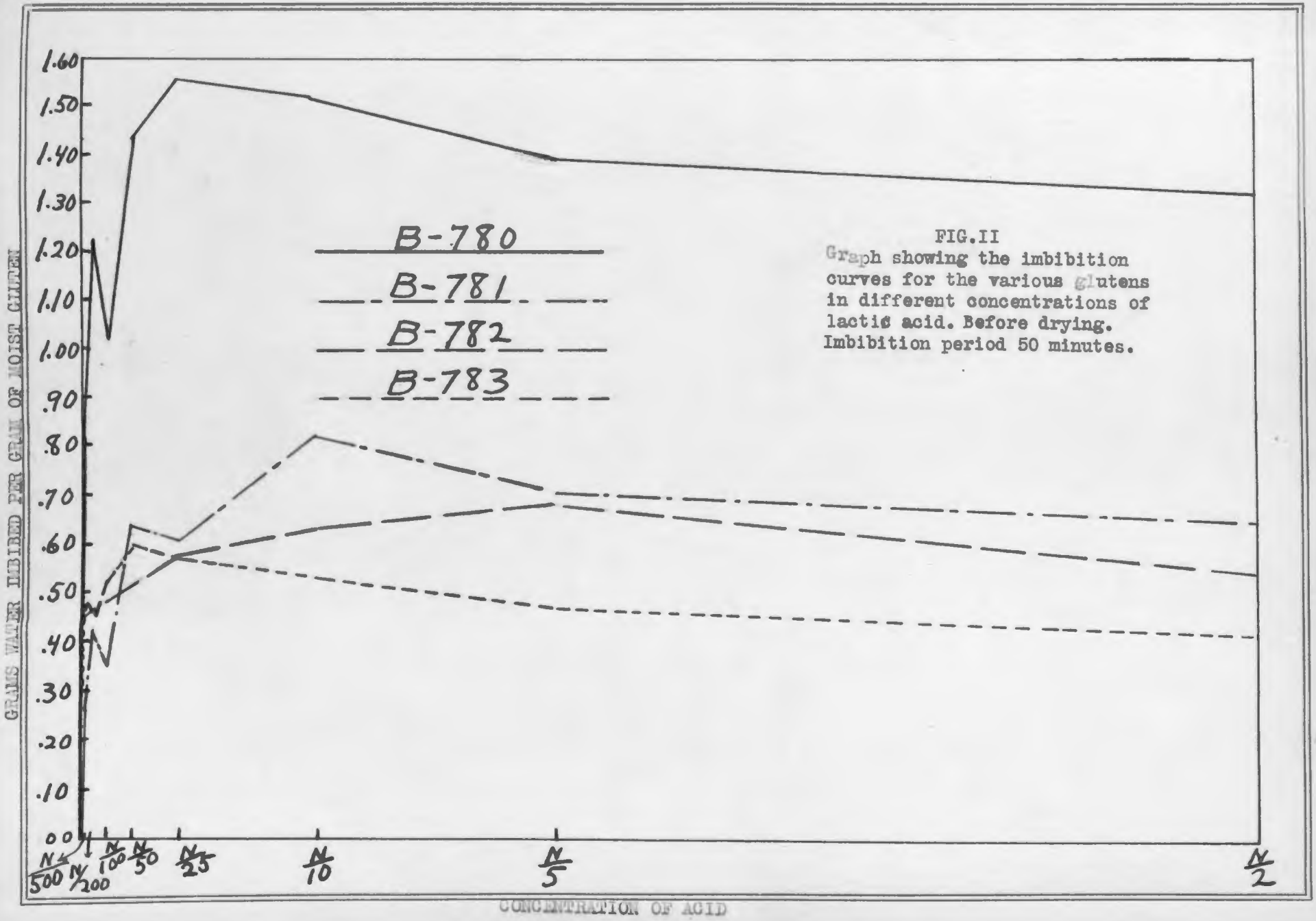


FIG.II
Graph showing the imbibition curves for the various glutens in different concentrations of lactic acid. Before drying. Imbibition period 50 minutes.

applied and the sheet could then be pressed a little thinner. This process was repeated until sheets of the desired thickness were obtained. Discs were then cut but they were not placed in water but went directly into the solutions. A more careful study of the dried material was carried out in the case of alkalies and data will be presented under that head showing more of the effects of drying.

The swelling capacity of the dried B-780 was tried in hydrochloric acid and it was found that the dried material did not differ much from the original in the case of this acid. The sample of dried B-780 used in this case, however, was dried at a lower temperature than the rest. It showed evidences of decomposition. The data is presented in Table VI.

Table VI.

B-780 in hydrochloric acid before and after drying. Imbibition period 50 minutes. (average of five).

Strength of Acid.	Stood 18 hours in water.	Used immediately.	Dried.
N/2	-.0115	-.0347	-.0398
N/5	+.0354	+.054	+.111
N/10	+.190	+.225	+.172
N/25	+.685	+.686	+.613
N/50	+.890	+.923	+.763
N/100	-----	+.938	+.952
N/200	+.733	+.773	+.847
N/500	+.645	+.727	+.584

As the differences found in hydrochloric acid between the glutens before drying were not very great the imbibition of the dried material in hydrochloric was not investigated further. In lactic acid, however, where the differences in the original glutens were great, the differences in the glutens before and after drying were also quite marked. The results obtained

with the dried material in lactic acid are given in Table VII, and Figure III.

Table VII.

Lactic acid after drying on glass plates. Imbibition period 30 minutes. (average of five).

Acid	B-780	B-781	B-782	B-783	B-783
water	+.039	+.063	+.026	+.047	+.037
N/500	+.320	+.453	+.424	+.404	+.303
N/200	+.647	+.365	+.381	+.543	+.488
N/100	+.791	+.435	+.389	+.620	+.452
N/50	+.920	+.556	+.409	+.675	+.508
N/25	+.887	+.625	+.407	+.771	+.597
N/10	+1.015	+.628	+.501	+.861	+.666
N/5	+.972	+.625	+.508	+.728	+.652
N/2	+.801	+.731	+.469	+.773	+.606

The main thing that was noted in this case is that the B-780 has decreased in its imbibition capacity in lactic acid to an appreciable extent, also B-781 has decreased somewhat but the other two did not change or if anything increased. This seems to indicate that both glutens were tending to become alike especially as the only marked change was in the one with the greatest imbibition power.

2. Imbibition in the presence of different strengths of the following alkalies: potassium hydroxide, sodium hydroxide, calcium hydroxide, barium hydroxide, and ammonium hydroxide.

(a) Before drying.

Fisher and found that when animal proteins were treated with different concentrations of alkali imbibition effects similar to those in acid were obtained. It was thought wise, therefore, to see if the glutens from the strong and weak flours showed the marked imbibition differences noted in acids when the glutens were treated with alkalies of different concentrations.

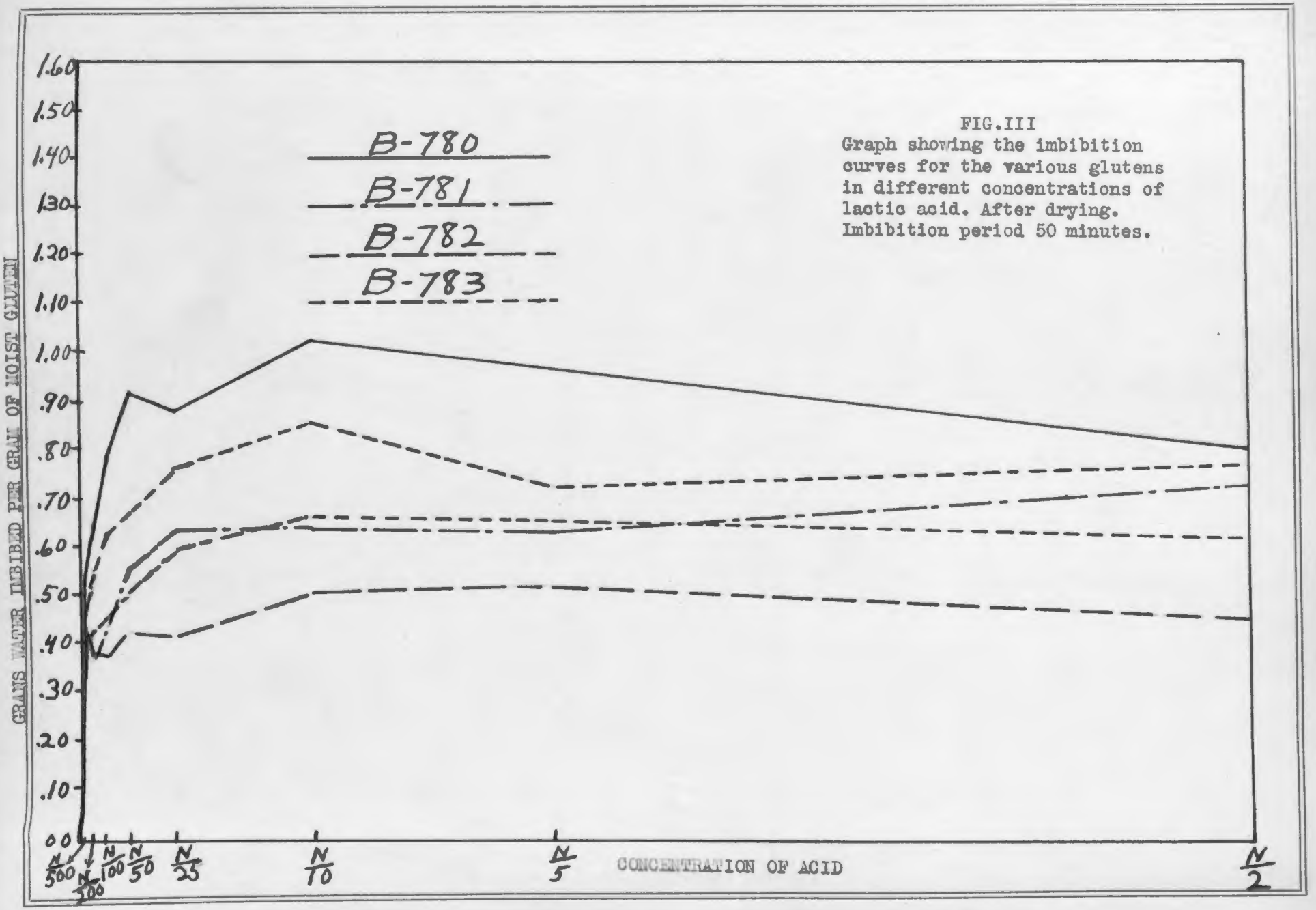


FIG.III
Graph showing the imbibition curves for the various glutens in different concentrations of lactic acid. After drying. Imbibition period 50 minutes.

A series of sodium hydroxide solutions was made up of the same strengths as those employed in the study of acids namely N/2, N/5, N/10, N/25, N/50, N/100, N/200, and N/500. The experiment was first tried with B-780, imbibition period 50 minutes. The discs placed in N/2 solution became colored a light yellow, they did not swell but seemed to grow smaller the rough edges becoming rounded off. When the attempt was made to remove them for weighing they went to pieces. The discs placed in the concentrations ranging from N/5 to N/25 did not have such a yellow color and the swelling was at first perceptible, the discs were not coherent enough to be removed for weighing. In the case of the remainder the discs were coherent enough to be removed for weighing. The results obtained are found in Table VIII.

Table VIII

B-780 in sodium hydroxide before drying, imbibition period 50 minutes. Average of five determinations.

Conc. of NaOH	N/50	N/100	N/200	N/500
Increase in gms. per gm. moist gluten.	-.191	-.086	+.133	+.201

We thus see that apparently the glutens swell more in the dilute alkali than they do in the more concentrated solutions. The time in alkali was the same as was used for the acids, i.e., 50 minutes. In all solutions but N/500 there was a perceptible cloudiness and a ring of white solid material (probably starch) settled around the discs. This ring was not present in the N/500 solution. In order to ascertain whether or not this was a case of complete dispersion or whether the alkali was merely causing loss of coherence the solutions of alkali used in the imbibition experiments were neutralized. A curdy precipitate

appeared in each solution excepting N/500. The quantity was roughly proportional to the concentration of alkali. This showed conclusively that the gluten was really being dispersed and by the amount of precipitate one would conclude that the process was going on rather rapidly.

It would seem that the action of alkalies was somewhat different from the action of the acids on gluten. In the case of acids the first step was the swelling of the gluten with no apparent dispersion, as (at most only a slight milkiness was shown) shown by bringing the solution to the isoelectric point of the gluten. In the case of acid there was marked imbibition as shown by appearance and by the increase in weight. In the case of alkali however the action seemed to be somewhat different the two steps imbibition and dispersion following one another almost immediately so that in some concentrations they compensated each other. In the more dilute solutions the imbibition factor was the more prominent, in the more concentrated solutions the dispersion factor was the most apparent.

As the imbibition in the case of alkali seemed to be greater in the more dilute solutions, concentrations of sodium hydroxide were tried ranging from N/1000 to N/10000. No imbibition was noted in these cases however, in fact there was always a slight loss in weight this loss was so slight that it is doubtful if it is signifigant.

In an attempt to make the imbibition factor more prominent the time for the swelling determination in the presence of alkalies was shortened from 50 minutes to 25 minutes. A comparison of the results obtained in these two time intervals

is given in Table IX and Fig IV.

Table IX

B-780 in sodium hydroxide before drying, comparison of imbibition periods of 50 minutes and 25 minutes. Average of five determinations.

Conc.of alkali	50 min.	50 min.	25 min.	25 min.
N/100	-.006	-.045	+.077	+.099
N/200	+.099	+.099	+.214	+.268
N/500	+.122	+.057	+.034	+.032
N/1000	-.036	+.015	-.010	+.003

It was concluded from Table IX and the swelling size as shown by appearance, that 25 minutes would indicate the imbibition factor more accurately

The imbibition of the different glutens was studied in connection with the following alkalies; potassium, sodium, barium, calcium, and ammonium hydroxides.

The results obtained with potassium hydroxide are found in Table X and are expressed graphically in Fig V.

Table X

Comparison of the imbibition of the different glutens in potassium hydroxide, imbibition period 25 minutes. Average of five determinations.

Conc.of alkali	B-780	B-781	B-782	B-783
N/100	+.138	+.208	-.084	-.109
N/200	+.328	+.072	+.008	-.011
N/500	+.042	.000	+.018	+.017
N/1000	+.001	-.023	-.012	-.029

In the case of potassium hydroxide the differences between the glutens from the different flours is vary apparent, the gluten from the strong flour having by far the greatest imbibition.

The results obtained with the different glutens in

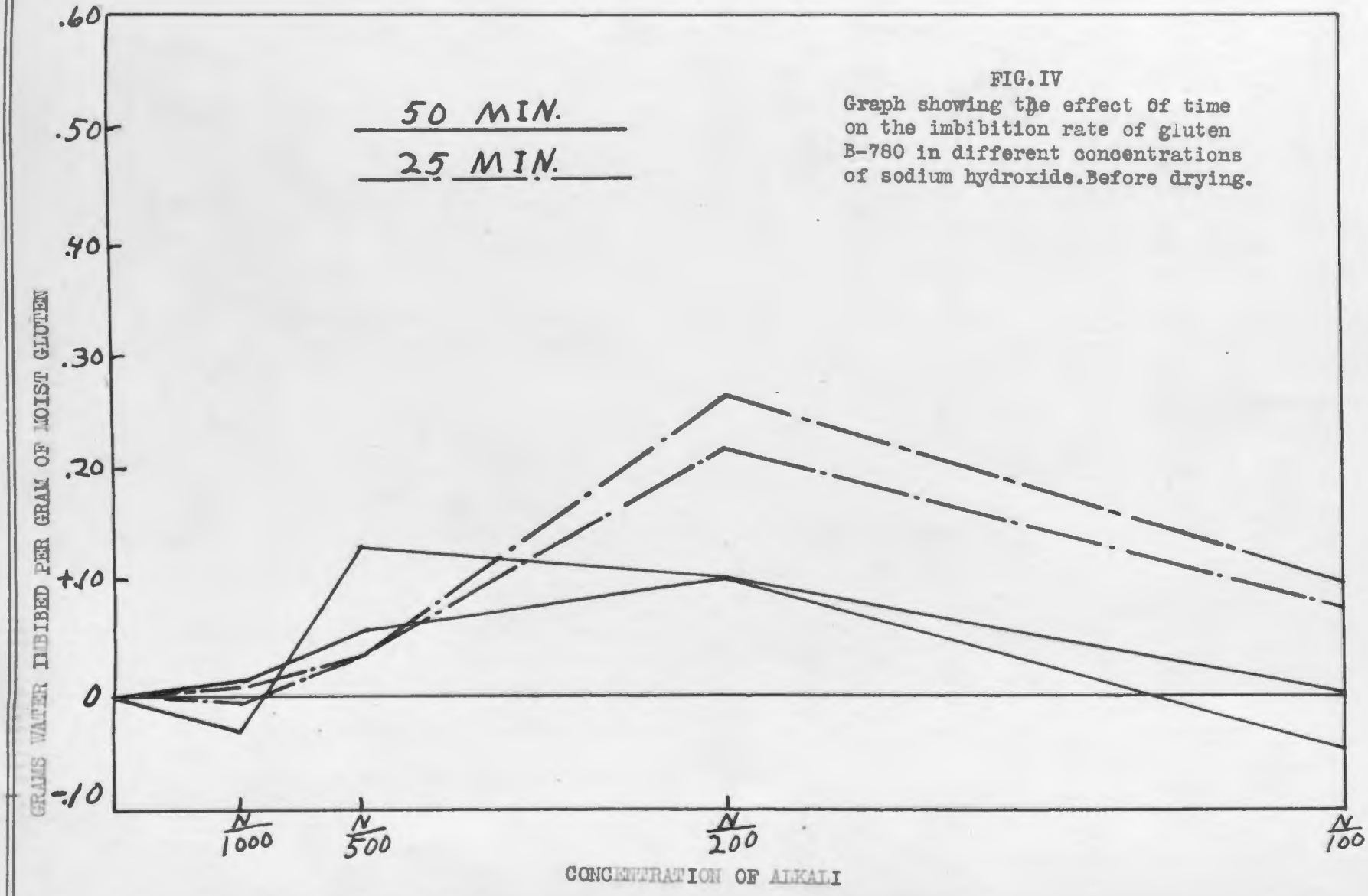


FIG. IV
Graph showing the effect of time on the imbibition rate of gluten B-780 in different concentrations of sodium hydroxide. Before drying.

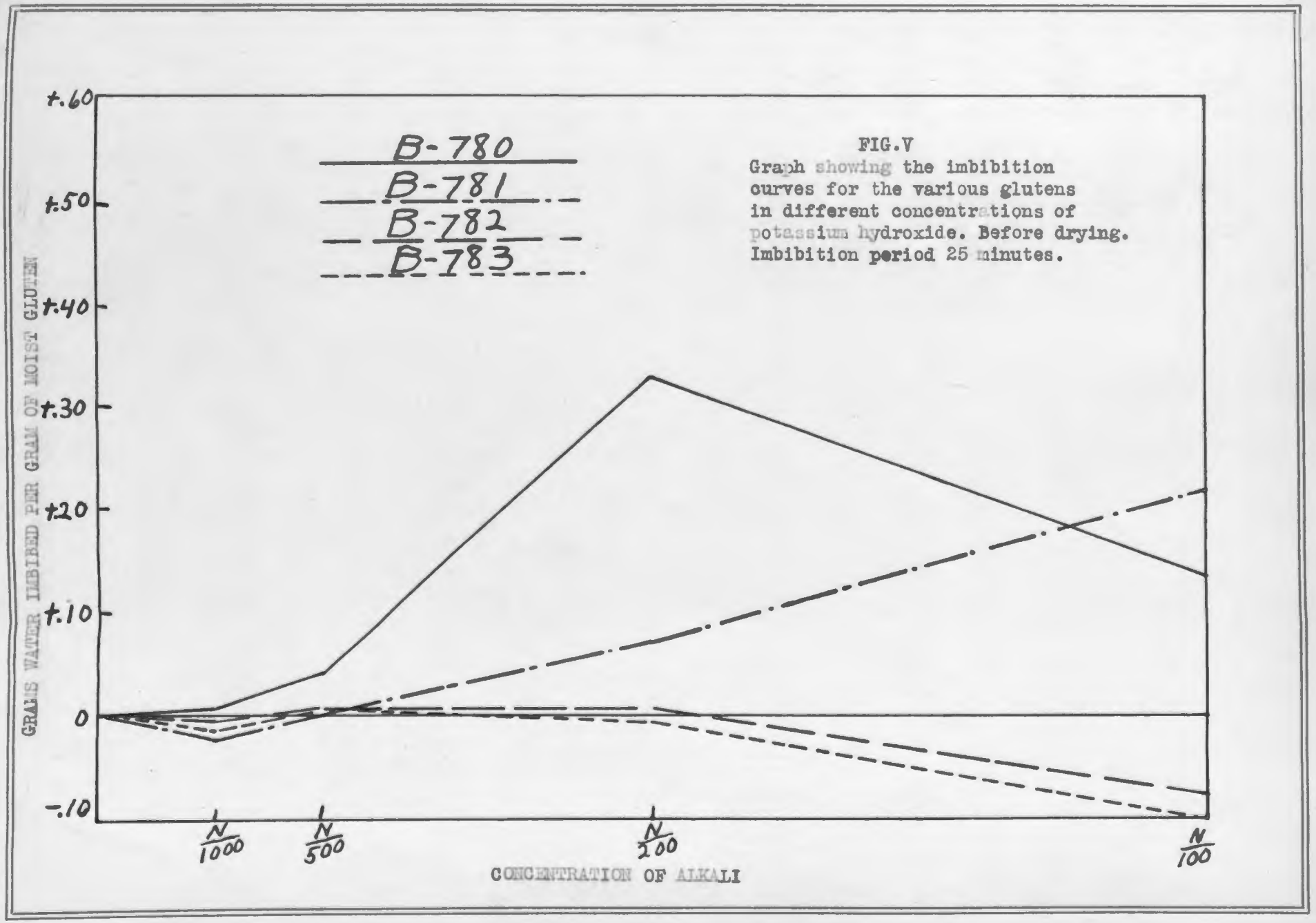


FIG.V
Graph showing the imbibition curves for the various glutens in different concentrations of potassium hydroxide. Before drying. Imbibition period 25 minutes.

sodium hydroxide are found in Table XI and Fig.VI.

Table XI

Comparison of the imbibition of the different glutens in sodium hydroxide, imbibition period 25 minutes. Average of five determinations.

Conc.of alkali	B-780	B-780	B-781	B-782	B-783
N/100	+.077	+.099	+.237	-.039	-.092
N/200	+.214	+.269	+.061	+.059	+.025
N/500	+.034	+.032	-.001	-.002	.000
N/1000	-.010	+.003	-.005	-.014	-.019

The results obtained in barium hydroxide are found in Table XII and Fig.VII.

Table XII

Comparison of the imbibition of the different glutens, before drying, in barium hydroxide imbibition time 25 minutes. Average of five determinations.

Conc.of alkali.	B-780	B-781	B-782	B-783
N/100	+.065	+.142	+.001	-.055
N/200	+.114	+.021	+.045	+.023
N/500	+.023	-.021	-.022	-.018
N/1000	+.016	-.011	-.022	-.009

The results of the measurement of the imbibition of the different glutens in calcium hydroxide are found in Table XIII and in Figure VIII.

Table XIII

Comparison of the imbibition of the different glutens, before drying, in calcium hydroxide imbibition time 25 minutes. Average of five determinations.

Conc.of alkali.	B-780	B-781	B-782	B-783
N/100	+.125	+.070	+.025	-.057
N/200	+.108	+.046	+.041	+.018
N/500	-.003	+.008	-.011	-.013
N/1000	-.015	+.002	-.021	-.016

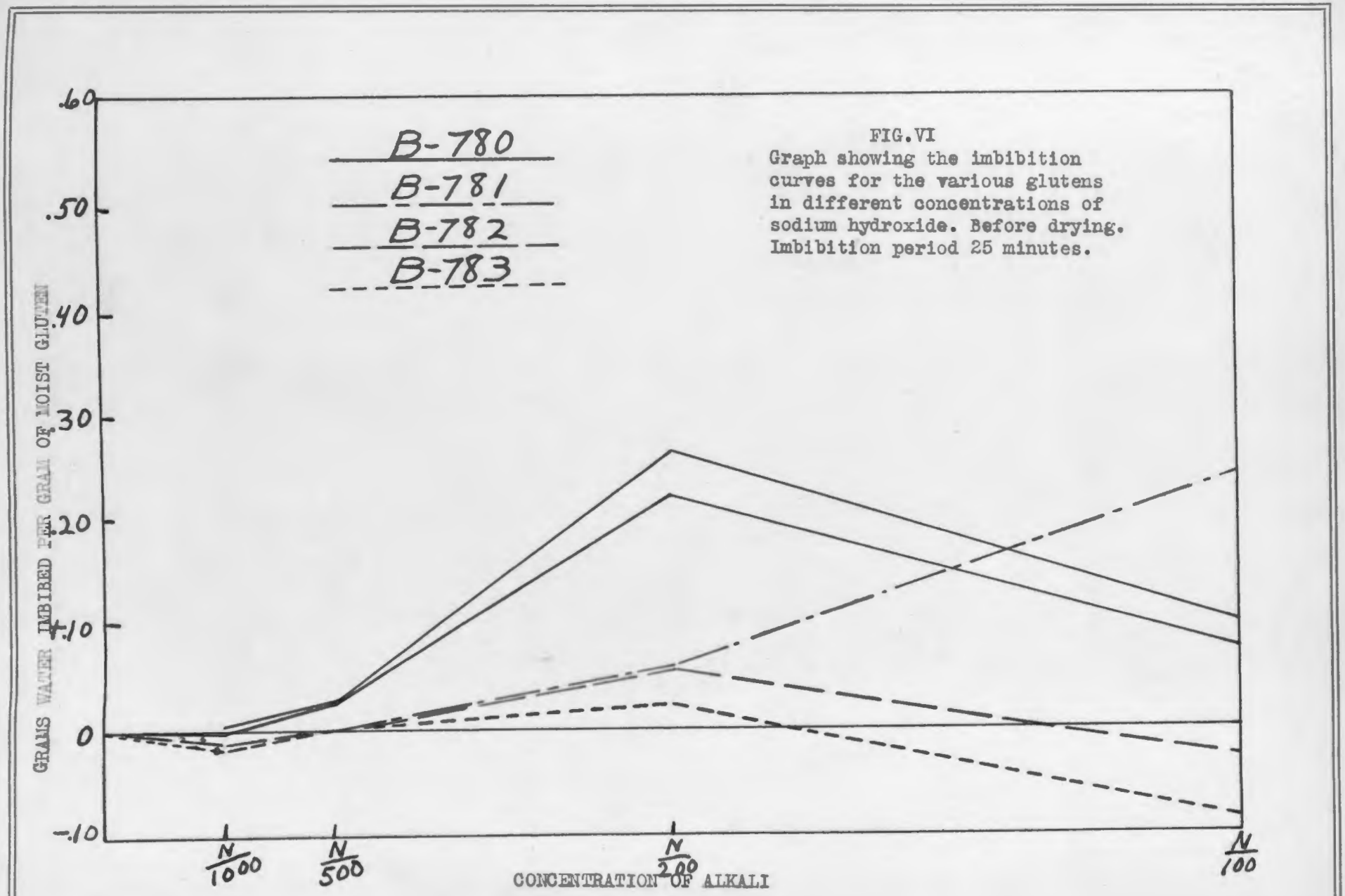


FIG.VI
Graph showing the imbibition curves for the various glutens in different concentrations of sodium hydroxide. Before drying. Imbibition period 25 minutes.

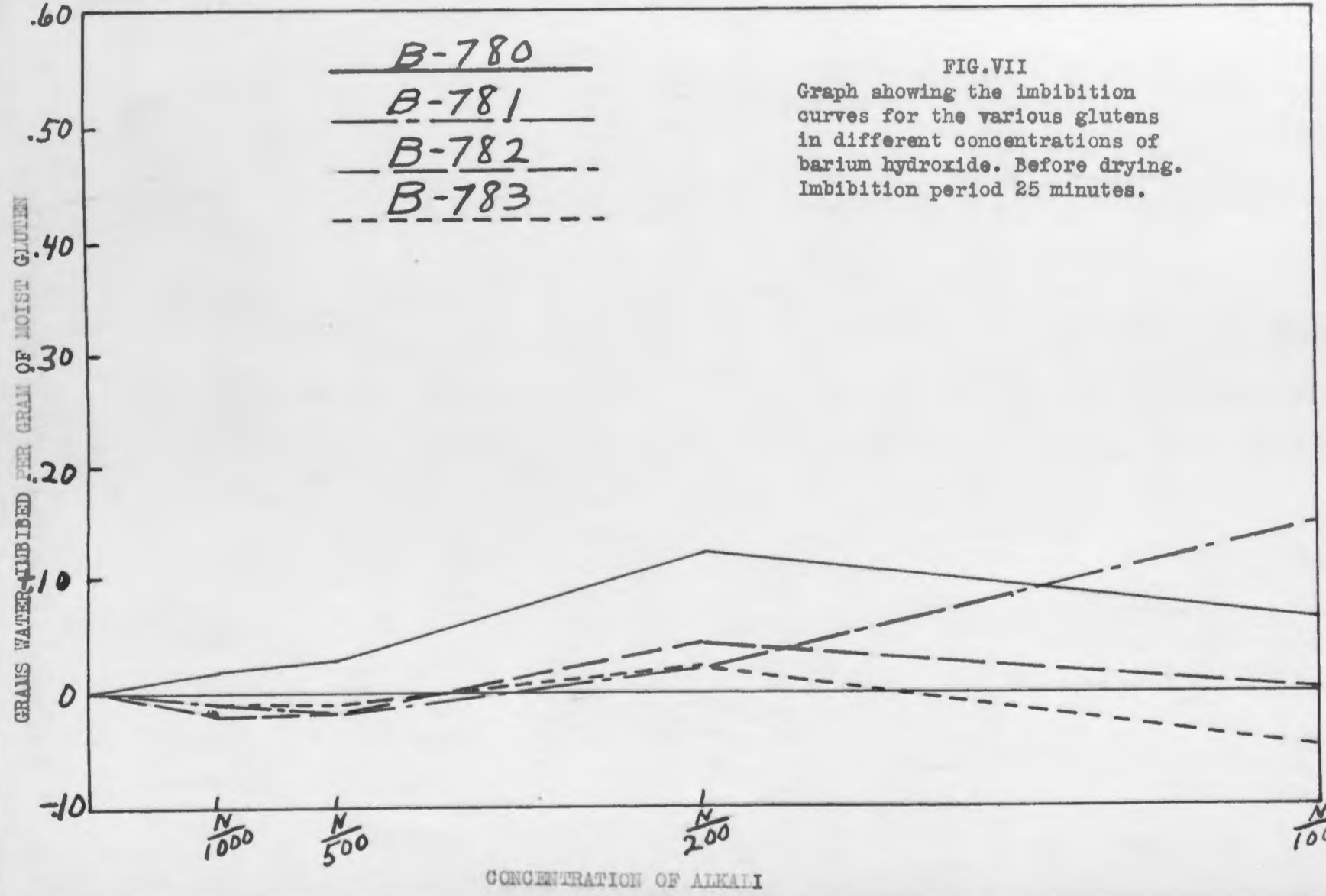


FIG.VII
Graph showing the imbibition curves for the various glutens in different concentrations of barium hydroxide. Before drying. Imbibition period 25 minutes.

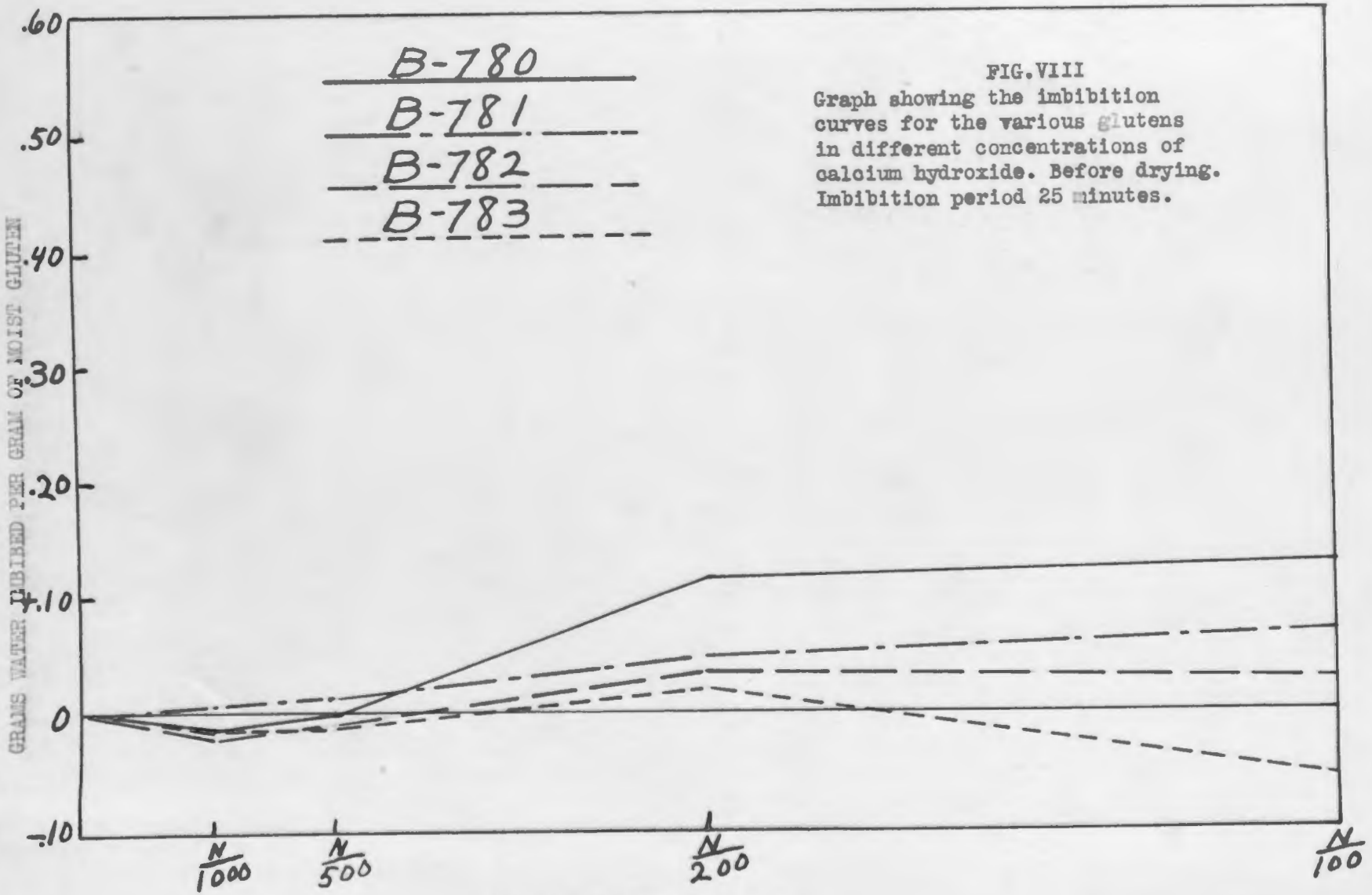


FIG.VIII
Graph showing the imbibition curves for the various glutens in different concentrations of calcium hydroxide. Before drying. Imbibition period 25 minutes.

The results obtained with ammonium hydroxide are found in Table XIV and Figure IX.

Table XIV

Comparison of the imbibition of the glutens before drying, in ammonium hydroxide imbibition period 25 minutes. Average of five determinations.

Conc. of alkali.	B-781	B-782	B-783
N/100	+.070	+.067	+.053
N/200	+.021	+.012	+.013
N/500	-.002	-.015	-.020
N/1000	-.014	-.020	-.012

These results on the imbibition of the different glutens in alkali show clearly that the strong gluten has a higher rate of imbibition than the other three. The differences are more apparent in the N/200 concentrations. In the higher concentration the dispersion factor is more prominent as shown by the fall in the curve. The clear flour B-781 does not behave like the others in this respect.

The discs from gluten B-780 that were placed in the N/100 alkalies dispersed more than these placed in the other concentrations. The discs placed in N/200 dispersed somewhat, those in the other two concentrations hardly at all. B-782 and B-783 behaved in all respects like the gluten B-780 with the exception that the dispersion was more marked in the case of B-782 and B-783. The clear B-781 did not disperse as readily as did the other three glutens. This would be indicated by the above Tables and is shown very clearly in Figures V toVIII where the curve for B-781 rises above the others in the N/100 concentration.

(b) Imbibition of the dried glutens in alkali.

In the first attempts to prepare wet gluten from the

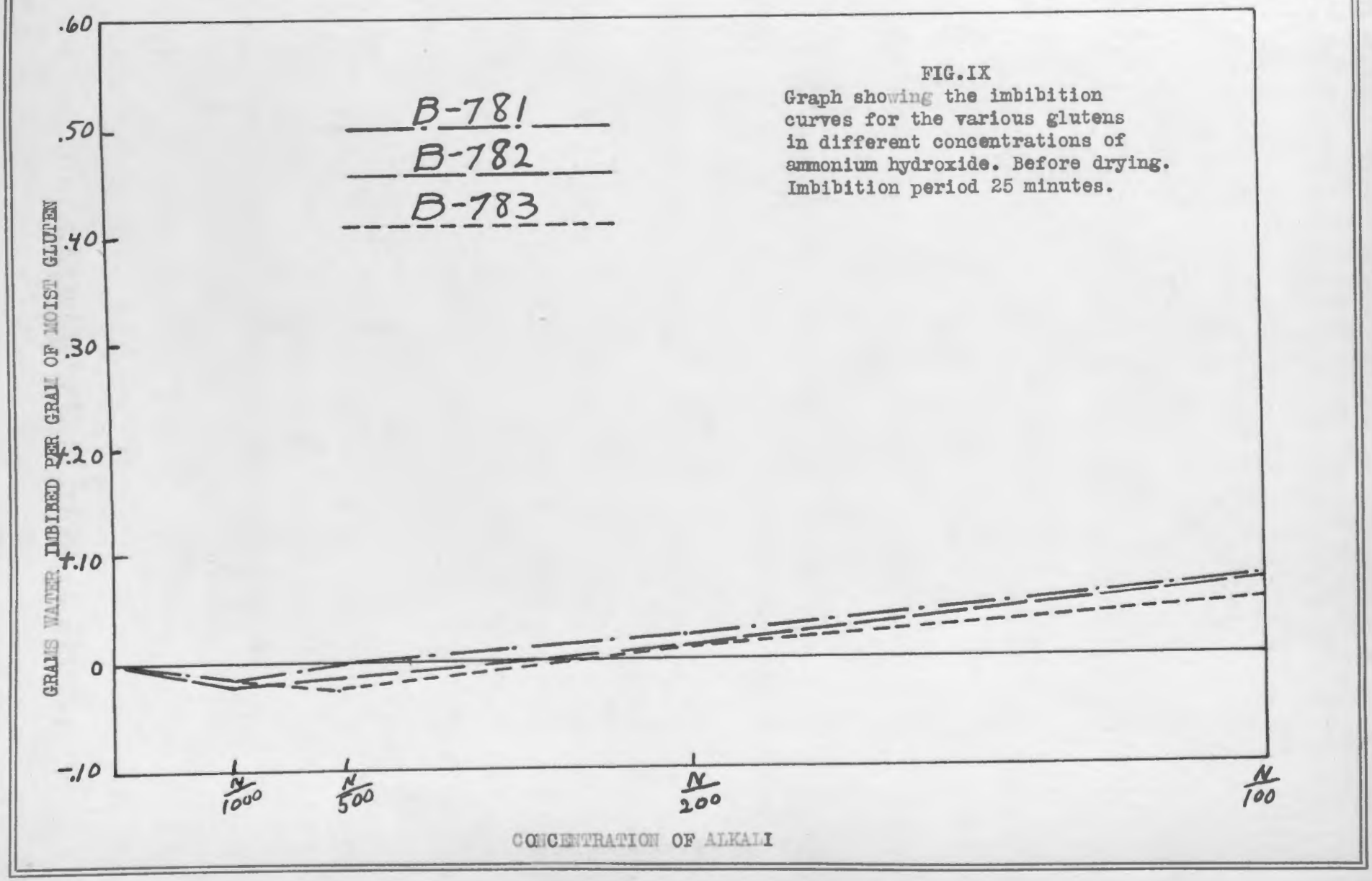


FIG.IX
Graph showing the imbibition curves for the various glutens in different concentrations of ammonium hydroxide. Before drying. Imbibition period 25 minutes.

dried material the dried gluten was treated in the same way that the original flour was treated, that is, enough water was added to theddried material to form a stiff dough, this dough was allowed to stand under distilled water for one hour and was then washed in a stream of distilled water. As soon as the washing was begun however the gluten began to disperse and in only one instance (the dried gluten used in the imbibition experiment with hydrochloric acid Table VI) was it possible to again work it into a coherent mass while washing with distilled water. This was tried repeatidly with the dried glutens from the different flours. It was found however, that if a very small amount of sodium chloride was added to the wash water the gluten immediately came together in a coherent mass which showed greater elasticity than the gluten before drying. If the coherant mass obtained by washing in sodium chloride solution was washed in distilled water it began to disperse but was easily brought to a coherent mass by again washing in the salt solution. This process was repeated several times with the same sample and probably could be repeated indefinitely. The method finally adopted was to add enough water to make a dough and let this dough stand under distilled water for one hour. The material was then pressed into sheets without working.

It was found that discs cut from gluten prepared in this manner varied in their behavior to the alkali according to the length of time the cut discs stood in water before placing them in the alkali solutions. The results obtained under the following conditions are found in Table XV and in Figure X.

A. Discs stood in water over night before placing in the alkali. B-780.

B. Discs stood in water one hour before placing in the alkali. B-780.

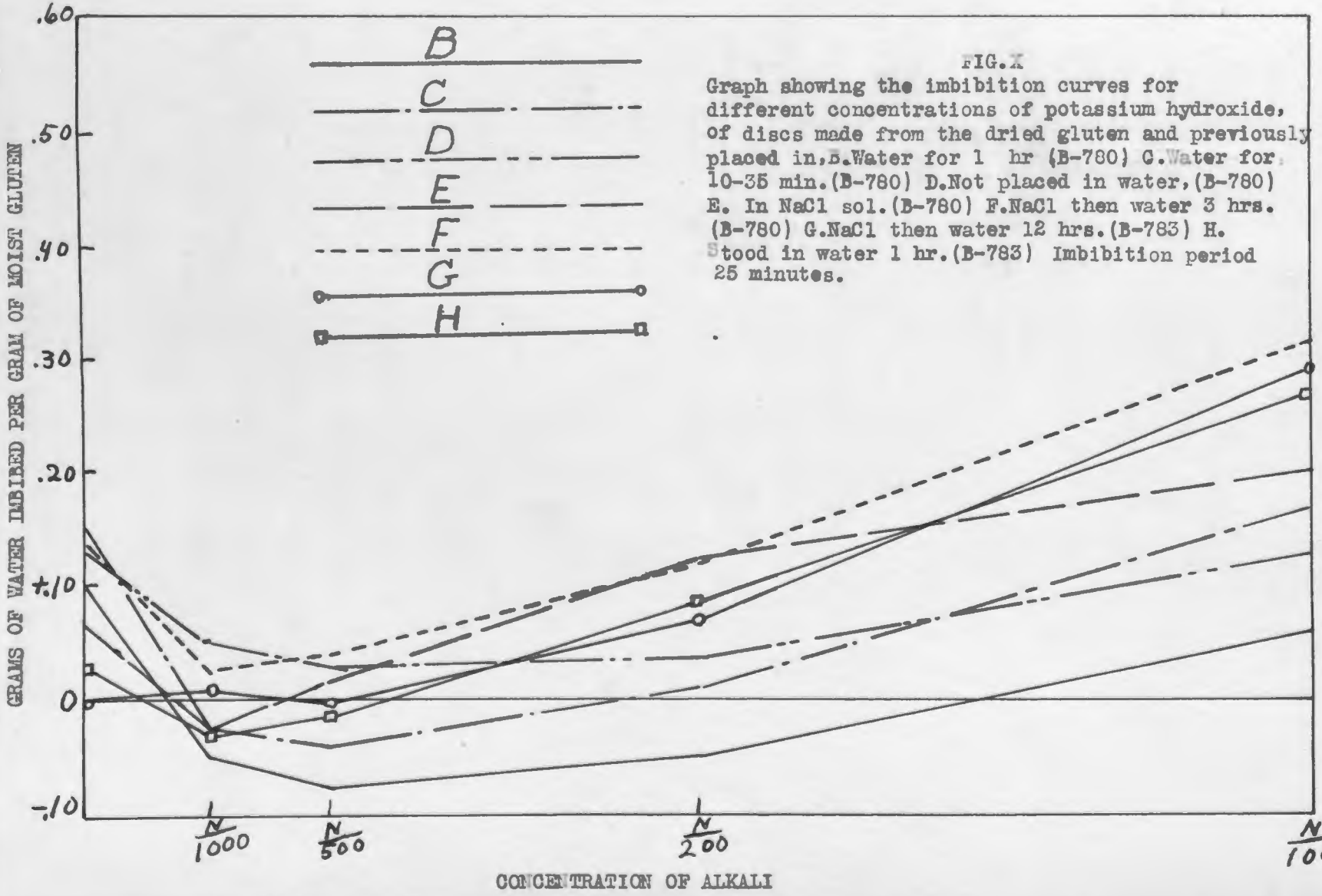


FIG.X

Graph showing the imbibition curves for different concentrations of potassium hydroxide, of discs made from the dried gluten and previously placed in, B.Water for 1 hr (B-780) C.Water for 10-35 min.(B-780) D.Not placed in water,(B-780) E. In NaCl sol.(B-780) F.NaCl then water 3 hrs. (B-780) G.NaCl then water 12 hrs.(B-783) H. Stood in water 1 hr.(B-783) Imbibition period 25 minutes.

C. Discs stood in water 10-35 minutes before placing in the alkali. B-780.

D. Discs were not placed in water at all. B-780.

E. Added sodium chloride to wash water after trying to wash in in distilledwater. B-780.

F. Added sodium chloride to wash water after trying to wash in distilled water, the cut discs were allowed to stand in distilled water three hours before placing in the alkali. B-780.

G. Washed with sodium chloride solution the cut discs were allowed to stand in distilled water over night. Considerable swelling took place in these discs on standing in water over night. B-783.

Table XV

Gluten, dried, imbibition in potassium hydroxide showing the effect of placing the discs in water for various lengths of time before placing in the alkali. Imbibition period 25 minutes. Average of five determinations.

Conc. of alkali.	B-780						B-783	
	A	B	C	D	E	F	G	H
H_2O		+.103	+.160	+.124	+.077	+.137	-.003	+.025
N/100		+.052	+.158	+.120	+.193	+.207	+.188	+.163
N/200	-.111	-.056	+.007	+.033	+.119	+.112	+.072	+.088
N/500		-.087	-.045	+.026	+.007	+.036	-.001	-.010
N/1000		-.054	-.019	+.051	-.019	+.025	+.008	-.021

H. Discs stood in water one hour before placing in alkali. B-783.

The effect of placing the discs of dried gluten from flour B-780 in water before making the determination of imbibition rate is shown very clearly in Table XV. in the N/200 concentration. The method finally adopted for the study of imbibition of the dried glutens was not to place the discs in water at all before placing them in the solutions to be tested. The losses in all cases with alkalies are not attributed to the dehydration of the gluten but rather to its dispersion. After the discs had been removed

for weighing the alkali was always neutralized and a precipitate was formed which appeared to be approximately proportional to the concentration of the alkali and therefore more or less proportional to the loss in weight of the discs.

Table XVI and Figure XI contain the results of the dried glutens with potassium hydroxide.

Table XVI

Imbibition of the dried glutens in potassium hydroxide. Imbibition period 25 minutes. Average of five determinations.

Conc. of alkali	B-780	B-781	B-783
H_2O	+.124	.000	+.045
N/100	+.120	+.068	+.175
N/200	+.033	+.017	+.061
N/500	+.026	+.025	+.018
N/1000	+.051	-.003	+.030

In order to directly compare the action of acids and alkalies the swelling in acids was carried out for 25 minute period in the same concentrations that were used for the alkalies. The results obtained with lactic acid are given in Table XVII and in Figure XII.

Table XVII

Imbibition of the different glutens in lactic acid before and after drying. Imbibition period 25 minutes. Average of five determinations.

Conc. of acid	B-780 Before drying.	B-780 After drying.	B-781 After drying.	B-782 Before drying.	B-783 Before drying.	B-783 After drying.
H_2O	-.007	+.201	+.024	-.031	-.014	+.044
N/100	+.509	+.411	+.195	+.329	+.259	+.427
N/200	+.458	+.311	+.127	+.234	+.255	+.231
N/500	+.332	+.253	+.071	+.184	+.207	+.142
N/1000	+.277	+.196	+.043	+.135	+.149	+.103

The results obtained with hydrochloric acid for

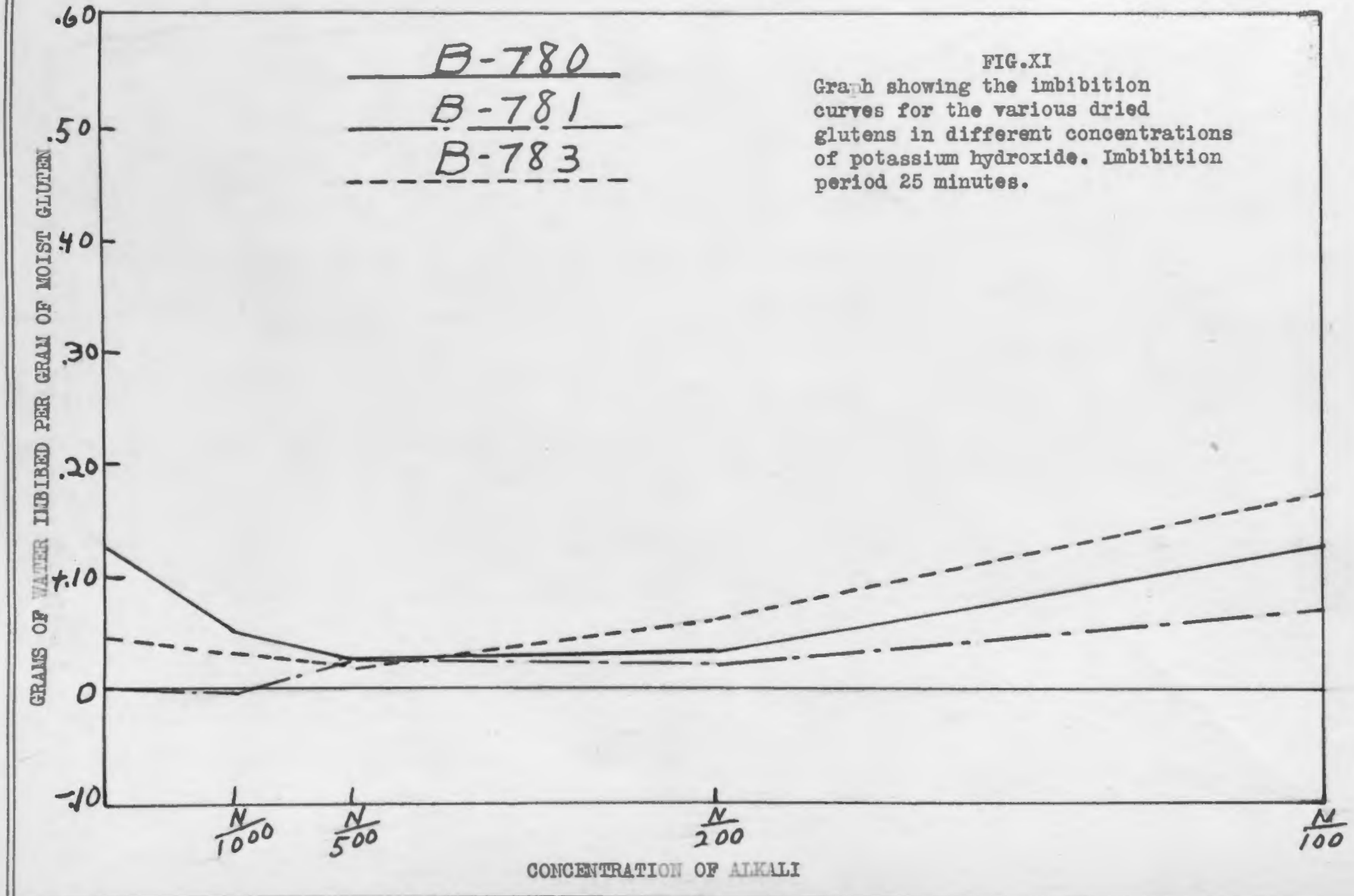
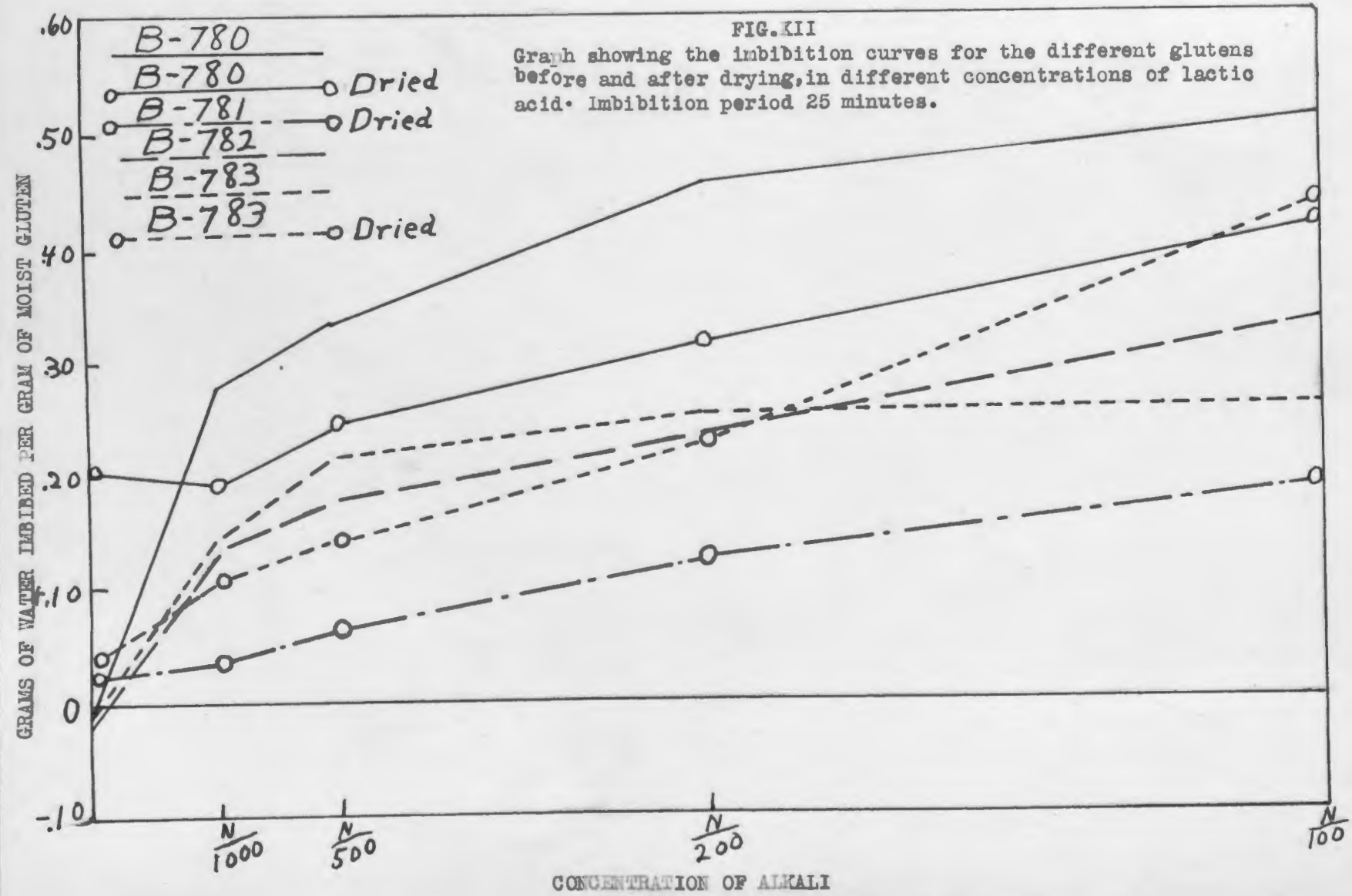


FIG.XI
Graph showing the imbibition curves for the various dried glutens in different concentrations of potassium hydroxide. Imbibition period 25 minutes.

FIG. XII

Graph showing the imbibition curves for the different glutens before and after drying, in different concentrations of lactic acid. Imbibition period 25 minutes.



25 minute periods of imbibition are found in Table XVIII and in Figure XIII.

Table XVIII.

Imbibition of the glutens in hydrochloric acid before and after drying. Imbibition period 25 minutes. Average of five determinations.

Conc. of acid.	B-780 Before drying.	B-780 After drying.	B-782 Before drying.	B-783 Before drying.
H_2O	-.007	+.020	-.031	-.014
N/100	+.687	+.378	+.375	+.247
N/200	+.501	+.227	+.317	+.222
N/500	+.346	+.129	+.223	+.185
N/1000	+.259	2.091	+.198	+.176

3. The effect on the imbibition rate of calcium, and potassium hydroxides containing N/200 sodium sulphate.

It was shown by Gortner and Doherty, Fisher and others that the swelling in acids was considerably reduced in the presence of dilute salt solutions. In order to ascertain whether or not the same relative effect held true with imbibition in alkali in the presence of salts, experiments were carried out with glutens B-780 and B-783 in the presence of N/200 sodium sulphate in potassium, and calcium hydroxides. The results obtained are found in Table XIX and XX and are expressed graphically in Figures XIV and XV.

The results are in good accord with the depressing effect of salts upon imbibition in acids and it was not thought worth while to conduct any further experiments to show the effect of salts inasmuch as their effect has been shown on so many proteins especially by Hofmeister, Fisher, Gortner and Doherty, etc.

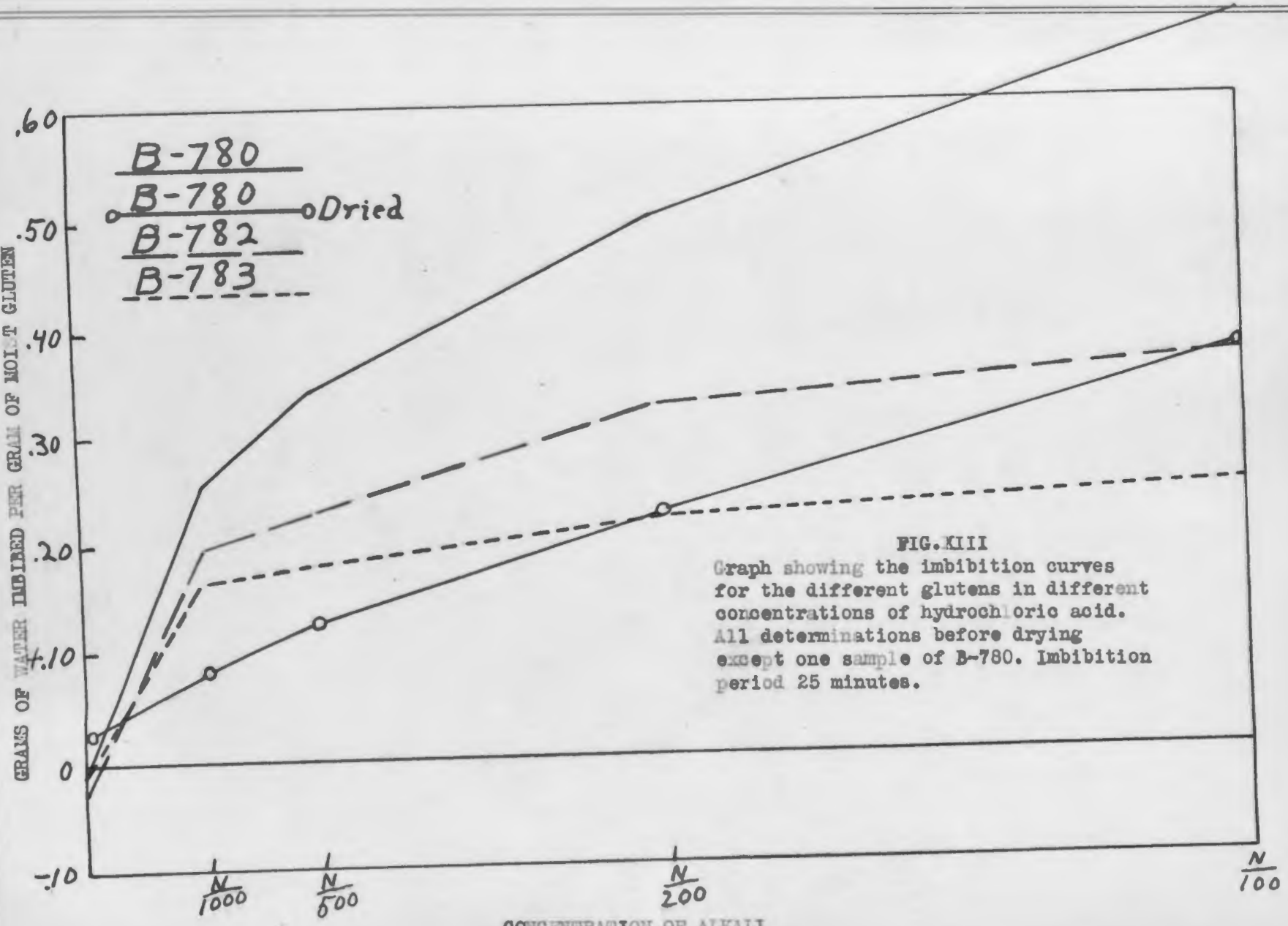


FIG. XIII

Graph showing the imbibition curves for the different glutens in different concentrations of hydrochloric acid. All determinations before drying except one sample of B-780. Imbibition period 25 minutes.

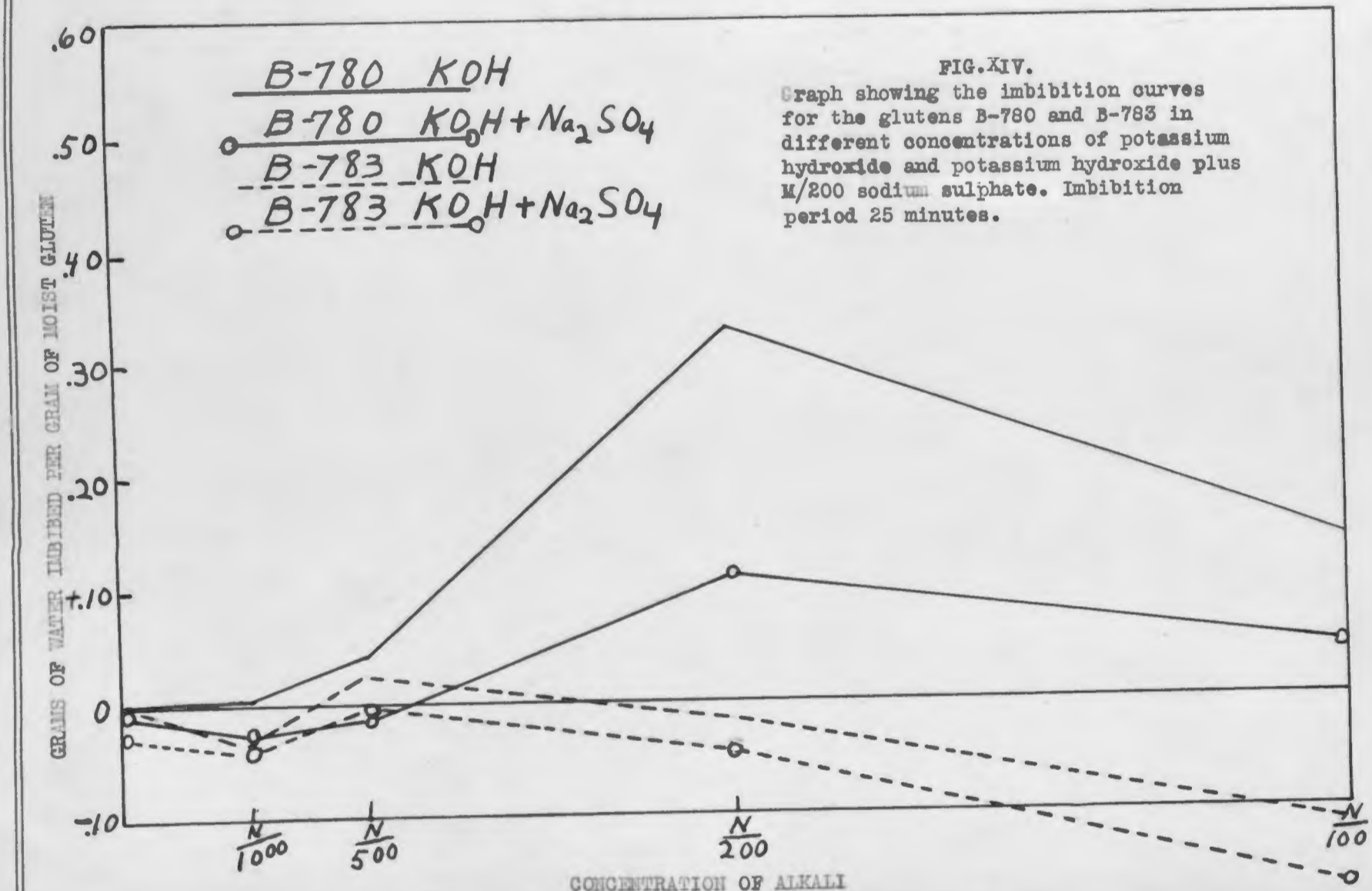


FIG.XIV.
Graph showing the imbibition curves for the glutens B-780 and B-783 in different concentrations of potassium hydroxide and potassium hydroxide plus M/200 sodium sulphate. Imbibition period 25 minutes.

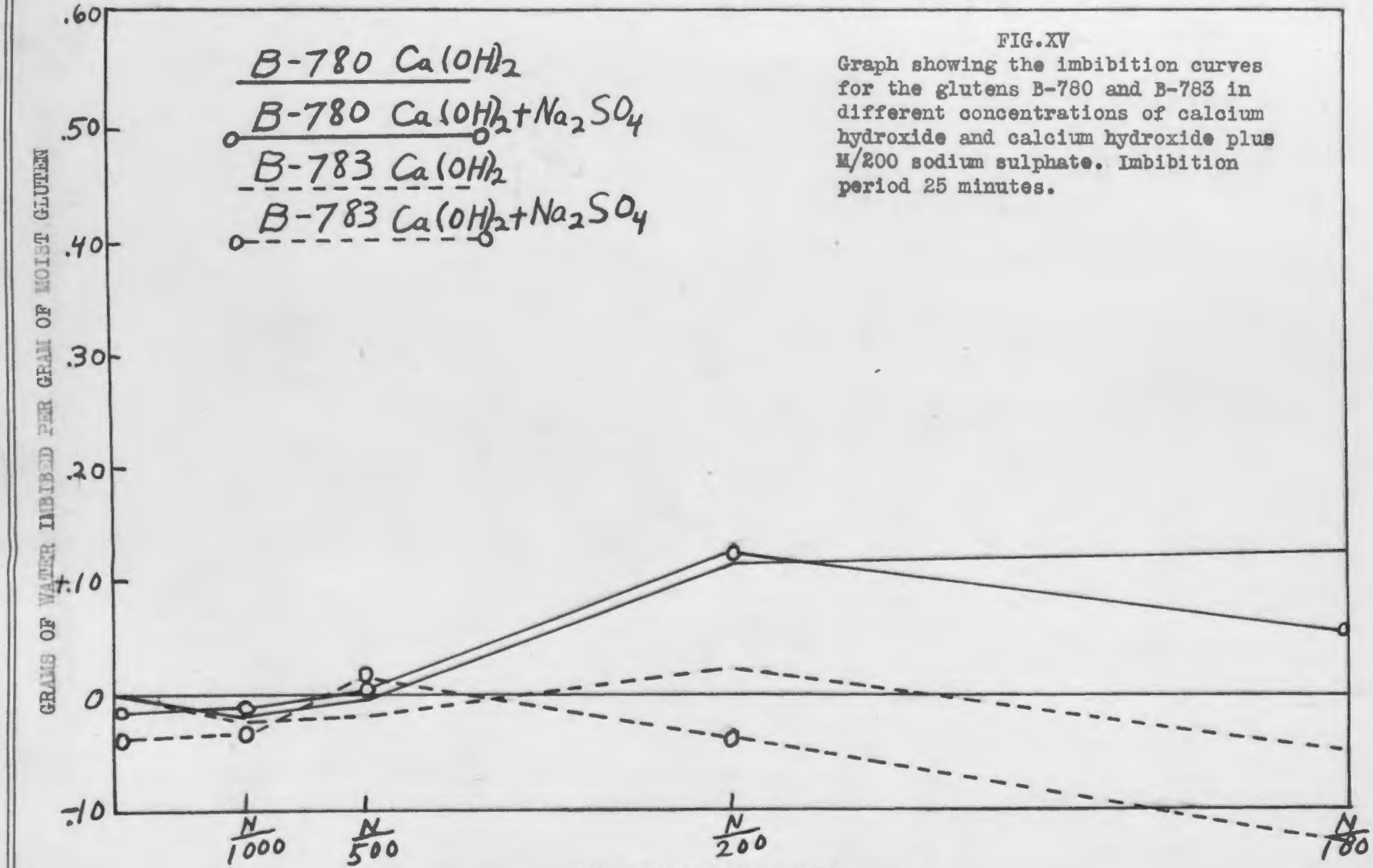


FIG.XV

Graph showing the imbibition curves for the glutens B-780 and B-783 in different concentrations of calcium hydroxide and calcium hydroxide plus M/200 sodium sulphate. Imbibition period 25 minutes.

Table XIX

Imbibition of the glutens B-780 and B-783 (before drying) in potassium hydroxide and in potassium hydroxide plus N/200 sodium sulphate. Imbibition period 25 minutes. Average of five determinations.

Conc. of alkali	B-780 KOH only	B-780 KOH + Na_2SO_4	B-783 KOH only	B-783 KOH + Na_2SO_4
H_2O	---	-.911	---	-.031
N/100	+.138	+.059	-.109	-.170
N/200	+.328	+.196	-.011	-.043
N/500	+.042	-.013	+.017	-.005
N/1000	+.001	-.020	-.029	-.039

Table XX

Imbibition before drying of glutens B-780 and B-783 in calcium hydroxide and calcium hydroxide plus N/200 sodium sulphate. Imbibition period 25 minutes. Average of five determinations.

Conc. of alkali	B-780 $Ca(OH)_2$ only	B-780 $Ca(OH)_2$ + Na_2SO_4	B-783 $Ca(OH)_2$ only	B-783 $Ca(OH)_2$ + Na_2SO_4
H_2O	---	-.011	---	-.031
N/100	+.125	+.055	-.057	-.140
N/200	+.108	+.131	+.018	-.033
N/500	-.003	+.002	-.013	+.017
N/1000	-.015	-.011	-.016	-.022

The experiments thus far indicate that there is a marked difference between the dried gluten and the original moist gluten as washed from the flour, and that results obtained for the dried material might not be comparable to those obtained on the original moist gluten. The object of this investigation was not to investigate differences between glutens before and after drying but to study the glutens from the strong and weak flours, so the drying experiments were not carried further. It is possible that if the drying could be accomplished at a lower temperature the glutens might not be so markedly altered.

4. The gold number of solutions of the different glutens in 0.005 normal potassium hydroxide.

Zsigmondy(1917) defines the gold number as the maximum number of milligrams of protective colloid that may be added to 10 cc. of gold solution without preventing a change of color from deep red to violet shades by 1 cc. of 10 per cent solution of sodium chloride.

It is only the emulsoid colloids that show this protective action. There is good evidence that in many cases the size of the colloid aggregate is indicated by the gold number. this Zsigmondy found to be true in the case of starch which has greater protective value than its split products. Schulz and Zsigmondy (1903) found that the different fractions obtained in the crystallization of egg albumin showed different protective values. Some of the latest results appearing in the literature are by Gortner (1920) where he shows that Protalbinic acid and Lysalbinic acid show slightly less protective action than the value found for egg.albumin. The gold number is therefore inversely proportional to the "protective" value or in other words to the purely emulsoid character of the colloid.

In the light of the available literature it was thought that the gold number might give an indication as to the size of the colloid aggregate in the glutens from the different flours or at least be a measure of their emulsoid character.

The gluten was washed from approximately 15 grams of each of the four flours (no attempt was made to make this quantitative)and was then dispersed in 500 cc. of 0.005 normal potassium hydroxide this was about five times the combining

capacity as shown later by the potentiometric method (See section 6). The amounts of protein actually contained in the 500 cc. on the basis of Kjeldahl nitrogen determination (nitrogen x 5.7) were:

B-780	1.315 grams.
B-781	1.350 "
B-782	0.992 "
B-783	1.222 "

A series of about 200 determinations were made with all concentrations of the above solutions. There seemed to be no marked end point, the completely precipitated gold gradually shading into a purple as the concentration of protective agent increased and the purple in its turn shading into red. Readings were also taken at various times of standing with no better result. While the material did show a protective action it was not very great and there was no sharp end point. The glutens did not differ among themselves to a noticeable extent. In the experiments the tests with the four glutens at any particular concentration of protective agent were all run at once so that the comparison would be direct. In some cases B-781 seemed to show slightly greater protection than the others but this could be accounted for on the basis that that solution actually contained more gluten.

The technique and reagents used when applied to gelatin gave a very sharp end point. The sample of gelatin used was one that probably in its previous history had had some drastic treatment as a 5% solution would not set at laboratory temperatures and the gold number obtained with it was 0.13 whereas high grade gelatin has a gold number of about 0.005.

5. The specific conductance of the gluten ions.

5. The combining capacity in equivalents per gram of gluten.

Because these two values are calculated from the same determinations, it is almost necessary to discuss them together.

A great volume of work has been done on the specific conductance of protein solutions and on their binding capacity for acids and alkalies. It has been established that the proteins actually do carry an appreciable amount of electricity when a current is passed thru their solution and that they actually do enter into combination with acids and alkalies.

Robertson (1918) has been the pioneer in this field and in his recent book treats the subject extensively and gives a complete literature list. Robertson worked principally with casein. In order to become familiar with the methods used and in order to establish a basis for comparison with the gluten determinations an attempt was made to repeat some of Robertson's determinations on casein. (p.172).

The conductivity measurements were made at 30° ± 0.05° and in each instance no less than four duplicate determinations were made with each solution and in most cases the determinations were made with two different Frens conductivity cells. A ten meter bridge was used. The current was supplied with a Leeds and Northrop high frequency generator which was governed to deliver 1000 alternations, located in a different part of the building and no sound of it could be heard except thru the tunable telephone.

The amount of acid or alkali present in the solution was calculated from the determination of the hydrogen ion concentration. The hydrogen ion concentration was determined by the electrometric method using a Leeds and Northrup potentiometer.

The balance was obtained by the use of the Type R high sensitivity galvinometer which gave a deflection on the scale of about one centimeter for each quarter of a millivolt. The millivolt reading of the potential between a N/10 potassium chloride calomel electrode and a hydrogen electrode as described by Hall (1920) was determined at room temperature, care being taken that the room temperature did not change markedly for some time before the readings were made. The millivolt reading was converted into pH, C_{OH}, or C_H at 25° by the use of the conversion tables of Schmidt and Hoagland (1920). At least four duplicate determinations were made with each solution.

The method used in these determinations will be described in detail in the case of casein. Two grams of the dried casein were added to each of four 200 cc. volumetric flasks. The flasks contained 20.00, 40.00, 60.00, and 80.00 cc. of N/10 potassium hydroxide respectively plus enough water to make the total volume 120 cc. The flasks were placed in a shaking machine and were vigorously agitated for four hours at 35-40°, this treatment was found necessary in order to get the protein in solution. After the dispersion of the material the flasks were cooled to room temperature and hydrochloric acid added to flasks 2,3, and 4 making them all N/100 with respect to potassium hydroxide and 0, 0.01, 0.02, 0.03 normal respectively in reference to potassium chloride. This necessitated the addition of 20 cc. to the second flask, to the third 40 cc. and to the fourth, 60 cc. of N/10 hydrochloric acid. Enough water was then added to make the final volume of each 200 cc.

In order to form a basis for the calculations in the

presence of proteins it was necessary to make determinations on the various solutions in the absence of proteins. In Table XXI are found the results obtained under the conditions described above when no protein was present.

Table XXI

Values obtained for the specific conductance and pH of N/100 potassium hydroxide and N/100 potassium hydroxide in the presence of varying concentrations of potassium chloride. No added protein. Each determination the average of four.

Normality of KCl	Sp. Cond. of solutions	Sp. Cond. of KCl *	Sp. Cond. per N/100 KCl **	M.V. at 25°	pH
.00	.002818	.00000	.0000	1034.	11.80
.01	.004277	.001459	.001459	1033.	11.76
.02	.005657	.002839	.001380	1031.	11.749
.03	.007066	.004248	.001409	1031.	11.749

* obtained by subtracting the first value in column 2 from the values for the remaining solutions in column 2.
** obtained by subtracting each two successive values in column 3.

The amount of alkali bound by the protein was determined by subtracting from the total amount of alkali added, the amount of alkali remaining free in the protein solution, as indicated by the hydrogen ion determination. The specific conductance of the protein ions was found by subtracting from the total specific conductance of the solution, first, the specific conductance of the potassium chloride present as shown in Table XXI and, second, the specific conductance of the potassium hydroxide which was not bound by the protein and which could be calculated from the hydrogen ion determination.

In order to determine from the hydrogen ion concentration the amount of unbound alkali and the specific conductance which the unbound alkali would contribute to the solution, a series of solutions were investigated containing varying amounts of N/10 potassium hydroxide in 200 cc. of water. The results obtained

for such a group of determinations are found in Table XXII.

Table XXII

Values obtained for the specific conductance, pH, C_{OH}, and a conductivity to C_{OH} ratio, of 200 cc. of solution containing various amounts of N/10 potassium hydroxide. Each determination the average of four.

cc. of N/10 KOH added.	Specific conductance.	M.V. at 25°	pH	C_{OH}	Conductivity / C_{OH}
20	.002818	1032	11.766	.00592	.4762
10	.001439	1016.8	11.510	.00292	.4926
(?) 5	.0007328	1001.2	11.246	.00178	.4117*
2.5	.0003627	978.1	10.890	.000726	.4995
1.25	.0001751	959.4	10.540	.000352	.4974

* obviously in error. Not included in the average.

The specific conductance of the alkali present as shown by the pH determination may now be calculated by taking the product of the C_{OH} concentration and the factor 0.4915. By plotting the logarithms of the number of cc. of added alkali against the logarithms of the C_{OH} concentrations a straight line was obtained and the values in cc. of N/10 potassium hydroxide not bound were determined from this chart.

Determinations were made with two different samples of casein from cows milk. The first sample used was of unknown purity the water extract giving a specific conductance of .001122 mohs the pH of the solutions was 4.07. Two different determinations were made with this sample, the first in .01 normal potassium hydroxide and the varying concentrations of potassium chloride indicated above, and the other in .011 normal potassium hydroxide and the varying concentrations of potassium chloride. In both cases practically all of the alkali was bound. The conductivity of the casein was obtained by subtracting the specific conductance of the water extract from the total specific conductance attributed to

the added protein. The second sample of casein had been especially purified by other workers in this laboratory. The water extract of this sample gave a specific conductance of .000353 mhos and the pH of the solution was 4.58. No attempt was made to further purify it. Two sets of determinations were made with this sample in .0125 normal potassium hydroxide and the varying concentrations of potassium chloride - (1) the determinations were made immediately after the four hour shaking period; (2) the determinations were carried out on the same set of solutions after they had stood 24 hours. The results are tabulated in Table XXIII.

In this table we find that the values for the specific conductance of casein in any particular set of solutions are fairly constant and that this value increases with an increase in concentration of the alkali, the specific conductance seems also to be affected by the length of standing, that is, it takes time for the solutions to come to equilibrium. In no instance was enough potassium hydroxide added to give an excess over the binding capacity of the casein.

Robertson (p. 172) gives results for a 1 per cent casein solution in N/100 potassium hydroxide plus various concentrations of potassium chloride. His values are shown in Table XXIV.

Table XXIV.

Specific conductance of a 1 per cent solution of casein in potassium hydroxide in the presence of varying concentrations of potassium chloride. Robertson p. 172.

Normality of KCl present	Sp. cond. without casein	Sp. cond. with 1% casein	Sp. cond. of 1% casein
0.00	.002774	.000812	.000838
0.01	.004170	.002226	.000856
0.02	.005480	.003605	.000918
0.03	.006980	.004945	.000761
			average .000843

Table XXIII

Specific conductance and combining capacity of two different samples of casein in the presence of slightly different amounts of potassium hydroxide and varying concentrations of potassium chloride. Average of four determinations.

Normality of KCl	Sp.cond. of solution	Sp.cond. of KCl present	Sp.cond. of casein & KOH	Sp.cond. due to casein	Millivolt reading at 25°	pH	C_{OH} x 10^{-5}	Combining capacity in equivalents x10^{-5}
Sample No.1. in 0.01 N KOH, readings immediately after solution.								
.00	.001672	.000000	.001672	.001670	841.9	8.552	.362	99.9
.01	.003163	.001459	.001704	.001703	834.8	8.433	.274	99.9
.02	.004571	.002839	.001732	.001731	830.4	8.357	.227	99.9
.03	.006036	.004248	.001788	.001787	833.0	8.402	.256	99.9
pH of water extract was 4.07				.001725-.001122(sp.cond.water extract)=.000601 Sp.cond.casein				
Sample No.1. in 0.011 N KOH readings immediately after solution.								
.00	.001817	.000000	.001817	.001816	832.8	8.400	.254	109.9
.01	.003256	.001459	.001797	.001795	839.8	8.518	.332	109.9
.02	.004700	.002839	.001861	.001860	833.6	8.412	.262	109.9
.03	.006118	.004248	.001870	.001869	828.4	8.325	.214	109.9
				.001835-.001122(sp.cond. water extract)=.000713 Sp.cond.casein				
Sample No.2. in .0125 N KOH readings immediately after solution.								
.00	.001261	.000000	.001261	.001238	907.3	9.657	4.61	124.2
.01	.002733	.001459	.001274	.001267	876.5	9.137	1.39	124.7
.02	.004171	.002839	.001332	.001327	867.7	8.989	.987	124.8
.03	.005622	.004248	.001374	.001372	848.0	8.656	.458	124.8
pH of water extract was 4.58				.001301-.000353(sp.cond. water extract)=.000948 Sp.cond.casein				
Sample No.2. in .0125 N KOH readings of the above solutions after standing 24 hours.								
.00	.001269	.000000	.001269	.001240	914.0	9.772	5.59	124.0
.01	.002767	.001459	.001308	.001301	877.4	9.153	1.44	124.7
.02	.004240	.002839	.001401	.001400	872.8	9.074	1.20	124.8
.03	.005667	.004248	.001619	.001618	828.0	8.318	.210	124.8
				.001389-.000353(sp.cond. water extract)=.001036 Sp.cond.casein				

Robertson states that the normality of the neutralized potassium hydroxide was .00993, this would make the combining capacity in this instance about 99.3×10^{-5} equivalents. He states (p.93) that the constant maximum binding capacity of casein is 180×10^{-5} equivalents of potassium hydroxide. It will be noticed that the results obtained for specific conductance as shown in Table XXIII are in good agreement with Robertson's data, and that the figures for combining capacity are also approximately identical.

The determinations were repeated in essentially the same manner with the dried glutens from the flours B-780 and B-783. In this case the concentration of potassium hydroxide used was N/100 which gave a slight excess over the binding capacity. In both cases two grams of the dried material on the dry basis were dispersed in a total volume of 200 cc. after the dispersion was complete, a considerable amount of starch separated out in the bottom of the flask showing that the impurity in the dried gluten was principally starch.

The results obtained for the two dried glutens are found in Table XXV. The good agreement which was found in the case of the casein was not found in these two glutens. The higher concentrations of alkali (before neutralization with hydrochloric acid) seemed to affect both the binding capacity and the specific conductance of both glutens. Perhaps some hydrolysis of the protein took place. It must be remembered that the proteins of the wheat gluten are decidedly different in chemical composition from animal protein such as casein and it is entirely probable that the amide linkages in the gliadin and glutenin are altered by the higher concentration of alkali. Some evidence of

Table XXV

Specific conductance and combining capacity of dried glutens B-780 and B-783 in the presence of N/100 potassium hydroxide and varying concentrations of potassium chloride. Average of four determinations.

Normality of KCl	Sp.cond. of solution	Sp.cond. of KCl present	Sp.cond. of gluten & KOH	Sp.cond. of KOH present	Sp.cond. due to gluten	Millivolt reading at 25°	pH	COH x 10^{-5}	Combining capacity in equivalents x 10^{-5}	On a N x 5.7 basis.
B-780, a 1 % solution on the moisture free basis.										
.00	.001693	.000000	.001693	.001046	.000647	1005.8	11.320	213.	63.	79.3
.01	.002911	.001459	.001452	.000590	.000862	991.13	11.070	120.	79.7	100.3
.02	.004185	.002839	.001346	.000300	.001046	975.7	10.780	61.1	89.6	112.8
.03	.005437	.004248	.001189	.000106	.001083	947.0	10.530	21.6	92.5	116.5
Sp. cond of water extract of the gluten was .0000489										
B-783 , a 1 % solution on the moisture free basis.										
.00	.001885	.000000	.001885	.001706	.000179	1018.3	11.535	347.	40.	60.7
.01	.003148	.001459	.001689	.001233	.000456	1010.0	11.394	251.	56.2	85.3
.02	.004399	.002839	.001560	.000786	.000774	998.5	11.199	160.	72.5	109.
.03	.005629	.004248	.001381	.000414	.000967	982.0	10.921	84.	85.2	129.

ammonia formation was observed in these samples.

As a method of checking the specific conductance of the various solutions, the difference between each two succeeding values, in the columns headed specific conductance of the solutions in Tables XXI, XXIII, XXIV, and XXV, was found. This difference should represent the conductivity of a N/100 solution of potassium chloride in each case and should be approximately constant. The values obtained from the above mentioned Tables are found in Table XXVI.

Table XXVI

The values obtained for the conductivity of N/100 potassium chloride obtained by subtracting each two succeeding values for the specific conductance of the solutions in Tables XXI, XXIII, XXIV and XXV.

Solutions with no protein Table XXI	Solutions of casein sample #1 from Table XXIII	
	First determination	Second.
.001459	.001491	.001439
.001380	.001408	.001444
.001409	.001465	.001418

Robertson's data: Table XXIV		Solutions of casein sample #2 from Table XXIII	
No protein	1% casein	Deter. Immediately.	Deter. after 24 hrs.
.00140	.001414	.001472	.001498
.00131	.001379	.001438	.001473
.00140	.00134	.001451	.001627

1% glutens from Table XXV	
#-780	#-783
.001218	.001263
.001274	.001251
.001252	.001230

It is difficult to draw conclusions from this data; the determinations appear to be at least as accurate as Robertson's. The method of subtracting the two successive determinations really shows up the errors in their maximum light. The potassium chloride

seems to have its normal conductivity excepting in the case of the two gluten solutions. In these cases the protein appears to have combined with some of the potassium chloride for the values are consistently lower than those obtained for the casein solutions or for the solutions containing no protein. The indications are that the gluten combines with or adsorbs the potassium chloride and the casein does not. According to this, there should be more alkali bound in the first case where no potassium chloride was present, this did not seem to be the case. More alkali appeared to be bound where the gluten was treated in the higher concentrations of alkali than where it was treated with the lower concentrations.

It seemed advisable to determine the combining capacity by direct titration using phenolphthalein as an indicator. The binding capacity of the glutens for potassium hydroxide was determined. Solutions were made up representing 0.5%, 0.75%, 1.0%, 1.5% of the moisture free dried gluten in 200 cc. of N/100 potassium hydroxide prepared by adding 20 cc. of N/10 potassium hydroxide to the gluten and making up to a volume of 200 cc. The results obtained are found in Table XXVII.

In the titration determinations, it was very difficult to get the exact end point, the results are in excellent agreement when the experimental conditions are taken into account. It is noticeable that when the data is calculated on the nitrogen basis, B-783 has a slightly greater binding capacity than has B-780. The difference is, however, probably not greater than could be accounted for by the experimental errors.

Table XXVII

The combining capacity of dried glutens B-780 and B-783 (on a moisture free basis) for potassium hydroxide as determined by titration to phenolphthalein with N/10 hydrochloric acid.

% of gluten in solution	Gms. gluten in sol. on dry basis	Cc. of N/10 KOH bound	Equivalents of KOH bound per gram
B-780			
0.5	1.000	2.50	25.0×10^{-5}
0.75	1.500	3.55	23.7
1.0	2.000	4.80	24.0
1.5	3.000	6.70	22.3
			23.8 average

$23.8 \times 10^{-5} \times 100/79.42 = 29.97 \times 10^{-5}$ equivalents of KOH on a Nx5.70 basis.

B-783			
0.5	1.000	2.10	21.0×10^{-5}
0.75	1.500	3.15	21.0
1.0	2.000	4.25	21.3
1.5	3.000	6.35	21.2
			21.1 average.

$21.1 \times 10^{-5} \times 100/65.86 = 32.04 \times 10^{-5}$ equivalent of KOH on a Nx5.70 basis.

The experiment was repeated in the same manner except that the amount of alkali present was determined by the potentiometric method. See Table XXVIII.

In this Table B-783 again appears to have a slightly higher binding capacity calculated on the nitrogen basis than B-780 but the difference is not sufficient to warrant a correlation between the imbibition capacity and the combining capacity.

It is interesting to note that the value of equivalents bound per gram of gluten on the nitrogen basis in the case of the potentiometric method is about twice what it is in the case of titration. The pH of the solution when titrated to a very slight pink as the end point was found to be 8.94. As yet no definite reason has been assigned to this observation.

It was shown by the experiments on the imbibition rate

Table XXVIII

The combining capacity and specific conductance of dried glutens B-780 and B-783 (on a moisture free basis) in N/100 potassium hydroxide as determined by the potentiometric method, using .5%, .75%, 1.0% and 1.5% solutions. Average of four determinations.

Gms. in 200 cc. of solution	Millivolt reading at 25°	pH	C_{OH}	Cc. N/10 KOH bound	Equivalents KOH bound per gram	Sp. cond. of solution	Sp. cond. of KOH present	Sp. cond. of the gluten	Sp. cond of gluten basis 1% sol.
B-780									
1.000	1025.2	11.650	.00452	4.40	44.0×10^{-5}	.002281	.002221	.000060	.000120
1.500	1020.8	11.576	.00383	6.70	44.7	.002023	.001882	.000141	.000188
2.000	1015.8	11.492	.00315	8.90	44.5	.001830	.001548	.000282	.000282
3.000	1004.12	11.291	.00200	13.10	43.7	.001456	.000983	.000473	.000315
					44.2 x 100/79.42=55.6 x 10^{-5} Equiv. on Nx5.70 basis/				
B-783									
1.000	1028.2	11.702	.00508	2.50	25.0×10^{-5}	.002297	.002497	------	-------
1.500	1019.26	11.548	.00360	7.50	66.6	.002073	.001769	.000304	.000405
2.000	1015.1	11.480	.00331	8.50	42.5	.001872	.001626	.000246	.000246
3.000	1003.7	11.288	.00196	13.25	44.2	.001519	.000963	.000556	.000371
					43.4 x 100/65.89=65.89 x 10^{-5} Equiv. on Nx5.70 basis.				

of the dried and undried material that there was a marked difference in some of the properties. The conductivity and binding capacity experiments up to this time had all been carried out with the dried material.

The following experiment was carried out with B-780 before drying. The method of getting the exact concentration of the gluten in solution was to add approximately the quantity desired of the wet gluten and then after the completion of the experiment to determine the protein (N x 5.70) in an aliquot. The results are found in Table XXIX.

Table XXIX

Combining capacity of gluten B-780 (before drying) for potassium hydroxide as determined by titration to phenolphthalein with N/10 hydrochloric acid.

Gms. of gluten N x 5.79 basis	Cc. of N/10 KOH bound	Equivalents of KOH bound per gram
0.971	2.07	21.3 x 10^{-5}
1.903	4.08	21.4
2.908	6.10	21.0
3.872	7.90	20.4
		21.0 average.

The value of the combining capacity of B-780 as shown by titration before drying is 21.0 x 10^{-5} equivalent of potassium hydroxide per gram of gluten and the material after drying gave a higher result i.e. 29.9 x 10^{-5}. Here the differences are probably significant.

7. The viscosity of the different glutens dispersed in both N/100 potassium hydroxide and N/100 lactic acid.

The literature on the viscosity of emulsoids is quite voluminous and no attempt will be made to summarize it. It is rather generally conceded, however, that emulsoids do

not show very constant values when subjected to viscosity measurements. This is due to the fact that the effect measured is not due to a single factor but to several factors. The literature of viscosity determinations of emulsoids is reviewed by Rothlin (1919) and some additional data on the effect of pressure in the determination of viscosity of emulsoids by the capillary tube method, is given. Hess (1910) pointed out that, in addition to the internal friction, a force which he called "displacement elasticity" or "cohesion power" had to be overcome. Rothlin confirmed the findings of Hess by observing that sos colloids do and some do not follow the Poiseuille law. Those that do not follow it showed a marked decrease at higher pressure. Those emulsoids that do not follow this law were found to be those that upon standing change from sol to gel. It is probable, therefore, that the determinations made with the gluten dispersions would follow the Poiseuille law. Even if they did not, the results would at least be comparative no matter what the pressure. While the following determinations are spoken of as viscosity determinations, it is recognized that what is really being measured may be the sum of two or more factors.

Levene and Van Slyke (1909) gave a small series of determinations on glimdin and gluten. In each case 0.400 grams were dissolved in 10 cc. of N/1 sodium hydroxide. The results are given in percentage increase over the dispersion medium at 25°. They found that the viscosity gradually fell after the solution was made up. It took four hours to get the material in solution.

Hours after making	Viscosity in % increase over the medium	
9	287.8	Gluten
26	229.1	"
8½	181.4	Gliadin
24	165.1	"
47	155.5	"

An attempt was first made to study the viscosity of the protein solutions by means of a MacMichael viscosimeter, a torsion viscosimeter of the rotating disc type. The degrees MacMichael decreased with each subsequent reading with the same material, due to precipitation of the protein on the disc. Other investigators have found the same difficulty when dealing with emulsoid colloidal solutions and say that the torsion type cannot be used for this reason. (Rothlin 1919).

The form of viscosimeter finally adopted was of the Ostwald type made with a large reservoir at the bottom so that there would be very little change in pressure due to a rise in this end of the apparatus. The level of the liquid in the lower reservoir never reached the level of the bottom end of the capillary. The upper reservoir held about 8½ cc. A total volume of 10 cc. of the solution to be tested was always introduced. The large volume was used because it would show very small differences in viscosity. The time of outflow was taken with a stop-watch. The time for water was over 9 minutes. The determinations were all carried out at constant temperature maintained by placing the viscosimeter in a thermostat. The bath was regulated to 20° as near as could be obtained with a 1/10 degree thermometer and the corresponding reading on a Beckman thermometer taken as the standard for future regulations of temperature. The bath was then held at this temperature ± .01°. Care was taken to see

that the contents of the thermostat were efficiently stirred.

The solutions were prepared in the same manner as previously described, the material being dispersed in 200 cc. of N/100 potassium hydroxide. A preliminary experiment, the results of which are found in Table XXX, was carried out with the dried glutens, 1 percent solutions on the dry basis being used. The viscosity results are expressed in terms of fifths of seconds.

Table XXX brings out very clearly the effect of time of standing on the amount of alkali bound. It also shows that the viscosity changes with the length of time of standing. On the other hand, these same samples when allowed to stand at room temperature for several days began to give off ammonia although there was no indication of bacterial action. The material had, however, begun to precipitate out. If the same material was allowed to remain in the viscosimeter and a series of readings taken, each succeeding one was lower than the preceding one showing a gradual fall in viscosity. If an entirely fresh sample was placed in the viscosimeter, the first reading would compare very closely with the first reading obtained with the previous aliquot.

The viscosity values for the dispersion liquids were determined (see Table XXXI). The individual determinations are given to show how the results agree for pure liquids. The values are in fifths of seconds.

Table XXX

Conductivity, combining capacity(average of four determinations),and viscosity determinations (average of three) of disperdions of the dried glutens in N/100 potassium hydroxide after various intervals of standing. Viscosity readings in fifths of seconds. 200 cc. of a 1 % solution was prepared in each case.on the moisture free dry basis.

Sp.cond. of solution	Sp.cond. of KOH present	Sp.cond. of gluten	Millivolts reading at 25°	pH	C_{OH}	Co.N/10 KOH bound	Equivalents of KOH bound per gm.	Viscosity in fifths of sec. after 7 hrs	after 24 hrs	after 53 hrs.
B-780 .001479	.000939	.000540	1002.95	11.275	.00191	13.42	6.71x 10-5	------	3453	3411
B-783 .001584	.001180	.000404	1008.9	11.376	.00240	11.60	5.80	------	3296	3272
B-780 .001711	.001808	-----	1019.8	11.560	.00368	7.25	3.63	.3559	3497	3446
B-781 .001450	.000968	.000482	1003.7	11.288	.00197	13.20	6.60	3448	3396	3379
B-782 .001908	.002276	-----	1025.7	11.660	.00463	4.20	2.10	3308	3268	3250
B-783 .001817	.002074	-----	1023.4	11.624	.00422	5.40	2.70	3362	----	3284

Note. The millivolt and the conductivity determinations in the first two cases were made 48 hours after the preperation of the solutions. In the last four cases the determinations were made 4 hours after the gluten was first put in the alkali. In these preliminary experiments the specific gravity of the solutions was not determined, it being assumed that they would be nearly the same and so would not affect the comparative results.

Table XXXI

Viscosity values in terms of fifths of seconds for water, N/100 potassium hydroxide and N/100 lactic acid.

Cond. water just boiled.	Cond. water not boiled.	N/100 KOH	N/100 lactic acid.
2766	2772	2773	2766
2764	2772	2768	2766
2766	2770	2769	2767
2764	2774	----	----
2765	2772	2770	2766

The specific gravity of N/100 potassium hydroxide at 20° compared with water at 20° was 1.00061. In order to make the viscosities comparable with water, the reading of the time of outflow is multiplied by the specific gravity in each case, 1.00061 x 2770.= 2772. viscosity for potassium hydroxide. The specific gravity of the lactic acid 1.00026 x 2766= 2767. the viscosity value for the lactic acid.

In calculating the part contributed to the viscosity of the solution by the various glutens the viscosity of the particular dispersion liquid used, was taken as the standard, in the case of the KOH it was the same as the water used. The solutions in the following Tables were prepared from the fresh washed gluten that had not been dried, the determinations were carried out in four different concentrations obtained by dispersing 3, 6, 9, and 12, grams of the moist gluten in 200 cc. of N/100 concentration of either potassium hydroxide or lactic acid. The dispersion was accomplished in the case of the potassium hydroxide by shaking in the shaking machine at 40° for four hours. In the case of the lactic acid the procedure was the same only treatment in the shaking machine for seven hours instead of four was necessary to accomplish dispersion. (Dispersion of the dried glutens

could not be accomplished in lactic acid, the treatment used with them was much more vigorous, even after shaking eighteen hours only dispersion to the form of a coarse flocculent precipitate could be obtained, the pH determination showed there was plenty of lactic acid present to accomplish dispersion, adding additional quantities of lactic acid did not help).

In the following cases the conductivities and pH determinations were made 24 hours after the solution was added to the gluten. The viscosities were determined thirty hours after making the solution. After dispersion was complete, the material was allowed to stand twelve hours. It was then centrifuged for 15 minutes and the liquid used for the determinations. Nitrogen was determined on aliquots of this liquid by Kjeldahl method and the protein content is based on this nitrogen determination (N x 5.70).

The results obtained with potassium hydroxide are found in Table XXXII.

In the case of B-783 in Table XXXII, the higher values for conductivity and the fact that they were not constant was investigated to see if it could be due to soluble material added, or derived from the gluten itself without the potassium hydroxide. In order to check this up, two samples were made up, the one containing approximately 6 grams of the wet gluten, the other 12 grams. The gluten was broken into small pieces and was allowed to stand for 24 hours being shaken at intervals. At the end of this time, the conductivity of the two solutions was determined. The values obtained are found in Table XXXIII.

Other determinations on the conductivity of water extracts of the fresh washed gluten indicated that the specific conductance

Table XXXII

Conductivity and combining capacity of various amounts of undried glutens B-780 and B-783 dispersed in potassium hydroxide, determinations made 24 hours after preparing the solution. Each determination the average of four. Also the viscosity of these solutions determined 30 hours after preperation, each value given is the average of at least three determinations.

Gms in 200 cc N x 5.70 basis	Sp.cond of solution	Sp.cond of KOH present	Sp.cond of prot. present	Sp.cond prot.on basis of 1% sol	M.V	pH	C_{OH}
B-780							
.7966	.002168	.001990	.000178	.000447	1022.7	11.604	.00405
1.602	.001755	.001400	.000355	.000443	1013.3	11.450	.00285
2.582	.001374	.000713	.000661	.000512	995.9	11.158	.00145
3.608	.001178	.000406	.000772	.000428	991.5	10.914	.00083
B-783							
.7401	.002184	.001686	.000498	.001345	1017.9	11.530	.00343
1.550	.001647	.001012	.000635	.000819	1004.9	11.310	.00206
2.200	.001308	.000624	.000684	.000622	992.5	11.097	.00127
2.581	.001161	.000382	.000779	.000604	980.0	10.887	.00078

Table XXXII continued.

Cc.of N/10 KOH bound	Equiv.per gm gluten x 10^{-5} bd.	Sp.gr. water 20/20°	Viscosity reading reading	corr.for sp.gr.	increase over sed.	per gm. gluten
B-780						
6.00	75.3	1.0017	3110	3115.3	343	431.
10.00	62.4	1.0034	3506	3517.9	746	466
15.05	58.3	1.0045	4076	4094.3	1322	512
19.72	54.7	1.0060	4667	4696.0	1924	533
B-783						
8.20	111.	1.0016	3040	3044.8	273	369
12.88	83.1	1.0026	3396	3404.8	633	408
15.65	71.1	1.0035	3696	3708.9	937	426
19.74	76.5	1.0042	3869	3885.2	1113	431.2

Viscosity of the medium 2772.

contributed by dissolved salts was not an important factor in the conductivity results.

Table XXXIII

The conductivity per gram (N x 5.70) of the water extract from gluten B-783 before drying.

Gms. of gluten in 200 cc. water	Conductivity	Conductivity on basis of 1 gram.
1.7305	2.044×10^{-5}	1.701×10^{-5}
2.6448	4.53	1.64

Average .0000167 per gram.

Determinations carried out on the water used in washing out the gluten gave the following results:

3.49×10^{-6} mohs.
3.12×10^{-6} mohs.

This value while being somewhat high for conductivity water would not be high enough to affect any of the results on the conductivity determinations of the dispersed glutens.

In the case of lactic acid little can be learned from the conductivity determinations and the acid is so slightly dissociated that its combining power is hard to determine. The viscosity determinations seem to be significant. These are shown in Table XXXIV.

The viscosity of solutions of B-780 is clearly higher than solutions of B-783 in both potassium hydroxide and lactic acid. This seems to be a rather definite difference between the two indicating that the physico-chemical properties of their solutions differ as well as the imbibing capacity of the glutens.

8. The isoelectric point of the glutens.

Michaelis and his co-workers (1909, 1910, 1911, 1912)

Table XXXIV

Conductivity and pH of various amounts of undried glutens B-780 and B-783 dispersed in 200 cc. of N/100 lactic acid. The determinations were made 24 hours after the preperation of the solution. Each value given is the average of four determinations. Also the viscosity of these solutions determined 30 hours after preperation, each value is the average of at least three determinations.

Gms.in 200cc. N basis	Sp.cond. of solution	Millivolt reading at 25°	pH	Viscosity reading	Sp.Gr. water 20/20°	Viscosity corrected for sp.gr.	Increase over medium	Viscosity per gram of gluten
B-780								
.7706	.000371	535.0	3.364	4131	1.0016	4137.6	1370.6	1778.
1.530	.000344	546.5	3.558	4717	1.0025	4728.8	1961.8	1282.
2.217	.000351	554.5	3.693	5227	1.0033	5244.2	2477.2	1117.
2.949	.000369	562.5	3.829	5605	1.0047	5631.3	2864.3	971.
B-783								
.667	.000405	532.5	3.411	3654	1.0013	3658.7	891.7	1336.7
1.530	.000353	550.5	3.627	4140	1.0023	4149.5	1382.5	974.
1.878	.000357	557.0	3.770	4344	1.0031	4357.4	1590.4	847.
2.513	.000362	564.6	3.864	4731.	1.0041	4750.4	1983.4	789.

Viscosity of the medium was 2766.

have been the most extensive investigators on the isoelectric point of proteins. They studied different methods of determining the isoelectric point and presented data to show that the coagulation optimum hydrogen ion concentration and the isoelectric point as determined by the migration experiments are identical.

Michaelis and Rona (1910) give the isoelectric point of gliadinas 6.0×10^{-10} or pH 9.22.

The experiments on the determination of the isoelectric point of the glutens from the strong and weak flours were modeled somewhat after those of Michaelis and his co-workers. It was found that the mixtures given by them did not have the pH range that was desired.

The method finally adopted for the determinations in the case of the glutens was as follows: Approximately .2 and .4 grams (on the dry basis) of the wet gluten were dissolved in 100 cc. portions of N/10 potassium hydroxide. After the solution was complete, solid salts of tri basic sodium phosphate and sodium tetraborate were added in amount sufficient to make the solution N/10 with respect to these substances. Three cc. of this solution were placed in test tubes and varying amounts of either N/30 or N/10 acetic acid were added and the mixture made up to a total volume of 30 cc. with water. Thus making them N/100 with respect to the original salts. By adding various amounts of acetic acid it was possible to obtain buffer solutions ranging in pH from 10.93 to 4.31 with the only variable the amount of acetic acid. In those tubes where either a precipitate or a cloudiness was formed, the pH was determined by the electrometric method. The results are given in Table XXXV.

Table XXXV

The isoelectric point of glutens B-780 and B-783 (before drying) as shown by the coagulation optimum hydrogen ion concentration for two different concentrations of each gluten. pH determined electrometrically. The + signs stand for cloudiness and precipitation, the degrees of cloudiness increasing with the number of + signs up thru three, the begining of precipitation is indicated by four + signs, the amount increasing with the number of + signs.

.02 % gluten solution			.04 % gluten solution		
Result at end of 10 min.	Result at end of 24 hrs.	pH of solution	Result at end of 10 min.	Result at end of 24 hrs.	pH of solution.
B-780					
---	++	6.269	---	---	6.100
+	+++	6.005	---	+	5.765
++	++++++	5.664	+	++	5.664
+++	++++++	5.605	++	+++	5.503
+++	++++++	5.351	+++	+++++++	5.334
+++	++++++	5.232	+++++	+++++++	5.232
+++	++++++	5.139	+++++	+++++++	5.139
++	++++++	4.877	++++	+++++++	4.894
+	+++	4.656	+++	+++++++	4.657
5.664+4.877/2= 5.27			5.334 + 4.657/2 = 5.00		
B-783					
+	++	6.185	+	++	6.252
+++	+++	5.985	++	++++	5.850
+++	++++++	5.613	+++	+++++++	5.731
+++	++++++	5.308	+++	+++++++	5.359
+++	++++++	5.181	+++	+++++++	5.198
+++	++++++	4.987	+++	+++++++	4.987
++	++++++	4.707	++	+++++++	4.725
+	+	4.453	+	++	4.470
5.613 x 4.707/2= 5.16			5.731 + 4.725/2 = 5.23		

BAKING TESTS.

The baking tests as carried out with the different flours are recorded in Table XXXVI.

Table XXXVI

Laby. no of sample	Ash content of flour.	Crude protein in flour N x 5.70	Water added to 100 gms. of flour to make dough	Volume of fermented dough given by 150 gms. of flour
B-780	.435%	11.09 %	60 cc.	900 cc.
B-781	.90	13.74	66	850
B-782	.49	9.46	60	740
B-783	.53	10.15	53	810

Volume of loaf	Color score of baked bread B-780 as 100.	Texture score of baked bread B-780 as 100
1580 cc.	100	100
1680	90	96
1540	102	94
1660	86	90

The flour B-780 had been used as a standard flour in baking tests, the loaf volume as shown in this particular set of experiments is lower than the volume obtained previously.i.e. 1640 cc. The maximum volume reached by the fermented dough is greater in the case of B-780 than it is in the other three cases. The loaves differed markedly in appearance, there were also great differences in texture B-780 having by far the best texture.

IV. DISCUSSION.

Physico-chemical studies were made on the glutens from four flours— One a high grade patent made from No.1 northern spring wheat (B-780), one a clear flour made from No.1 northern spring wheat (B-781), and two patent flours milled from soft western wheats.(B-782) and (B-783).

The baking tests are given in Table XXXVI. The loaf volumes are very nearly the same, the expansimeter tests on the dough undergoing fermentation show some differences the B-780 expanding the most, the greatest differences were found in the appearance and texture of the loaf B-780 having a markedly better texture than the others.

The rate of imbibition of the different glutens.

The results on the rate of imbibition of the different glutens in the presence of hydrochloric and lactic acids confirm the findings of Gortner and Doherty. The differences as shown in Table IV and Fig.I for hydrochloric acid compare very favorably with the results they obtained with this acid. The results obtained show a somewhat greater variation between the glutens of the strong and weak flours than the differences found by Gortner and Doherty. In the case of lactic acid, see Table V and Figure II, the differences between the glutens are much more marked than any found by Gortner and Doherty, the gluten B-780 having almost three times the rate of imbibition of the gluten B-783, indicating that the "strong" flour is stronger and the "weak" flour weaker than the samples with which they worked.

The imbibition rates of the various glutens in alkalies show marked differences. The behavior of the glutens to alkalies is somewhat different from the behavior in the presence of acids, disperaion beginning almost coincident with hydration in the case of the alkalies. If we compare the results given with potassium and sodium hydroxide in the N/200 concentrations, see Figure V and VI, we find differences greater than any found in the case of the acids. When we compare the reaction of the glutens to the various alkalies, we find that they follow the allitropic series, that is, potassium > sodium > barium and calcium > ammonium.

The imbibition rate of gluten S-780 is markedly affected in potassium hydroxide plus N/200 sodium sulphate as compared with potassium hydroxide alone, see Table XIX and Figure XIV. S-783 is also affected but to a less extent. In the case of calcium hydroxide plus N/200 sodium sulphate, the effect with both glutens is less marked. See Table XX and Figure XV. The effect of the sodium sulphate on the swelling of gluten in potassium and calcium hydroxides is in agreement with the findings of Fisherand others for the effect of salts on the swelling of animal colloids in alkalies, and of Gortner and Doherty for the effect of salts on the swelling of gluten in the various acids.

The studies with the dried glutens indicate that a marked change has taken place. This was shown by the appearance of the wet gluten made from the dried material, it being tougher and more elastic than the fresh washed gluten. The results obtained for the imbibition rate of the dried material with lactic acid (see Table VII and Figure III) show this very clearly, the

most pronounced change being in the case of the strong flour gluten B-780. In this instance the imbibition rate has been materially altered. It is of interest to note that the various glutens tend to become more alike in imbibition capacity, etc., when the glutens have been previously dried. The strong gluten in particular shows a lowered imbibition capacity. This is what might be expected if the imbibition capacity is due to marked colloidal properties, for we know that alternate freezing and thawing or subjection to alternate moist and dry conditions tends to break up the colloid complexes of a soil, and approximately the same factors are operating in the present instance.

Also the glutens after drying show a great similarity in their behavior towards potassium hydroxide. See Table XVI and Figure XI.

In order to more directly compare the effect of acids and alkalies on the imbibition rate, the imbibition experiments were carried out for 25 minutes in the case of lactic and hydrochloric acids. See Tables XVII and XVIII and Figure XII and XIII. These results indicate that under the same imbibition conditions the effect of the acids is somewhat greater than the effect of alkalies. Here also the difference between hydrochloric and lactic acids is not so marked as it is in the higher concentrations and the longer intervals of time.

The gold numbers of the different glutens.

In the experiments with the gold numbers of solutions of the various glutens (before drying) in .005 normal potassium hydroxide, the results obtained were inconclusive inasmuch as the exact end point could not be obtained.

Combining capacity of the various glutens for potassium hydroxide and the conductivity of the gluten ions.

In the experiments carried out with casein (see Table XXIII) the results agree very well with the results obtained by Robertson (see Table XXIV). The method was found to give fairly concordent results with this protein.

In similar experiments, with dried glutens B-780 and B783, the results are given in Table XXV. The agreement is not so good. One thing of interest in this experiment is brought out in Table XXVI, i.e. the conductivity which is attributable to the potassium chloride is consistently less in the case of the two glutens than in the case of casein or in the case of the solutions without added protein indicating combination with or adsorption of the potassium chloride.

The determinations of the binding capacity by the use of various amounts of the dried glutens B-780 and B-783 in the same volume, i.e. 200 cc. of N/100 potassium hydroxide gave results which were in good agreement. The binding capacity of both glutens on a protein (N x 5.70) basis when the amount bound was determined by titration with phenolphthalein (see Table XXVII) was about the same, B-783 binding 32.04 x 10^{-5} equivalents and B-780 binding 29.97 x 10^{-5} equivalents of potassium hydroxide per gram of protein.

When the amount of alkali bound was determined by the potentiometric method Table XXVIII B-780 bound 55.6 x 10^{-5} equivalents while B-783 bound 65.89 x 10^{-5} equivalents. It is of interest that the values given by the potentiometric method are approximately twice the binding capacity as shown by titration. An experiment Table XXIX, carried out with B-780 before drying, using the titration method, gave slightly lower results than

those obtained for the dried material on the protein basis, namely, 1.0 x 10^{-5} equivalents of potassium hydroxide per gram of gluten protein.

Viscosity determinations of the glutens from flours B-780 and B-783 dispersed in potassium hydroxide and lactic acid.

The results obtained with the dried material (see Table XXX) dispersed in potassium hydroxide did not show any significant differences. By the treatment used, it was not found possible to disperse the dried glutens in lactic acid. When the moist glutens were dispersed in potassium hydroxide or lactic acids, the results were much more encouraging and are probably significant. By reference to Tables XXXII and XXXIV, it will be seen that the gluten from B-780 shows a considerably higher viscosity than does that from B-783. The differences are not so great in the case of potassium hydroxide as they are in the case of the lactic acid. The binding capacities for potassium hydroxide as shown in the Table are probably significant, B-783 binding more alkali than does B-780. The specific conductivity attributed to the gluten is likewise greater in the case of B-783, than it is in the case of B-780.

The isoelectric point of the glutens.

The results indicate that the isoelectric points of the glutens as determined by the optimum hydrogen ion concentration for coagulation are so nearly the same that by the method employed no significant difference could be detected.

V. SUMMARY AND GENERAL CONCLUSIONS.

Physico-chemical studies were made on the glutens from four flours— one a high grade patent made from No.1 northern spring wheat (B-780), one a clear made from No.1 northern spring wheat (B-781) , and two patent flours milled from soft western wheats (B-782) and (B-783). The baking tests show that these flours are distinctly different, the difference being most apparent in the texture of the loaf.

The data presented seem to warrant the following conclusions.

(1) The rate of imbibition determined in hydrochloric and lactic acids is in agreement with the findings of Gortner and Doherty, i.e., the "strong" flour gluten has a much higher rate of imbibition than has a "weak" flour gluten.

(2) There is a marked difference in the rate of imbibition of the different glutens in potassium and sodium hydroxides, the difference while not so apparent is noticable also in the case of barium and calcium hydroxides. Here again in the case of alkalies the "strong" flour gluten has a higher rate than the "weak" flour gluten.

(3) The reaction of glutens to alkalies appears to be somewhat different from the reaction of the glutens to acids, dispersion taking place much more rapidly and at lower concentrations in the case of alkalies, indeed dispersion and imbibition are here almost coincident.

(4) The addition of sodium sulphate to the alkalies of potassium and calcium markedly lowered the imbibition rate.

(5) Drying the glutens washed from the different flours in a vacuum oven at 40° C. markedly altered the physico chemical properties of the glutens; the properties of the different glutens studied becoming more nearly alike.

(6) No material difference in the gel numbers of the different glutens was detected.

(7) In the study of the binding capacity of the dried glutens for potassium hydroxide, the value obtained for B-783 was slightly higher than the value obtained for B-780 both by titration with phenolphthalein and by the potentiometric method.

(8) The binding capacity of the dried glutens as shown by the potentiometric method is about twice that shown by titration, i.e. 35.6×10^{-5} and 29.97×10^{-5} for B-780 and 65.89×10^{-5} and 32.04×10^{-5} for B-783 respectively.

(9) The binding capacity of B-780 as determined by titration was about one-third greater after drying than it was before drying.

(10) The binding capacity of the fresh washed gluten B-783 for potassium hydroxide was somewhat greater than the binding capacity found for B-780.

(11) The specific conductance due to the glutens before drying as shown by their sols dispersed in potassium hydroxide was slightly different, B-783 showing the greater specific conductance.

(12) There is a marked difference in the viscosity of the acid glutens dispersed both in potassium hydroxide and in

lactic acid, the glutens from B-780 showing the greatest viscosity.

(13) These results indicate that there are marked differences in the physico-chemical colloidal properties of the glutens washed from the "strong" and "weak" flours, in addition to the differences noticed by other investigators on imbibition capacity and rate of imbibition.

VI. LITERATURE CITED.

Bailey, C. H.

1920 A Simple Hydrogen Electrode. J. Amer. Chem. Soc. v. 42, p. 45-48.

Blish, M. J.

1916 On the Chemical Constitution of the Proteins of Wheat Flour and its Relation to Baking Strength. Jour. Ind. & Eng. Chem. vol.8, No.2, p. 138-144.

Fisher, M. H.

1915 Oedema and Nephritis. ed.2, 695 p., 160 fig. New York. Bibliography, p. 671-673.

Fleurent, M. E.

1896 Sur une method chimique d'appreciation de la valeur boulangere des farines de ble' Compt. rend. 123 p.755-758.

Gortner, R. A. and Doherty, E. H.

1918 Hydration Capacity of Gluten from "Strong" and "Weak" Flours. Jour. Agri. Research. Vol. 13, No.8, p. 389-418.

Gortner, R. A.

1920 The Gold Numbers ofProtalbinic and Lysalbinic Acids. J. Amer. Chem. Soc. v.42, p. 595-597.

Gröh, J. and Friedl, G.

1914 Beiträge zu den physikalisch-chemischen Eigenschaften der Alkohollöslichen Proteine des Weizens und Roggens. Biochem. Zeit. v.66, p.154-164.

Hess, W. R.

1910 Reibungswiderstand des Blutes und Poiseuillesches Gesetz entnommen. Zeit. f. klin. Med. v.71,p.422.

Hofmeister, Franz.

1890 Zur Lehre von der Wirkung der Salze. V. Untersuchungen über den Quellungsvorgang. In Arch. Expt. Path. u. Pharmakol., Bd. 27, Heft 6, p. 395-413,2 fig.

Humphries, A. E. and Biffen,R. H.

1907. The Improvement of English Wheat. In Jour. Agri. Sci. v.2 pt. 1, p. 1-16.

Jago, William and Jago, W. C.

1911. The Technology of Bread Making. p.291. London

Levene, P. A. and Van Slyke, D. D.

1909 Über Plastein. II Mitteilung. Biochem. Zeit. v. 16, p. 203-206.

Michaelis,L.

1909 Elektrische Überführung von Fermenten. I. Das Invertin. Biochem. Zeit. v.16, p. 81-86.

Michaelis, L. und Davidsohn, H.

1910 Die isoelektrische Konstante des Papains. Biochem. Zeit. v. 28, p. 1-7.

Michaelis, L. und Rona, P.

1910 Beiträge zur allgemeinen Eiweisschemie. II. Mitteilung. Über die Fällung der Globuline im isoelektrischen Punkt. Biochem. Zeit. v. 28, p. 193.

Michaelis,L. und Davidsohn,H.

1910-11 Zur Theorie des isoelektrischen Punktes. Biochem. Zeit. v. 30, p. 143-150.

Michaelis, L und Davidsohn,H.

1910-11 Trypsin und Pankreasnucleoproteid. Biochem. Zeit. v.30, p. 481-504.

Michaelis,L. und Pechstein,H.

1912 Der isoelektrische Punkt des Caseins. Biochem. Zeit. v. 47, p. 269-268.

Olson,Geo. A.

1912 The Effect ofModifying the Gluten Surrounding the Flour. Eighth International Cong. ofApplied Chemistry. v.XVIII, p. 283-296.

Robertson, T. B.

1918 The Physical Chemistry of The Proteins. Longmans, Green and Co., New York, 483 p.

Rothlin, E.

1919 Über die Methodik der Viscositätsbestimmung bei organischen Kolloiden. In Biochem. Zeit. v. 98, p.34-91.

Schmidt, C.L.A. and Hoagland, D.R.

1919 Table Of pH, H^+, OH^- Values Corresponding to Electromotive Forces Determined In Hydrogen Electrode Measurements, With a Bibliography. Uni. of Calif.Pub. v.5, No. 4, p. 23-69.

Schulz, W. and Zsigmondy, R.

1903 Die Goldzahl und ihre Verwertbarkeit zur Charakterisierung von Eiweisstoffen. Beitr. Chem. Physiol Pathol., v.3, p. 137-169.

Snyder, H

1899 The Proteids of Wheat Flour. Minn. Exp. Sta. Bull. 62.

Snyder, H.

1901 Studies On Bread and Bread Making. Office of Exp.St.Ser. U. S. Dept. of Agri. Bull 101

Upson, F. W.,and Calvin, J. W.

1915 On the Colloidal Swelling of Wheat Gluten. In Jour. Amer. Chem. Soc. v.37, No.5, p. 1295-1304, 3 fig. 2 pl.

Upson, F. W. and Calvin, J. W.

1916 The Colloidal Swelling of Wheat Gluten in Relation to Milling and Baking. Nebr. Agr. Exp. Sta. Research Bul. 8, 26 p., 5 fig.

Wood, T. B.

1907 The Chemistry of Strength of Wheat Flour. I. The Size of theLoaf. InJour. Agri. Sci. v.2, pt.2,p.159-170, 2 fig.

Wood, T. B.

1907 The Chemistry of Strengthof Wheat Flour. II. The Shape of the Loaf. In Jour. Agri. Sci. v.2, pt.3, p. 267-277, pl. 5-6.

Wood, T. B. and Hardy, W. B.

1908 Electrolytes and Colloids. The Physical State of Glu - ten. In Proc. Roy. Soc. (London) B. v81, No. 545, p. 38-43,2 fig.

Wood, T. B.and Hardy, W. B.

1909 Elektrolyte und Kolloide. Der Physokalische Zustand Des Glutins. In Kolloid Zeit. Bd. 4, Heft. 5, p. 213-214.

Zsigmondy, R. and Spear, E. B.

1917 The Chemistry of Colloids. Wiley and Sons, N.Y. 1917.