STUDIES ON THE NATURE OF "KOJI-DIASTASE"

BY

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Contents

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Introduction

Enzyme chemistry, one of the branches of biochemistry, seems to be an important key to the study of various vital phenomena in both the animal and botanical worlds.

The extent of investigation, however, is far and wide, and, although considerable endeavour in various respects has been made in order to determine the nature of the enzymes by many scholars since IRVINE (1785) and KIRCHHOFF (1815) discovered saccharification in the process of malt manufacture, yet there remain questions unanswered, even as in diastase, in regard to the principles governing their actions and explaining the essential natures of the same.

It has been about 140 years since the enzyme first attracted attention, but it was not till about 50 years ago that it came to be explained somewhat scientifically. And so far, most of the investigations concerning enzyme have been made with regard to its action rather than to the natures of the enzyme itself. The studies on its physico-chemical nature and structure were initiated only 20 years ago, being most strenuously pursued in about 1910, and then gradually waning in the few years before and after 1920. But they have been pursued with a renewed enthusiasm in recent years. As to the progress of enzyme chemistry, it may be said that it began with the discovery of the enzymic action in the vital phenomena of the biological world, then the existence of enzyme came to be recognized by the actual proof of enzymic action, and finally it has been followed by studies on the chemical and physical nature of the enzyme itself.

Now the preparation or purification of the enzyme used for its investigation is very important and has a direct relation to the study of its nature. As for the preparation methods of diastase in various origins, there are PAYEN and PERZOZ's method (Ann. d. Chim. et Phys. 53, 78, 1833), DUBRUNFAUT's method (Driyler's Palyt Jour. 187, 491, 1868), BARANETZKY's method (Die Stärkeumbilden den Ferment in d. Pflan. Leipzig, 10, 1878), ZULKOWSKY's method (Wiener Acad. Sitzb. Math. Nat. cl 71, II, 453; 77 II, 68), LINTNER's method (Jour. Prak. Chem. 34, 378–394, 1886; 36, 48-498, 1887), PETER's method (Jour. of Biol. Chem. 5, 367, 1908), PRIBRAM's method (Biochem. Z. 44, 293, 1912), SHERMAN and SCHLESINGER's method (Jour. Americ. Chem. Soc. 35, 1617-1923, 1913), WILLSTÄTTER's method (Zeitsch. f. Phys. Chem. 123; 45, 1922), MICHAELIS and EHRENREICH's method (Biochem. Z. 10, 283–299, 1908), FRICKE and KOJA's method (Ber.

Deutsch. Chem. Ges. 57, 310; 755, 1924), etc. However, no sufficiently pure diastase has ever been obtained notwithstanding many and continual endeavours.

Observing the literature regarding the nature of diastase, the "single enzyme theory of diastase", which has been argued about since malt-diastase was discovered at the end of the 18th century, is not generally believed by many scholars at present. As the "two enzyme theory of diastase", there is the theory of WIJSMAN (Rec. d. Trav. Chim. des Pays-Bas IX, I, 1889) that the diastase is composed of two kinds of enzyme, one of which decomposes starch into maltose and erythrogranulose, and the other decomposes the latter into maltodextrine. POTTEVIN (Ann. Inst. Past. 13, 665, 1899) stated also this sort of theory that one enzyme makes dextrine from starch and the other decomposes the dextrine into maltose. BEYERINCK (Zbl. Bakt. (2), 1. 221, 1895) approves of WIJSMAN's opinion, but this is not generally accepted; while POTTEVIN's theory, though there are many doubts, is now commonly regarded as true by many chemists. Next, CHRZASZCZ (Ws. f. Brau. 28, 510, 1911) stated that he obtained both the liquefying and the saccharifying enzyme by partial separation with Ammonium sulphate. In recent years, EFFRONT (C. R. 174, 18, 1922) has stated that there is a great difference in the ratio between the saccharifying and the liquefying activity of the diastases, which are obtained from various origins. This is to say that he recognizes the existence of two enzymes in the diastase. More recently, FRICKE and KOJA (Ber. Deutsch. Chem. Ges. 57, 2, 310–313, 1924) have stated that, when the malt-diastase is dialyzed by electro-dialysis, the power of its starch liquefying action vanishes. So they could separate the saccharifying enzyme from the liquefying one.

From the "two enzyme theory of diastase", CHRZASZCZ (Biochem. Z. 80, 211, 1917) came to assert the "three enzyme theory", which means that there are in the diastase starch liquefying, dextrinizing and saccharifying enzymes. This is a convenient and popular view in explaining the diastatic action of starch decomposition.

Apart from the above opinions KUHN (Ber. Deutsch. Chem. Ges. 57, 1955, 1924) asserted that there are two kinds of amylase, the one is α-amylase and the other β-amylase.

But, most recently observing the attitude of OPPENHEIMER and other scholars towards the action of diastase upon the starch decomposition, it seems to be very complex, and it must not be recognized that the hydrolysis is only due to the character of one or more kinds of enzyme.

There have been many discussions as to whether the diastase is a protein-like substance or not. PRIBRAM (Biochem. Z. 44, 293, 1912), and SHERMAN and SCHLESINGER (1915) published their opinion that the malt-diastase is a protein-like substance, and SHERMAN and TENBERG (J. Am. Chem. Soc. 38, 1638, 1915) declared that the Taka-diastase indicates proteinous reactions like maltase or pancrease. On the other hand, FRICKE and KOJA (Ber. Deutsch. Chem. Ges. 57, 2, 310-313; 313-316, 1924) denied the proteinous nature of the malt-diastase, and KATO (Bull. Agri. Chem. Soc. Japan 1, 937-951, 1925) also denied the proteinous nature of the saliva-amylase and of Taka-diastase from the studies on the manner of action of protease on them. Even if it is granted that the diastase is not constituted only of a protein-like substance, there is no denying the existence of a nitrogenous substance as a constitutional ingredient of the diastase, in so far as there can be no absolutely pure diastase manufactured freely from nitrogen.

MATHEW and GLENN (J. Biol. Chem. 9, 25-56, 1911) published their opinion that the amylase is constituted of active-protein and carbohydrate. And BIEDERMANN (Fermentf. 4, 1920) published the opinion that amylase, especially the saliva-amylase, is a conjugated substance of albumose and mineral ingredient.

As for the studies on the electric cataphoresis of diastase, there are those of MICHAELIS (Biochem. Z. 17, 188, 1909) HALN (Zitungs ber. Ges. Morph. Physiol. München 31, 66, 1919) FRICKE and KOJA (Ber. Deutsch. Chem. Ges. 57, 2, 310-313; 313-316, 1924), etc. According to the results of their investigations, it seems to move mostly, to the negative pole in neutral or acidic medium, while in basic solution it moves to the positive pole.

BIEDERMANN (Arch. ue land-Physiol. 7, 151-156, 1922) recognized in his investigation the existence of diastatic action through albumoses and amino acids, and HAEHN and BERENTZEN mentioned that, by means of a combination of neutral salt, amino acid and peptone, the saccharification of soluble starch occurs. But KÖCHLING (Chem. Zelle u. Gewebe 12, 347-285, 1925) and TAKANE (Biochem. Z. 175, 241-251, 1926) published an opinion that is opposite to the above.

As seen in the literature cited already, the nature of diastase has not yet been definitely determined. Although much may be left to be studied in the future, it is hoped that, by the experiments carried out and the studies made upon this special subject set forth in this thesis, much will be contributed, not only to the chemistry of diastase but also to the understanding

of the enzyme in general.

The principal items of the thesis are "Resume of the preliminary investigations on diastase published already", "Dialysis of Koji-Diastase[1] Solution", "The relation between the action of starch decomposition of Koji-Diastase and its ash, protein and carbohydrate", and "On the enzymic action of mixed chemicals".

Now some modifications of the old idea of diastases must be made. The idea that diastase is a matter similar to protein can not be accepted; but there is no reason to declare it to be some carbohydrate. It seems that diastatic action is not caused only by an organic substance. Though there are still some doubts in regard to its nature, owing to an insufficiency of the data from which to draw a conclusion, the following may be deduced from many investigations that have been made hitherto and from our present studies on "Koji-Diastase": Diastase is made up of several constitutional ingredients which come from protein, carbohydrate, and inorganic substance related to one another. These constituents are exquisitely balanced. This balance of each constituent in a diastatic enzyme is easily broken by a change of outer circumstances. The degree to which this balance is broken seems to have a direct relation to that of the decrease of the enzymic activity.

Thus, the results obtained in our studies on "Koji-Diastase" seem to be much better than the old idea in explaining various phases of enzymic action which occur in the biological world as a vital phenomenon.

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(1) "Koji" means a steamed grain which is infected with *Aspergillus oryzae*. "Koji-Diastase" means an enzyme substance purified from a polished rice "Koji" by Lintner's method.

I. Resume of the Preliminary Investigations on Diastase Published Already

Here are mentioned the summaries of some literature that have been published already and are closely related to this present paper.

(I)

On Lethal Temperature of Koji-Diastase in Aqueous Solution and the Recovery of Its Action After Heating.[1]

The results obtained from the experiments for the lethal temperature of Koji-diastase in aqueous extract of polished rice Koji and the recovery of its action after heating, are as follows:

No influence of heating upon Koji-diastase in aqueous solution (pH= 6.50) is perceptible under 25°C.; but if heated 2 hours at 40°C., an injurious effect is obviously perceived. The degree of injurious effect on diastatic power by heating corresponds to the temperature and the time of duration. When the heat was applied at 140°C. for an instant, Koji-diastase in aqueous solution was entirely destroyed. It is to be called lethal temperature of a diastatic function. It was also observed that Koji-diastase in aqueous solution was destroyed by heating more than 30 minutes at 115°C., or more than 5 minutes at 130°C., while it was not destroyed at a temperature lower than 100°C. even when heated for one hour. It seems that the diastatic power of Koji-diastase solution injured by heating may be restored to some extent by preserving the solution at room temperature; but this recovery only occurs when the enzymic power is not destroyed entirely. The Koji-diastase solution heated at more than lethal temperature never recovers its power of saccharification even by preserving it for a long duration of time.

Experiment "On Lethal Temperature of Purified Koji-Diastase in Aqueous neutral Solution and the Recovery of Its Action After Heating"[2] was also made to discover whether the lethal temperature of purified polished rice Koji-diastase in neutral medium (pH=6.9) of aqueous solution and the recovery of its diastatic power after heating would coincide with the results of the experiment made with the diastase in aqueous solution of polished rice Koji. The purified Koji-diastase was manufactured by LINTNER's method; and the result of the experiment made with it shows that its lethal temperature is 137.5°C., and its recovery after heating is almost the same as that observed in the experiment of Koji extract solution.

(II)

Comparative Studies on Enzymic Powers of Koji Manufactured from Different Materials[3] (In Japanese).

Various kinds of "Koji", used in this experiment, were made from different materials by the same method of preparation.

The results of the investigation are summarised as follows:

(1) Power of the diastase is strongest in polished rice-Koji which contains much carbohydrate, while it is weak in unpolished rice-, soy-bean-, or maize-Koji which contains comparatively little carbohydrate and much protein.

(2) On the activity of maltase, it is difficult to find clearly any difference between polished rice-Koji, unpolished rice-Koji, and soy-bean-Koji; or between polished rice-Koji and maize-Koji.

(3) Power of invertase is strongest in soy-bean-Koji, next in unpolished rice-Koji, and weakest in polished rice-Koji.

(4) Pepsin is strongest in its action in soy-bean-Koji which contains much protein, and next in unpolished rice-Koji, and very weak in polished rice-Koji which contains comparatively little protein.

(5) Power of trypsin is the same in order as pepsin, being very weak in polished rice-Koji.

(6) Lipase is strongest in its power in soy-bean-Koji, next in unpolished rice-Koji, and weakest in polished rice-Koji, being almost proportional to the fat content of the raw materials.

(7) The diastase is one of the enzymes that is durable for a long keeping. It can be preserved for a long time in a dry state. Even in solution it also can be kept for several years, if toluene is added as an antiseptic. From the results of the experiment on keeping the aqueous extracts of polished rice-, unpolished rice-, and soy-bean-Koji, it is found that they have retained rather strong diastatic power even, after five years' preservation.

(8) Diastase, maltase, pepsin, and trypsin show some resistance against heating; of these, the diastase is the strongest and it does not always lose decidedly all its power, even after boiling for 1–2 hours. Maltase is weaker than the former, and it perishes after 20 minutes' boiling. Invertase and lipase can not stand a boiling. Lipase seems to be the weakest in this respect.

(9) The enzyme solution of soy-bean-Koji is not so badly injured in its action as that of polished rice- and unpolished rice-Koji at the be-

ginning of its boiling, but the former, after a long time of boiling, seems to be injured more than the latter two.

(10) As the results of this investigation, diastase of polished rice-Koji is found to be one of the most convenient materials for the purpose of studying various problems on diastase, especially on the nature of diastase, owing to easiness of preparation of a comparatively pure sample and also to its strength in power and its durability in keeping.

(III)

Influence of the Colloids upon the Action of Diastase and Its Resistance to the Heating of the Enzyme Solution, and also Its Recovery of Its Action After Heating [4]. (In Japanese).

The results of the investigation are summarized as follows:

(1) When the Koji-Diastase in almost neutral solution is heated, its enzymic power is always weakened; and some whitish turbidity appears, except when the solution is extremely diluted or heated at a very high temperature. But the degree of turbidity does not always agree with that of the change of the enzymic power. The injurious effects of heating seem to be due to the various physico-chemical changes of the materials in enzyme solution, which depend upon the nature of the enzyme solution and its concentration, degree of heating, etc.

(2) The influence of the added colloidal substances upon the enzymic action of Koji-Diastase in almost neutral solution is generally injurious. The substrate and the enzyme itself in a highly colloidal state also exert the same influence.

(3) The increase of enzymic power of the Koji-Diastase in almost neutral solution is not strictly proportional to its concentration; the more diluted the solution is, the stronger is the enzymic power comparatively. The chief cause of this disproportionality perhaps consists in the relation of the degree of dissociation of the enzyme in the solution.

(4) The speed of heating has a great influence upon its injurious effects on the action of enzyme. The higher the speed is, the greater the injuries are on a basis of the same degree of heating. The higher the concentration of the enzyme solution is, the greater is the influence of the heating speed.

(5) The influence of the colloidal substances added upon the effects brought by heating the enzyme solution may differ according to their nature and concentration, degree of heating temperature, time of heating, and

the reaction of the medium. It is protective till it reaches a certain degree of concentration, while it is injurious in the concentration above it. This is due to the protective action of colloid against the coagulation of enzyme and its adsorption. The concentration of colloid, degree of heating temperature and time of heating which control protectively or injuriously, and also their influence upon the heated enzyme solutions are seen as follows:

Pure neutral gelatine solution——In case of heating 0.056 % solution of Koji-Diastase up to 85°C. in 55 minutes, the protective action of gelatine on heat-injury as compared with the control appears in the concentration of gelatine from 0.001 to 0.5 %, and its maximum protective influence attains in the concentration of 0.1 %. But when the concentration of gelatine reaches from 1.000 to 5.000 %, it shows an injurious influence.

Alkali-albuminate (almost neutral solution)——In case of heating the 0.056 % solution of Koji-Diastase up to 85°C. in 50 minutes, the protective action of alkali-albuminate attains in the concentration from 0.0005 to 0.5 %, with a comparatively remarkable influence in the concentration 0.005 %.

Soluble starch (almost neutral solution)——In case of heating the 0.02 % Koji-Diastase in almost neutral solution at 85°C. for 5 minutes, the effect of soluble starch appears to be protective in the concentration from 0.01 to 0.10 %, its effect being greatest at 0.1 %, while it is injurious in the concentration from 0.25 to 5.00 %, particularly from 0.50 to 5.00 %. In case of heating the solution at 85°C. for 10 minutes, the protective effect of the soluble starch appears in the concentration from 0.00001 to 0.5 %, it being highest at 0.2 %. In case of heating at 85°C. for 15 minutes, it has the same tendency with that of the former case, it being highest at 0.01 %. In case of heating the solution at 75°C. for 15 minutes, the protective influence is found always in the concentration from 0.001 to 5.0 %, it being highest at 2.5 %. When 5 c. c. of 0.4 % Koji-Diastase solution at 25°C. is added to 20 c.c. of the soluble starch solutions in different concentrations at 100°C., the influence of soluble starch is protective to some degree in the concentration of 0.1 %, but it is remarkably injurious at the concentrations from 2.5 to 5.0 %.

(6) When the enzyme in almost neutral solution is heated, the colloid in the solution itself has a great influence upon the injury caused by heating, generally the lower the concentration of the solution, the

less the injury. However, the colloid exerts protectively on the enzyme against the injurious effect of heating in a certain concentration of the solution. The higher the temperature of heating, the greater the injury becomes. As for the protective action of the colloid in this case, it is seen at 0.45 % when heated up to 60°, at 0.09 % when heated up to 80°, and to 0.075 % when heated up to 100°C.

(7) As for the influence of added colloidal substance on the recovery phenomenon of the action of injured diastase by heating in almost neutral solution, it shows that the colloid has no influence on the recovery of enzymic action in spite of the method of its addition, that is both before and after heating. Sometimes, it tends rather to decrease the enzymic power. This is perhaps due to the coexisting colloidal substance that prevents enzymes once injured from regaining their active form, and to its adsorption of the enzymes.

(8) As for the relation between the concentration of the enzyme in almost neutral solution and the recovery phenomenon of the action of Koji-Diastase in the cooled solution after heating, it shows that the recovery is greater as the concentration is lower in a time of heating, or as the heated solution is diluted earlier after cooling. It may be said conclusively that, on heating the enzyme solution, the difference in a concentration of the enzyme solution causes a difference in the degree of association of the enzyme and other substances, accordingly there is a difference in the degree of dissociation of enzyme substance after its cooling, thus differentiating the enzymic power.

(IV)

Studies on the Electric Cataphoresis of Koji-Diastase in Aqueous Solution[5]. (In Japanese).

The aqueous solution of polished rice Koji-Diastase purified by LINTNER's method was used and the phenomena of electric cataphoresis of enzyme was examined by means of flowing the electric current into the solution in various concentrations of hydrogen ion (pH 6.9, 9.8 and 2.6).

The results obtained in the experiments are as follows:

(1) The diastase is moved by the flowing current, changing the pH value, to the negative pole in the neutral and acidic solutions, while it is moved to the positive pole in the basic solution, as in the case of the experiment with vegetable amylase performed by MICHAELIS[6]. So it can be recognized that the isoelectric point lies rather in the side of

basic nature. And the moved diastase returns to the original position by exchanging the two connected poles of the battery. Moreover, there also can be seen the same phenomena of cataphoresis in other enzymes.

(2) The enzymic power is decreased when Koji-Diastase is prepared in an acidic solution (pH 2.6); but is not decreased when it is prepared in a basic solution (pH 9.8), and it may be deduced from this fact that Koji-Diastase does not suffer easily in a basic solution. It is almost positively charged in neutral solution (pH 6.9), and it has a tendency to move to the negative pole. This tendency is more remarkable in an acidic solution. On the contrary, in a basic solution it has mostly the negative charge, and so it moves to the positive pole. The relation between both the positive and negative charge of electricity, which is considered as one of the controlling factors of the strength of the enzymic power, is also understood to exist in the other enzymes, as in the case of the diastase.

(3) When the electric current is passed through the aqueous neutral solution (pH 6.9) of Koji-Diastase, the cathodic portion gradually becomes turbid, and at last a coagulated precipitate is produced. Consequently, the transparent enzyme solution obtained by mixing the two filtrated anodic and cathodic solutions is decreased in its viscosity and surface tension as compared with the original enzyme solution, but on the contrary, the enzymic power of Koji-Diastase is gradually increased. However, if the flowing of the current lasts for a much longer time, the enzymic power will be at last decreased.

(4) When the coagulated substances produced in the cathodic portion are removed, the power of the enzyme becomes greater, as compared with that of the unfiltrated solution, in case its action on starch is for a short time, but with a long time of action the power becomes weaker than that of the unfiltered solution.

(5) In spite of the fact that diastase has a tendency to move mainly to the negative pole in the neutral aqueous solution of Koji-Diastase, the anodic portion of the solution after a short time of flowing the current has a tendency to increase its enzymic power as compared with that of the original enzyme solution, and it decreases its power after a long time of flowing.

(6) There are at least two different kinds of enzyme in the so-called diastase of Koji, the one resembles malt-diastase which decomposes starch into maltose, and the other decomposes maltose into glucose.

(7) Maltase in Koji-Diastase moves to the positive pole in a neutral solution, while it moves to the negative pole in a HCl-acidic solution. It also moves to the positive pole in a NaOH-basic solution, when the current is passed through the solution of Koji-Diastase.

(8) The power of maltase in the acidic solution which is made with HCl (pH 2.6) is increased as compared with that of the neutral solution (pH 6.9), but it is quickly decreased when the electric current is passed through the solution. On the contrary, it is decreased when the enzyme solution is made alkaline with NaOH (pH 9.8). But it is extremely increased when the current is passed through the solution, as it becomes at last greater than that of the neutral solution. It is possible to state that maltase has much of a negative charge, except in the case of acidic solution, as compared with diastase, and also that it is in a state of dissociation in the acidic medium, it being very susceptible to acid, while it is in a state of nondissociation in the basic medium. Therefore, the effect of the current on the maltase is great in an acidic solution, as in the case of diastase.

(9) Invertase in Koji-Diastase moves to the positive pole by the current in the neutral and acidic solutions as in the experiment by MICHAELIS; but the power of the invertase is very weak. In the case of the basic solution, it is very difficult to find clearly the electrical property of the invertase, since its power is very much weakend in basic solution.

(10) Pepsin in Koji-Diastase seems to have a tendency, though not clear, to move by the current to the negative pole in the neutral solution (pH 6.9), to the positive pole in the HCl-acidic solution (pH 2.6) and also to the negative pole in the NaOH-basic solution (pH 9.8).

(11) Trypsin in Koji-Diastase seems to have a tendency to move to the negative pole in the neutral solution (pH 6.9), and also in the HCl-acidic solution (pH 2.6), and to the positive pole in the NaOH-basic solution (pH 9.8).

(12) The filtrate of the cathodic solution which is obtained after about 100 hours' passing of the electric current through the 0.45 % aqueous neutral solution of Koji-Diastase is powerful in diastatic activity as compared with the original solution. On the contrary, the powers of maltase, pepsin, invertase and trypsin are comparatively weak. The cathodic solution thus obtained is a very good sample for the study of the diastase of Koji.

The filtrate of the cathodic solution of Koji-Diastase obtained in basic reaction by the flowing of the current for 87 hours contains

only a little maltase, invertase, pepsin and trypsin, but the power of diastase is very strong. The filtrate is comparatively poor in the reaction of protein.

(13) The power of resistance of enzymes in the solution of Koji-Diastase against the injury caused by the electric current differs according to the nature of the solution. In the neutral solution, the power of the resistance is in the order of diastase, maltase, pepsin, trypsin and invertase. This is the same as in the order of heat resistance which has already been mentioned in the preliminary investigation on the diastase (II). This also is the same as in the order of the diffusion velocity of enzymes in dialysis with the exception of pepsin and trypsin, which will be mentioned in a later chapter.

(14) The solutions of Koji-Diastase, from which the precipitates produced by the passing of the current of electricity are removed, show all the protein reactions viz. MILLON's, BIURET's and Xanthoprotein reactions. And these reactions are more obvious in the cathodic solution in the case of the neutral and acidic reactions than that of the anodic, while in a basic reaction they are more obvious in the anodic solution than that of the cathodic.

(15) The viscosity of the enzyme solution is always high as compared with that of the distilled water. When the electric current is passed through the solution, it generally becomes low, and the lowest viscosity appears in the anodic solution in the case of neutral reaction. And when the viscosity of the solution is decreased the enzymic power of it seems to be increased.

(16) The surface tension of the solution of Koji-Diastase is always low as compared with that of distilled water. After the flowing of the electric current it shows lower tension than that of the original enzyme solution, being the lowest one to appear in the anodic portion of neutral solution. The relation of the surface tension to the change of the enzymic power of the solution is almost the same as in the case of the viscosity.

(17) The specific conductivity of the solution of Koji-Diastase is lowest in the neutral solution, next in the basic solution (pH 9.8) and highest in the acidic solution (pH 2.6). By the flowing of the electric current into the solution, its specific conductivity becomes gradually higher than that of the original enzyme solution. This seems to be due to the increased dispersion or ionization of the enzyme and other impurities.

(18) When the electric current is passed through the 2 % solution (pH 6.1) of soluble potato starch which is a substance to be acted upon by diastase, it moves always to the positive pole, contrary to the diastase. Therefore, it seems to be charged mostly with negative electricity.

(V)

On the Comparison of Carbohydrate Splitting Activity in Each Part of Koji-Diastase Treated Fractionally with Alcohol, and the Relation Between Diastase and Its Ash Component[7]. (In Japanese).

The results obtained in the experiments are as follows:

(1) The yield of diastase obtained by fractional precipitation with various concentrations of alcohol from Koji-extract, is greatest at 70 % concentration of alcohol. The enzymic power is highest in the portion precipitated in 80 % alcohol, and next in 70 %. The yield and intensity of maltase which coexists with diastase are also highest at 70 %.

(2) The yield of diastase obtained by fractional precipitation with various concentrations of alcohol from dialyzed solution of Koji-Diastase by means of a shark's air bladder is greatest at 82 % concentration of alcohol. The power of diastase is highest in that part of the precipitation. The total enzymic power of each fraction, calculated by multiplication of the power to yield, is also highest in the part precipitated with 82 % alcohol, with decreasing order of the power of the fraction obtained in both sides of that concentration of alcohol. Also the behaviour of maltase and invertase which coexist with the diastase are almost similar to that of the latter enzyme. In dialyzed solution it is very difficult to precipitate the enzymes with alcohol from the solution as compared with that which is not dialyzed. The ash ingredient contained in the diastase has a very important relation to the character of precipitation of enzyme substance caused by the addition of alcohol.

(3) Diastase of Koji is very permeable through a shark's air bladder from the beginning of the dialysis. The amounts of the precipitates produced from the permeated enzyme solution renewed successively by the addition of alcohol in various concentrations became gradually small as the dialysis proceeded. The total diastatic power of the precipitate has also a tendency to become gradually less by the successive renewals. But the powers of maltase and invertase are weak in the

precipitate obtained from the permeated solution at the beginning of dialysis, with gradual increase of its power up to a certain renewal and then gradually decrease again. The saccharifying power of diastase in the permeated solution with the same concentration increases distinctly in an extent from the first up to the forth of the renewals. And the powers of maltase and invertase of the permeated solution are both weak at the beginning of the dialysis and gradually increase and then decrease again. Thus, the purity of the diastase solution seems to be increased after the renewals of the permeated solution have been made successively several times.

(4) Koji-Diastase dialyzed by shark's air bladder in its aqueous or 10 % alcoholic solution is able to permeate "De HAEN's Membran Filter" ("2 M-F, Fein Portig"); and even when its solution is made to 60 % of alcohol concentration in order to make its precipitate, there is still some part of it in soluble form which can be permeated through the membrane filter.

(5) The starch liquefying and saccharifying powers of each fraction obtained from the polished rice Koji-Diastase with various concentrations of alcohol (65, 75, 85 %), do not necessarily coincide. The quality and quantity of the ash contained in each fraction of the enzyme has a correlative influence upon its starch liquefying power. At least, the ash contained in the fraction precipitated by 65 % alcohol seems to be more suitable for starch liquefying action than that separated by 85 %.

(6) The relation of malt-diastase to its ash almost resembles that in the case of foresaid polished rice Koji-Diastase. But the injurious effect on its power of preservation for a long time seems to be greater than that in the diastase of Koji.

(7) The relation between each diastatic power of white earthy Koji, red rice Koji and polished rice Koji and their ash containing ingredients, is as follows: The order of the saccharifying power of the diastase prepared from the three kinds of Koji stated above is highest in polished rice Koji, next in red rice Koji and lowest in white earthy Koji. But there can not be found any relation between the ash of purified enzyme and its power of starch saccharification. The starch liquefying power of the diastase comes in order of polished rice Koji, white earthy Koji and red rice Koji. The ash of white earthy Koji is quantitatively highest, but as it seems to have comparatively many parts which are not directly related with enzymic action, its liquefying

power is superior to that of red rice Koji, but inferior to that of polished rice Koji. Although the ash of polished rice Koji-Diastase is a little superior to red rice Koji-Diastase, its liquefying power is very superior. From these facts it is recognized that the quantity and quality of ash of polished rice Koji enzyme are always in good condition. The ash of red rice Koji is inferior to that of the polished rice Koji in quality and quantity, and that of the white earthy Koji is superior in quantity but inferior in quality to that of the polished rice Koji. These are the most interesting facts in the chemistry of enzyme.

(8) The relation between the change of starch liquefication which results from the germination of unhulled rice and its ash is as follows: The ash content is highest in the original unhulled rice and next in unhulled rice at a later stage of germination, and least in unhulled rice at an early stage of germination. The quantity of purified enzyme obtained from unhulled rice, is in the order of unhulled rice at later stage of germination, unhulled rice at an early stage of germination and the original unhulled rice; and the quantity of the ash is in the order of unhulled rice at a later stage of germination, unhulled rice at an early stage of germination and the original unhulled rice. And the power of the enzyme substance is the same as the order of the above mentioned ash. In other words, unhulled rice, in which its ash has been decreased by means of steeping of the original unhulled rice, is changed qualitatively in its proper material by germination and produces the enzyme substance in which the quantity of ash is comparatively great.

(9) According to the results of the precipitation experiment with various concentrations of alcohol on the inorganic compounds, such as phosphates, sulphates and chlorides of Mg, Ca, K and Na which seem to be important constituents of the ash in Koji-Diastase, at the point of 63.3 % alcohol which is suitable to precipitate the diastase almost all of the phosphates or sulphates of Mg and Ca and a part of K or Na are precipitated, while the chlorides of Mg, Ca, K and Na are not precipitated at all. Therefore, it naturally will be considered that the ash ingredients which especially relate to starch liquefying action are Mg, Ca, K, Na, PO_4 and SO_4.

(10) Taking the results of various investigations in regard to the diastase into consideration, the ash ingredients, protein-like substances and carbohydrates, which are supposed to be important constituents of Koji-

Diastase are mixed. From the solution of these mixed chemical a precipitate is made by the 63 % alcohol. The power of the starch liquefying action of the precipitate is then treated and found to be very strong, though it is less powerful than that of the polished rice Koji-Diastase while it is more powerful as compared with that of the red rice Koji-Diastase.

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2) K. MIYAKE and M. ITO:—Jour. of Biochem. Vol. III, No. 2, pp. 177-194, 1924.
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II. Dialysis of Koji-Diastase Solution

The purpose of the dialysis of enzymes up to this time, as in the case of purification for general proteins or other substances, has been no more than an attempt to remove the containing ash impurities. Our purpose of the dialysis is entirely different from that of the ordinary one mentioned above. It is to find out the nature of the substances contained in the inner or outer solutions after the dialysis of Koji-Diastase, in which the outer liquid was renewed successively.

The results of this experiment are important data in elucidating the essential nature of the diastase and the mechanisms of its action. Heretofore, there has been no experiment of this sort made in regard to Koji-Diastase. But there is only an investigation made by R. FRICKE and P. KOJA[8] on purifying malt-diastase by means of electric dialysis and electric diffusion, which are somewhat related to the present investigation. The original malt-diastase used in their experiment showed a strong protein reaction, its nitrogen and ash contents being 6.12 and 5.05 % respectively.

After the electric dialysis, there was produced a precipitate of a protein-like substance in the center chamber, so the dry matter which corresponded to 13-18 % of the original enzyme substance remained in the filtrate of the dialyzed solution. The power of the enzyme which remained was increased up to 2.1-2.5 times the original, while its nitrogen and ash con-

8) R. FRICKE and P. KOJA:- Ber. Deutsch. Chem. Ges. 57, 2, 310-316, 1924.

tents were decreased to 3.5 % and 1 % respectively. It showed therefore no more protein reaction, but MOLISCH's reaction of carbohydrate was shown clearly. By the electric diffusion the substance which amounted to 16 % of the original enzyme was diffused out in the liquid of the cathodic side.

The diastatic power of this substance was increased up to 4.6 times that of the original enzyme. It showed no MILLON's protein reaction, but MOLISCH's reaction of carbohydrate was evident.

The constitution of it was C,56.4 % ; H,7.9 % ; and N,3.28 %. Moreover, they mentioned that the power of starch liquefying was always accompanied by the power of producing maltose. Therefore there must not be recognized the existence of two different enzymes in malt-diastase. They noted that diastase was not damaged by protein splitting enzyme, and that the saccharifying power was not changed by the removal of its contained protein by means of adding Uranil acetic salt. Thus they denied the protein nature of diastase, because protein is not needful for its action.

In spite of their opinion, it can not yet be believed unreservedly that the diastase may not perhaps be related to some protein-like substance, because there still remains some nitrogenous material in their purified enzyme. And it must be considered that the power of enzyme should be influenced not only by its purity, but also by the state of the enzyme in its solution.

EXPERIMENT

A. Preparation of sample

The material used in this experiment for Koji-preparation was 3rd class rice produced in Asahigawa, Hokkaido. The material used for "Moto-Koji"[1] was 3rd class rice produced in Banshû, Honshû ; its loss in polishing was 25 %, and the polished rice after washing and steeping contained 0.435 % of ash (P_2O_5, 0.285 % ; MgO, 0.045 % ; CaO, 0.014 % ; K_2O, 0.102 % ; Na_2O, 0.025 %) in dry matter. The steeping time was 12 hours, and the steaming time was about 1.5 hours.

Preparation of Koji : Samples of Koji No. 1– No. 6 were obtained in the market. Moto-Koji was made by using 112.5 grms. of "Tane-Koji"[2] per one "Koku" (=180.4 litres).

(1) "Moto" is a source of yeast in the course of "Sake" brewing. "Sake" is Japanese alcoholic liquor brewed from polished rice.

(2) "Tane-Koji" is a source of Koji infected with *Aspergillus oryzae*.

Koji and its extract: The samples of Koji prepared were all of superior quality, and among them the Moto-Koji was the best.

The total dry matter of the extract of Moto-Koji was made as follows: 1500 c.c. of 20% alcohol were added to 760 grms. of Koji and extracted for three days and filtered, thus 1150 c.c. of the filtrate were obtained. Then it was concentrated at low temperature and dried for a long time in a sulphuric acid desiccator. 152 grms. of dry matter of the extract, corresponding to 20 % of the original Koji, were obtained. And its ash content was estimated to be 0.357 %.

Preparation of Koji-Diastase:

Koji-Diastase used for this experiment was purified from a Koji by LINTNER's method. The results are shown in table 1.

Preparation of semipermeable membrane:

The air bladder of a shark, and the intestine and bladder of a pig were used in this experimet. These semipermeable membranes were soaked and renewed for some days in distilled water, 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 %, 95 % alcohol and ether one after another; and next they were soaked and washed one after another in 80 %, 70 %, 60%, 50 %, 40 %, 30 %, 20 %, 10 %, alcohol, and distilled water. In this progression, the time spent for soaking these membranes in the 40-50 % alcohol was longer than in the other concentrations of alcohol, and they were washed in 80 %, 90 %, 95 %, 99 % alcohol and ether, then dried in the air. So five months were taken for these treatments and at last we could find absolutely no substances dissolved in 10 % alcohol or in distilled water.

B. Dialysis of enzyme solution.

Dialysis: To 0.5535 gram of the enzyme substance No. 1 were added 50 c.c. of 10 % alcohol solution, and the solution in a somewhat turbid state was poured into the shark's air bladder and dialyzed against 100 c.c. of 10% alcohol solution in a cool place; at certain intervals the renewal of the outer solution was made repeatedly; the removed solutions after being added with a little toluene were kept at room temperature for experiment. There was a suspense lest a change of the enzyme substance appear on account of the contamination of microorganisms in the course of the dialysis experiment for 10 months, but such a defect was not seen. The number of days after the beginning of dialysis, the time allowed for diffusion and the hydrogen ion concentration of the outer and inner (residual)

solution[1] are shown in table 2. It is provided that the number with dash in parentheses is that of the inner residual solution corresponding to the outer solution of the same number. I' is a sample of the outer solution taken in 30 hours of dialysis, and I is the outer solution at the time of first renewal.

In the course of dialysis, the renewed outer solution did not always hold the amount of 100 c.c. which was added at every time of renewal. From I to XXXV, the outer solution decreased and the inner solution increased remarkably; while from XXXVI to XL, the outer solution decreased slightly and the inner solution showed a slight tendency of increase. And after XLI, the osmotic pressures of both solutions seem to be nearly in equilibrium, showing almost no change in their quantities. As seen in the previous table, 10 c.c., 10 c.c., 15 c.c. and 15 c.c. of the inner solution were used respectively in XV, XXII. XXVI and XXXV renewals for the examination of enzymic power in a state of dilution or in its original state. And, there were 30 c.c. of inner solution remaining at the end of the dialysis. It is self evident that the inner solution which was 50 c.c. in the beginning has become in total 80 c.c. (30+10+10+15+15) in the course of dialysis. So the increasing of the inner solution seems to be 60 %.

From the results of this experiment, it can not be denied that not only inorganic substances, general crystalloids and other impurities but also some kinds of enzymes aré permeated from the membrane.

Moreover the diffusing phenomenon seems to occur very slowly, continuing for a long time, and, of course, the manner of changes of the enzymic activity of the outer and inner solution differs according to the different kinds of enzymes. So it is desirable to explain particularly about the manner of the dialysis on different kinds of enzymes.

1. Permeation of carbohydrate-splitting enzyme

To 5 c.c. of I'solution were added 20 c.c. of 2 % starch, soluble starch, maltose or sucrose solution and 2 c.c. of toluene, placed in the thermostat at 38°C., and after the digestion 5 c.c. of each solution were used for the determination of the carbohydrate splitting powers.

The starch used in this experiment is a purified kind prepared from potatoes produced in Sapporo, the soluble starch is made from the above

(1) "Outer solution" means an enzyme solution diffused through semipermeable membrane in dialysis of "Koji-Diastase".
"Inner (residual) solution" means a dialyzed enzyme solution remaining in semipermeable membrane in the course of dialysis of "Koji-Diastase".

starch, and the maltose and sucrose are all Merck's goods.

The saccharifying power of the enzyme solution is shown, for the sake of convenience, with the titrated number of c.c. of $KMnO_4$ solution by BERTRAND's method, and 1 c.c. of $KMnO_4$ solution corresponded to 10. 26376 milligrams of Cu. The specific viscosity was calculated from the flowing time of samples by means of OSTWALD's viscosimeter which took 48.75 seconds for the distilled water at 38°C.

In the following tables, "Control" means the experimental result without enzyme, using 10 % alcohol solution instead of the enzyme solution.

These remarks can also be applied to other experiments on the nature of Koji-Diastase (II, III, IV) in so far as no explanations are found there.

The results are shown in table 3.

According to the results of this experiment, it can be recognized that there are diastase, maltase and invertase in the "dialyzate"[1], especially the diastatic power predominating the others; and that its saccharifying activity for the soluble starch is more powerful than for starch paste.

2. Permeation of protein-splitting enzyme

The powers of pepsin and trypsin were estimated quantitatively by VOLHARD's casein method. The casein solution was prepared as follows: 20 grams of Merck's casein was mixed with 200 c.c. of distilled water and 16 c.c. of normal NaOH solution in a flask of 500 c.c. capacity, shaken well, and then 184 c.c. of distilled water was added to it. The mixture was dissolved by warming at 85°–90°C., and used after cooling.

Estimation of the power of pepsin

To 1.1 c.c. of normal HCl solution distilled water was added to make it 15 c.c. and then 10 c.c. of the casein solution was added to it (no precipitation of casein occured in this case); next 5 c.c. of the enzyme solution, 1 c.c. of distilled water and 2 c.c. of toluene were added. This mixture was digested for 40 hours in the thermostat at 38°C. After the digestion, the undigested casein was made to precipitate by adding 0.5 c.c. of normal HCl solution and 10 c.c. of 20 % Na_2SO_4 solution. The precipitate was filtered with tared filter paper, washed with water, dried and weighed.

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(1) "Dialyzate" means an enzyme substance (in its solution) permeated through semipermeable membrane in dialysis of "Koji-Diastase".

Estimation of the power of trypsin

To the mixture of 0.5 c.c. of normal NaOH solution, 15 c.c. of distilled water and 10 c.c. of casein solution were added 5 c.c. of the enzyme solution and 2 c.c. of toluene. The mixture was digested for 40 hours in the thermostat at 38°C., and the remaining casein was precipitated by adding 1.5 c.c. of normal HCl solution and 10 c.c. of 20 % Na_2SO_4 solution, and it was filtered, washed, dried and weighed in the same manner as above.

The results are shown in table 4.

According to the results, the protease such as so-called pepsin and trypsin, seems not to permeate the membrane.

3. Comparison of the enzymic powers between the outer and inner solution of dialysis

A comparative study of the enzymic powers was made out with the results as shown in table 5.

The powers of diastase, maltase and invertase of the outer solution are always inferior to those of the inner solution. But they are not always so, if the concentration of the solution is taken into consideration. Both the outer and inner solution at the 35th renewal are more decreased in their starch liquefying powers as compared with the saccharifying powers.

4. Changes of enzymic powers of the outer solution

(1) Diastase

The results are shown in table 6.

In this experiment the outer solution evidently had both the starch liquefying and saccharifying powers, but the former seems to be comparatively decreased after the 31st renewal.

The diastatic power, though it shows many changes probably caused by the state and concentration of the enzyme solution, seems to be gradually decreased in general. This point will be mentioned in detail in a later chapter.

(2) Carbohydrate-splitting enzymes.

The results are shown in tables 7 and 8.

The powers of maltase and invertase in the outer solution show many changes as seen in that of diastase. The solutions from the 36th to the 45th renewals are holding the power of decomposing starch, soluble starch

and dextrine, but are very feeble for the sucrose. The starch and soluble starch splitting powers of diastase and dextrine splitting power of dextrinase seem not always to accompany with each other owing to the condition of the enzyme solution. After the 47th renewal, the enzymic powers of in vertase, maltase and diastase all become very weak, and become almost invisible after the 50th. In the 52nd and 53rd renewals there is no starch liquefying power, consequently, no saccharifying action in either outer or inner solution. But there are found a slight saccharifying activity for soluble starch, a trace of enzymic action of maltase, and no action of invertase.

(3) Lipase, pepsin and trypsin

Of the 3rd renewal of dialysis, the power of lipase, pepsin and trypsin in both the outer and the inner solution was examined as follows:

Lipase..... 20 c.c. of olive oil emulsion was mixed with 10 c.c. of distilled water, 10 c.c. of enzyme solution and 2 c.c. of toluene, and the mixture was digested for 40 hours in the themostat at 38°C. After the digestion, the mixture was cooled, and the fatty acid which was produced from olive oil by the lipase was titrated with 1/10 normal NaOH solution, after adding 40 c.c. of 99 % alcohol, 5 c.c. of ether and 3 drops of phenolphthalein, till it indicated a rose colour. The olive oil emulsion used was prepared by heating and shaking the mixture of 270 c.c. of 2.5 % solution of traganth gum and 30 c.c. of olive oil.

Pepsin and trypsin..... The estimation of pepsin and trypsin was performed by VOLHARD's casein method as previously mentioned.

The results are shown in table 9.

According to this experiment, the power of pepsin and trypsin is invisible, and that of lipase is nonexistent.

5. Relation between the time of dialysis and the rate of diffusion of enzyme substance

It is already evident that at the dialysis of Koji-Diastase, diastase, maltase and invertase are able to permeate the shark's air bladder. It is not difficult to imagine that a certain ratio my be held between the amount of enzyme diffused out and the time of dialysis. But the rate of the diffused enzyme for the unit time will be gradually decreased in general in consequence of dilution of the inner solution caused by the repetition of renewal. To emphasize these facts, an experiment was tried, the results of which are shown in table 10.

According to this experiment, the powers of the outer solutions renewed successively have a tendency to increase and decrease periodically, though they decrease gradually. These results show only the power held by every enzyme solution at the time when the XVth renewal was made, but they do not show the absolute quantities of the enzyme. It may be believed at least that there would exist a proportional relation between the amount of the diffused enzyme and the time of its diffusion, if there were no dilution or changing character of the inner solution. These will become plain through further researches into the character of the enzyme.

C. The increasing phenomena of viscosity in the course of starch digestion by the dialyzate

In the course of the saccharification of starch or soluble starch by the dialyzate, the viscosity of the digested solution is often increased more remarkably than that of the original solution or of the solution of such as dextrine, maltose or glucose which is to be produced in the digested solution. This is a very interesting phenomenon for investigation of the mechanism of starch liquefying, dextrinizing and saccharifying action by so-called diastase.

The results of this experiment are shown in table 11.

The specific viscosity of the digested solution shows twice as compared with the original soluble starch as control, and also it is higher than that of the digested solution of the starch paste. But the quantity of reducing sugar produced is higher in the soluble starch than in the case of the starch paste.

To show the relations among the mechanisms of starch decomposition of diastase, the viscosity, the quantity of reducing sugar of the digested solution and the color reaction by iodine in the process of its action, further investigation was made, and the results are shown in tables 12 and 13. The iodine reaction is shown by MULIKEN's color standard sheet (Identification of pure organic compounds vol. 1, accessory color standard sheet A and B.) The iodine solution was 1/100 or 1/10 normal. The iodine reaction was tested by taking 2 c.c. of each sample for 63 hours digestion, and by taking 5 c.c. of each for 148 hours digestion.

It is noteworthy that the above results show a similar tendency with the previous experiment shown in table 11.

The power of diastase permeated through the shark's membrane is comparatively strong, though the concentration of its solution is very low.

And owing to the lapse of time after its permeation, it seems to attain a special state as compared with the ordinary enzyme. So, it seems to show an extraordinary phenomenon in its action, such as increasing of viscosity with much deposition of dextrine. This probably shows that the dextrine saccharifying action of the diastase seems to have occurred very slowly as compared with its starch dextrinizing action. The cause of such phenomenon is perhaps the lack of the diastatic enzyme substance.

Next, the relation between the saccharifying action of diastase and the viscosity of the digested solution must be considered. For example, when No. XXV of the outer solution is compared with No. XXXIII in case of 5 hours digestion for starch paste, the viscosity of the latter is about 3.5 times higher than the former, that is, the liquefying power of the latter must be thought comparatively low, while, on the contrary, the saccharifying power of the latter is more than twice the former. Therefore, the saccharifying action is not always accompanied by the viscosity or its liquefying action. Moreover, though the comparative number representing the production of reducing sugar of the latter is 5.35, the viscosity is 8.718, being higher than that (6.876) of the control test of starch paste that is not acted upon by enzyme. Thus, even in the case of starch, it is recognized that there are often cases in which the viscosity increases during the digestion as in the case of the soluble starch. This phenomenon is especially worthy of attention.

The degree of starch and soluble starch saccharifying action of diastase is not always accompanied by the degree of iodine colour reaction. The degree of the colour reaction is very different according to the nature of the remaining materials in the digested solution as well as to the quantity of iodine used, as seen in the above tables.

D. Change of the enzymic power of outer solution by its preservation

When the renewed outer solution was preserved successively and used for starch decomposition at the same time, there was often seen some unexpected difference in the enzymic power as shown in table 14.

According to this experiment, it will be recognized that even when the surface area of diffusion and the diffusing hours are the same, great changes appear in the powers of liquefaction, saccharification, maltose decomposition and sucrose decomposition, on account of the difference of the time preserved after the renewal of the solution. And the change of each

enzymic power seems to be somewhat periodical, according to the lapse of the time preserved as seen in the table. This point will be explained in detail in the following sections.

E. Periodical turbid phenomena of the enzyme substance in the outer solution renewed and preserved successively

When the outer solution is renewed and preserved, the solution becomes gradually turbid, though it is transparent at first. And the turbid solution becomes transparent again. This phenomenon was found to occur periodically for a long time.

The results are shown in table 15.

The cause of this phenomenon can not be easily explained. It has already been ascertained that such phenomenon does not occur in 10 % alcohol, toluene or air bladder's extract themselves. It must take place by changing the state of the diffused enzyme substance of Koji-Diastase. In order to explain this fact, a comparative study of the enzymic powers of diffused enzyme solutions before and after the turbidity, in turbid state, and immediately after the renewal was carried out.

The results are shown in table 16.

The samples XXIII–XXVI are obtained successively by dialysis for about 96 hours, and the concentration of them will be about equal or rather decreased somewhat gradually. But the solution of 4 days after its renewal is lowest in its power and is turbid, that of 8 days is highest in power and is transparent, and the solution immediately after its renewal is high in power and is transparent. Furthermore, the solution of 8 days, after its renewal, was once turbid and decreased its power extremely, but after some days it recovered its soluble and dissociated state, and its enzymic power as well. Considering these facts, it may be said that the enzymic power is not only related to the concentration of enzyme, but also to the colloidal state of the enzyme itself.

F. Relation between the enzymic power and the periodical change of pH value of the outer solution renewed and preserved successively

When about 100 c. c. of the outer solution is renewed continuously in each certain time interval, the pH value of the diffused solution is nearly neutral at the beginning, but with a repetition of its renewal, the solution

gradually becomes acidic. When the pH value of the diffused solutions which have been preserved is measured at the certain time, though the value of them holds a tendency of gradual decrease, there is shown clearly a periodical change. These facts can also be presumed from the periodical transparency and turbidity, and the periodical increase and decrease of the enzymic power. These will be made plainer by the following experiments.

1. pH value of the outer solution

The indicator used in this experiment is brom thymol blue, and the results are shown in table 17.

According to the experiment, each permeated enzyme solution seems to become acidic gradually with repetition of renewal.

2. Change of pH value during the preservation of the outer solution

(1) The pH value of each outer solution which is diluted at the rate of the same diffusing time (19.2 hours).

The results are shown in table 18.

To show the state of the change of pH value of each solution put aside for different days, experiments were made with the sample which was diluted by 10 % alcohol solution at the rate of diffusion for 19.2 hours. From the results it can be recognized that the state of the enzyme substance in the solution is changing continuously, in spite of the fact that the pH value becomes gradually acidic with repetition of the renewals as seen in the previous experiment. The periodical change of the pH value must also unquestionably be a proof of this fact.

(2) Change of pH value of the outer solution in the course of its preservation (shown in table 19).

It is already obvious that the diffused solution of Koji-Diastase changes its pH value during preservation. It is also necessary to investigate the relation between the enzymic power of the solution and its hydrogen ion concentration.

3. Relation between the enzymic power and the change of pH value of the outer solution

It is generally known that the enzymic power has a close relation with the hydrogen ion concentration. Notice must be taken of this point in these experiments.

For convenience, the optimum pH value which has been published by

many investigators, and the pH value of the substrates used in this experiment will be tabulated in tables 20 and 21.

(1) Carbohydrate splitting enzymes, especially diastase, maltase and a little invertase, seem to exist comparatively largely in the dialyzate of Koji-Diastase. So the relation between the enzymic power and the change of the pH value was next investigated in detail with the following results in tables 22 and 23.

According to the results, the periodical change of the diffused solution has a most important relation with the liquefying and saccharifying powers on starch, soluble starch and dextrine, and with the decomposing power on maltose.

The saccharifying action is almost proportional to the increase of hydrogen ion concentration, but the liquefying power is not always accompanied by this relation.

Even when the pH value is equal, the power will be different, according to the position of the periodical changes, namely, it may be different depending upon whether the pH value is increasing or decreasing. The relation between the pH value and the saccharifying power of dextrine is the same as in the case of the soluble starch, and also is similar to that of the decomposition of maltose.

(2) To compare the relation between the enzymic activity and the change of the pH value, the next experiment was made, and the following results were obtained (in table 24).

From the results of the previous experiments it must be recognized that, although the strength of the enzymic power depends upon the concentration of the enzyme, it is rather closely relatde with the state of the enzyme itself in the solution. The phenomenon of the periodical change of the enzymic power occurs correlatively with the change of the pH value which is caused by the difference of dissociation of the enzyme. For example, Koji-Diastase has a tendency to become acidic in the dissociated state, and to become neutral in the comparatively undissociated state. It may be recognized that the enzyme substance raises its acidity by the decreasing of ash ingredients in it. And this ash ingredient of the enzyme seems to take an important role in its starch liquefying action, and by the mutual action of all the ingredients the saccharifying action of starch seems to be completed.

G. Change of the enzymic power of the outer solution by the dialysis of Koji-Diastase

According to the results of the dialysis experiment which has been tried as a method of enzyme purification, it has been generally recognized that the enzymic power decreases by the removing of ash ingredients. In fact, in the dialysis of Koji-Diastase performed with the shark's air bladder, the enzymic power is decreased gradually not only by the decrease of its ash ingredient but also by the decrease of the enzyme substance in the inner solution, until it disappears at last.

On the other hand, as it is seen in the above experiment, the outer solution has a comparatively strong diastatic power as long as the inner solution holds the diastatic enzyme substance, in other words, as long as the ash ingredient and other substances are in the dialyzate. So, necessarily, doubts occur about the idea which has been held up to the present time. In regard to the many dialysis experiments performed up to this time, it must be considered that the diffused substance is not ash ingredient only, which has no relation to the enzymic action, and that the decrease of the enzymic power in the inner solution is due not only to changing of the enzymic character, caused by the removing of the impurities, but rather by the decrease of the enzyme substance itself and the destruction of the internal parts of the enzyme substance.

Therefore, the next experiment was performed in order to compare the enzymic power between all the outer solution obtained by the dialysis experiment and the corresponding concentrated original enzyme solution prepared by dissolving the original purified enzyme substance. Here "a" is the solution containing 0.4974 gram of the diffused enzyme substance in 5 liters of 10 % alcohol solution, and the concentration of it is 0.009948 %. This 0.4974 gram of the diffused enzyme substance is obtained from the renewed solutions from I to XL, dialyzing 0.5535 gram of the original purified enzyme substance which is obtained from 159.5 grams of rice Koji. "b" is the solution containing 0.5535 gram of the original purified enzyme substance in 5 liters of 10 % alcohol solution, and its concentration is 0.01107 %. And "c" is the control, only a 10 % alcohol solution without enzyme. The results are shown in table 25.

According to the results of this experiment, Koji-Diastase is permeated almost perfectly through the shark's air bladder, and the power of the diffused enzyme solution is markedly increased as compared with that of the corresponding concentrated original enzyme solution. The cause of

the fact is supposed to be the result of diffusion from the membrane and of removal of the residual matter. So that the former is holding mostly the dissociated state, while the latter is naturally holding the associated form. This not only shows the relation betwen the activity and the physical and chemical forms of diastase, but establishes a fundamental suggestion for the nature of the general enzymes and their actions.

H. Dialysis of Koji-Diastase performed with various semipermeable membranes

The results of the dialysis experiment of Koji-Diastase performed with the shark's air bladder and 10 % alcohol solution were described above. Next will be reported the results of the further experiments tried, using three kinds of membranes (air bladder, intestine membrane and bladder) and 2 kinds of solutions (10 % alcohol solution and water solution).

Dialysis:—Began Jan. 18, 1927.

Sample used is the mixture of purified Koji-Diastase No. 2—No. 6, and the results are shown in table 26.

1. Renewal of the outer solution

The renewal of the outer solutions is performed as seen in table 27, making the various combinations (1-6) of membranes and solutions, and observation was made individually upon the diffused solutions divided into 3 periods (I-III).

Table 28 shows the change of volume of the inner residual solution which occurred from the difference of the osmotic pressure between the outer and inner solutions.

According to these results it is indicated in general that the increasing of volume of the inner solution ceases in the water solution of Koji-Diastase sooner than that in the 10 % alcohol solution.

2. Change of the enzymic power in the outer and inner solution

(a) Carbohydrate-splitting enzyme

There were mixed together 20 c. c. of the 2 % solution of substrate, 2 c. c. of toluene and 2 c. c. of the enzyme solution, placed in the thermostat at 38°C., and each 5 c. c. was taken as a sample for examination. Its enzymic power is expressed by c. c. of the titrated $KMnO_4$ solution,

here 1 c. c. of $KMnO_4$ solution corresponds to 9.945 mgs. of Cu. This solution is applied in all the following experiments. The results are shown in table 29.

From the results of this experiment, it is indicated that the order of diffusion of diastase, maltase and invertase is almost similar with the results of investigation previously mentioned. The velocity of permeation of the enzyme is generally greater in aqueous solution than in 10 % alcohol solution. This fact perhaps depends upon the nature of the membrane and the degree of dissociation of the enzyme in the solutions. Considering the several points, the diffusion velocity of enzyme substances by difference of semipermeable membranes is in the order of the air bladder, intestine membrane and bladder membrane. Besides, observing the enzyme substance of the inner solution, there is yet remaining the powerful activity of splitting carbohydrate even in the case of the 25th renewal.

From the above experiments, it is considered reasonable that the diffusion of ash as an impurity at first occurs and afterwards of the enzyme substances themselves. Furthermore, it may be considered that easily diffusible parts of the enzyme substance mainly pass through at first, according to the kind of membrane used and the state of its solution; and if to such an abnormal enzyme solution is added the material to be decomposed, there must occur an abnormal reaction. For instance, in the case of "6" which is the outer solution of the 8th renewal in the table, when it reacted to the maltose, the decreasing of the reducing power in comparison with the control was assured. And a new phenomenon, as an abnormal reaction which is difficult to explain, was observed from the results of the enzymic action of the 12th renewal dialyzates for glucose in every 21.5 hrs. and 67 hrs. That is, in the reaction of the 21.5 hours the values of the enzymic activity of each outer solution (1-6) expressed by $KMnO_4$ solution are from minimum 2.40 c.c. to maximum 3.00 c.c. against 14.20 c.c. in glucose solution used (control); while in the reaction of the 67 hours these are from minimum 13.60 c. c. to maximum 14.60 c.c. against 13.80 c. c. in glucose solution.

(b) Protease

VOLHARD's casein method is used in this experiment for the estimation of the power of protease, namely pepsin and trypsin as previously mentioned, and the results are shown in table 30.

According to the results of this experiment, as in the result of the previous case, neither pepsin nor trypsin exists in the dialyzate, but these

actions are to be recognized very slightly in the inner residual solution.

(c) **Lipase**

Ten c.c. of oil emulsion, 5 c.c. of enzyme solution, 5 c.c. of distilled water and 2 c.c. of toluene were mixed together, placed for 40 hrs. at 38°C. After it was cooled, 40 c.c. of 99 % alcohol, 5 c.c. of ether and 3 drops of phenolphthalein were added. The mixture was then used for titration by 1/10 normal NaOH solution until it became rose color. The results are shown in table 31.

According to the results of this experiment, the power of the lipase is almost negligible.

3. Change of pH value of the outer and inner solution

The results are shown in table 32.

The results obtained indicate that both inner and outer solutions have a tendency to increase the hydrogen ion concentration according to the repetition of renewals. Also by preserving these solutions the periodical change of pH value is brought about as a result of continuous associating and dissociating phenomena as mentioned already.

SUMMARY

(1) When 10 % alcohol solution of Koji-Diastase is poured into the shark's air bladder which is placed in 10 % alcohol solution, the diastase is diffused through the membrane.

When the outer solution is continuously renewed with the alcohol solution 35 times (for 110 days), though the enzymic power of the outer and inner solution shows a tendency of gradual decrease, it still holds powerful activity, and at the 40th renewal (130 days) the power is decreased remarkably, and disappeared entirely at more than 50 times (about 8 months).

So, the carbohydrate splitting enzyme substance seems to be diffused entirely in 47–50 times of renewal.

(2) Pepsin and trypsin in Koji-Diastase solution are not diffused through the shark's air bladder, invertase is slightly diffused, and maltase is also diffusible, but more slowly than diastase.

(3) The diastatic power of the diffused enzyme solution is increased as compared with the original enzyme solution of the same concentration.

(4) In the course of the saccharification of starch or soluble starch

by the diffused enzyme solution, the viscosity of the digested solution is frequently much more increased than that of the original solution or the solution of any substance which is supposed to be produced in the digested solution. In this case the content of sugar produced is not always small, but still deposits much dextrine, as compared with the ordinary case in which the viscosity is not increased. This seems to be a good phenomenon for research into the saccharifying mechanism of diastase. And this fact seems to be a certification showing the continuous phases of molecular association and dissociation between the enzyme substance and the substrate or the intermediate products in the saccharifying process.

(5) When every diffused enzyme solution, which is renewed and preserved successively, is observed on the whole, there occurs a periodical turbid phenomenon. This is perhaps due also to the periodical associating and dissociating character among the enzymic ingredients.

(6) The pH value of the diffused enzyme solution is nearly neutral at the beginning, and with repetitions of the renewal it becomes gradually acidic.

But the hydrogen ion concentration of every outer solution seems to express the periodical change according to the turbidity as previously mentioned, and when the turbidity is high the acidity is low, vice versa. Consequently, it is supposed that this is due to the difference of the intermediate product owing to the dissociation and undissociation degree of the enzyme substance.

(7) The diastatic power of the outer solution has a tendency to decrease gradually with repetition of the renewal, the solution being diluted gradually. There is also the periodical change in the enzymic power of the diffused solution, namely, when the solution is transparent and its hydrogen ion concentration is high, its activity is comparatively powerful, while it is weak in the contrary condition.

(8) When an outer solution is preserved for a long time, there occurs also a periodical change in its turbidity, hydrogen ion concentration and enzymic power similar to what occured in the solutions successively preserved. Also the enzymic activity is comparatively powerful when the hydrogen ion concentration of the solution is high and the solution is transparent.

Hereby, from the results of the previous investigations, it is assumed that diastase of Koji is a kind of coalescent substance; and that it contains at least, as one of its constitutional parts of the enzyme substance in the adsorbing state, a kind of amphoteric compound which has one or more

basic and acidic radicals.

From this assumption it can be easily understood, by the various phenomena previously mentioned, that, when the enzyme substance in its solution is present in the dissociated state, the solution is transparent, its hydrogen ion concentration is high and the enzymic power is strong. On the contrary when it is in the associated state, the solution is generally turbid, the hydrogen ion-concentration is low and at the same time weak in its enzymic power.

III. The Relation between the Action of Starch Decomposition of "Koji-Diastase" and its Ash, Protein and Carbohydrate

The opinion that the diastase may be a protein-like substance has been advocated by many scholars. SHERMAN's opinion[9] favouring this view depends on the increasing of the protein reaction by purifying diastase, and that of BIEDERMANN [10] [11] depends upon the fact that he found the action of starch decomposition by protein-like substances such as albumoses, polypeptides and amino acids. On the contrary, there are some scholars, like FRICKE and KOJA [8] and others, who assert the non protein-like substance of diastase from the appearance of the reaction of carbohydrate and the absence of any kind of protein reaction of the diastatic enzyme substance which was purified by a method different from the former authors.

But it has never been reported clearly that, when any purified enzyme substance which is carbohydrate-like or protein-like acts on starch, the ash ingredient or other matter which coexists with the enzymic substance or its substrate must rather be treated as an important part of the enzyme substance for the enzymic action.

Through present investigations on Koji-Diastase and the numerous works which have been published on enzymes, especially on diastase, it is to be considered that there are intimate relations between the action of starch decomposition of Koji-Diastase and its ash, protein, and carbohydrate. Furthermore, it is probable that the diastase is not merely a sort of protein or carbohydrate, but rather it should be considered that the diastase of Koji is a kind of coalescent substance consisting of several materials which

9) H. C. SHERMAN and A. P. TANBERG :- J. Am. Chem. Soc. 38, 1638, 1916.
10) W. BIEDERMANN :- Münch. med. Wochenschr. 68, 692–693, 1921 ; C. A. 17, 564.
11) W. BIEDERMANN :- Arch. ue Land-physiol. 7, 151–156, 1922; C. A. 17, 564.
8) R. FRICKE and P. KOJA :- Ber. Deutsch. Chem. Ges. 57, 2, 310–316, 1924.

are related with ash, protein, carbohydrate, and some other materials.

Here, the author wishes to propound the "Poly-constituent Theory" of Koji-Diastase, and in order to explain the foundation of the hypothesis experimentally, the nature of the ash, protein, and carbohydrate of Koji-Diastase must necessarily be examined. The purpose of this research is different fundamentally, to a great extent, from that of many other investigators. Though our opinion about the nature of diastase might be already inferred by what has been previously written, the effort was made to ascertain this view by the following detailed experiments.

Samples used in this experiment are the Koji-Diastase No. 2–No. 6 and that of *"Moto-Koji" purified by* LINTNER's *method.* But these are not still considered as perfectly pure enzyme substance.

A. Ash ingredient of "Koji-Diastase"

In Koji-Diastase that has been purified until now by any method, it is always recognized that the enzymic material which holds the power of starch decomposition contains ash ingredient. And as it is presumed that the ash is rather considered to be an important ingredient of Koji-Diastase according to many investigations, advanced researches are attempted here.

1. Percentage of ash in "Koji-Diastase"

It is naturally observed that there are two parts of ash in the material of Koji-Diastase. One of them is to be considered as an adsorbing constituent of the diastatic substance having a relation to enzymic action, or rather, as a chemical constituent of enzyme substance, and the other part is to be called an impurity.

(a) Percentage of ash in purified "Koji-Diastase"

The ash, in this case, means crude ash, and its percentage in various samples is shown in table 33.

From these results it can be seen that Koji-Diastase obtained from material sold on the market by LINTNER's method contains about 11 % of ash, and the one made from "Moto-Koji" contains about 4.7 %. Therefore, although the percentage of ash in purified Koji-Diastase is not always definite, from this table the outline of it can be seen.

(b) Distribution of ash in the dialyzate and dialyzed substance of "Koji-Diastase"

The distribution of ash in the diffused and the remaining substance in dialysis of Koji-Diastase is shown in table 34.

The ash content of each diffused enzyme substance classified by the method of dialysis shows an average 15.927 % in period I, 8.078 % in period II and 8.285 % in period III respectively as against 11.050 % of the original enzyme substance, and in the dialyzed enzyme substance it is 4.800 %, and also in the total dialyzate it is 12.592 %. The ash content of the diffused enzyme substance is very large against that of the dialyzed enzyme substance. But as long as the enzymic power of starch decomposition is not lost, the ash ingredient is contained in the substance. That is, observing the change of the ash content of the dialyzates in the periods from I to III of the dialysis experimet, it may be said that after the period II the diffusion of the enzyme substance is principally going on in the almost constant ratio of the enzymic constituents. And generally, it may be known that as long as the diastatic activity of dialyzate is strong, 7–8 % of ash is always contained as in the case of II and III periods.

(c) Relation between the ash content and the diastatic power of dialyzed enzyme solution

The purpose of this experiment is to know the relation between the diastatic power and the ash of enzyme substance in the inner solution after the renewals of the outer solution. The samples used in this experiment are the dialyzed solutions after the 25th renewal, which are obtained at the end of the experiment previously mentioned; and each of these solutions is diluted to the same degree in volume, using the case of "5" as the standard (distilled water and pig's bladder).

To 25 c.c. of 2 % solution of substrate were added 5 c.c. of enzyme solution and 2 c.c. of toluene. The mixture was digested at 38°C. for 24 hrs., and then 5 c.c. of the solution was used for the experiment.

The results are shown in table 35.

According to the results of this and previous experimets, it will be recognized that there is an important relation between the ash content and the action of starch decomposition of dialyzed enzyme solution. Actually, the solution containing the larger amount of ash is more powerful than that of the smaller one in respect to starch splitting activity. It is also to be said that the one which has the larger amount of ash contains the larger amount of total enzymic substance as well; and at the same time it shall be explained that the enzymic action will be able to occur as long as the enzymic constituents of diastase are still left in the dialyzed solution.

2. Quantitative analysis of the constituents of ash ingredient contained in "Koji-Diastase"

The results of quantitative analysis of the main constituents af ash contained in Koji-Diastase are shown in table 36.

According to this experiment, the largest constituent in total ash of Koji-Diastase is P_2O_5, and next CaO, K_2O and MgO, for instance, the percentage of mineral constituents in dialyzate II is P_2O_5, 19.885; CaO, 17.003; K_2O, 19.715; SO_3, 12.104; and MgO, 10.95. But among the main constituents of the ash in the inner solution, the contents of Fe_2O_3 and Al_2O_3 are much larger as compared with the other mineral constituents above mentioned. Therefore, it is possible to consider from the above experiments and others that P_2O_5, MgO, K_2O and CaO are very naturally to be cited as the ash components which have the most important relations to the starch decomposition of Koji-Diastase.

3. Influence of the mineral matters uppon the action of carbohydrate decomposition

Sample and process used for this experiment were as follows:

The inner residual solution.

Control 1. To 2.5 c.c. of the inner solution (LIII') which is in the case of "B-G" in chapter "II" after the renewal of the outer solution LIII, 2.5 c.c. of distilled water was added. (Original inner residual solution).

a) To 2.5 c.c. of the above mentioned (LIII') was added 2.5 c.c. of the mixture of the outer solution I-IV which had an enzymic activity, but became inactive after boiling for 5 hours, and was concentrated to half volume. This concentrated solution was used for supplying ash ingredient in the dialyzates I-IV, and, though other substances were present in the solution, there was no diastatic activity.

b) To 2.5 c.c. of the above mentioned inner residual solution was added 2.5 c.c. of the solution containing 0.2 % of Na_2HPO_4 and 0.125 % of $MgCl_2$.

The outer solution.

Control 2. To the mixture of the outer solutions I-IV which had been already concentrated to half volume by boiling as above was added 2.5 c.c. of 10 % alcohol solution.

c) To 2.5 c.c. of the outer solution XLV in chapter II was added 2.5 c.c. of the mixture of the solutions I-IV which had been already concentrated to the half volume as before mentioned.

d) To 2.5 c.c. of solution XLV above mentioned was added 2.5

c.c. of the solution containing 0.2 % of Na_2HPO_4 and 0.125 % of $MgCl_2$.

Control 3. To 2.5 c.c. of solution XLV was added 2.5 c.c. of 10 % alcohol solution.

Mineral salts (used instead of ash).

Control 4. To 2.5 c.c. of the solution containing 0.2 % of Na_2HPO_4 and 0.125 % of $MgCl_2$ was added 2.5 c.c. of 10 % alcohol solution.

To 25 c.c. of 2 % solution of substrate were added 5 c.c. of each sample and 2 c.c. of toluene. And after the digestion at 38°C. for 15 hrs., the sample 5 c.c. was taken for examination.

The results are shown in table 37.

According to the results shown by both the outer and the inner solution after the continuous renewal of dialyzate, even if it is so dilute in concentration of all ingredients of enzyme that the influence of ash, especially PO_4-ion and Mg-ion, upon the enzymic activity is very small, it will be recognized at least that there is a promoting influence upon the starch liquefying and saccharifying activity.

B. The protein-like substance contained in "Koji-Diastase"

As has already been described, it is impossible to accept the idea that diastase of Koji only a single compound which belongs to a protein-like substance, carbohydrate or any other substance. Most of the purified diastatic enzyme substances which are obtained by any method generally show some reactions of protein. Although some do not show the reaction, there has never been prepared any sample in which some nitrogenous substance is not contained.

From the above studies, it can be said that in diastase of Koji there is contained at least some protein-like substance as part of its constituent. To prove this conception detailed experiments were performed as stated in the following:

1. Protein reaction of purified "Koji-Diastase"

It has been recognized already that the Koji-Diastase purified by LINTNER's method and the substance obtained further by the electric cataphoresis or the substances which are purified fractionally from the original substance by alcohol of different concentrations have all the precipitation reaction by boiling or other methods and show color reactions for protein such as BIURET, Xantho-protein and MILLON's reactions. But more detailed experimets are made, using the original enzyme substance and samples related to this enzyme.

The results are shown in table 38.

In the table, the parenthesis means the reaction which did not distinctly appear (this is applied in all the following experiments). It is evident from the results that the purified Koji-Diastase and many other diastatic enzyme substances show many color reactions for proteins except lead sulphide reaction. The solutions of Koji-Diastase, malt-Diastase, germinated unhulled rice Diastase, Taka-Diastase and "Pepsinum" (a market article) yield the precipitation reaction by boiling; and also there occurs the precipitation by addition of sodium sulphate, basic lead acetate or others, but no HELLER's reaction at all.

2. Protein reaction of the dialyzate and dialyzed substance of "Koji-Diastase"

Samples used in this experiment are prepared as follows:—

Dialyzate No. 1 is prepared by gathering the dialyzates which are renewed continuously in the dialysis of Koji-Diastase No. 1 (same sample as in the case of "B—G" in chapter "II"), and by concentrating them at low temperature.

Dialyzate No. 2 is prepared by mixing I and II which are two of the three kinds of dialyzate I, II and III in the experiment of dialysis of Koji-Diastase of No. 2—No. 6 (same sample as in the case of "H" in chapter "II").

Dialyzed substance is prepared by continuous renewal of the outer solution I–II (after 25 times of renewal).

The results are shown in table 39.

In view of the qualitative reaction of the protein above mentioned, the precipitation reaction by boiling or chemical reagents is seen in the purified Koji-Diastase itself and in its dialyzed solution, but it is not shown in the portion which is diffused. Therefore, it is reasonable to consider that the protein-like substance having relation to Koji-Diastase must be at least a compound of a lower class than peptone.

As to the color reaction, the BIURET reaction is observed in every case. This reaction, it is said, is present in the polypeptides higher than tripeptide, and by SCHIFF it is said that the reaction is due to the radicals such as $-CONH_2$, $-CSNH_2$, $-C(OH)NH_2$, $-CH_2$ and $-NH_2$. A red color which is common for albumin and peptone is shown especially when the dialyzate was applied. Therefore, for the reasons above mentioned, the existence of a peptone-like substance can be recognized at least in a diastase solution. The MILLON's reaction is present in every case of the enzyme solu-

tion. As this reaction is thought to be produced by the oxyphenyl group, the existence of a substance such as tyrosine may be considered in the protein-like substance. Xantho-protein, LIEBERMANN's, NEUBAUER and ROHDE's and ADAMKIEWICZ's reactions, further HOPKINS and COLE's glyoxylic acid reaction are all observable. And according to HOPKINS and COLE there can be recognized the existence of a compound with an indole group such as tryptophane. The existence of a compound such as tyrosine or histidine can be recognized by the presence of PAULY's diazo and KNOPS' reactions; also the existence of protein, peptone, polypeptide or amino acid through Ninhydrin reaction is evident.

Therefore, as for the protein-like substance related to the starch splitting activity of diastase, it can be mentioned that monoamino-acid, diamino-acid or some kind of polypeptides lower than peptone is present in the enzyme substance.

3. Classification of nitrogenous substances of various forms in "Koji-Diastase" and its hydrolysis products

To find out the nature of the ingredient of the protein-like substance just described, the following quantitative analysis of amino acid and the other nitrogenous substance was carried out according to VAN SLYKE's method. Samples used in this experiment were the purified diastase of Moto-Koji, and the enzyme substances of inner and outer solutions in the dialysis of Koji-Diastase No. 2–No. 6 as previously described. And these samples were all treated as follows.

(a) The purified Moto-Koji-Diastase was subjected to hydrolysis. To 3 grams of the sample, 20 c. c. of 20 % HCl solution was added and hydrolysed for 20 hrs. in sand bath with a reflux condenser.

(b) To 0.7795 gram of the diffused enzyme substance (in the outer solutions I–III), 10. c. c. of 20 % HCl solution was added and hydrolysed.

(c) To 0.3633 gram of the dialyzed enzyme substance (in the inner solutions (1)–(6)), 10 c. c. of 20 % HCl solution was added and hydrolysed.

(d) As preliminaries for the quantitative analysis of non-protein nitrogenous substance, the solution of Moto-Koji-Diastase was heated, and the coagulated substance was filtered. The coagulum which was considered to contain the protein was decomposed by conc. H_2SO_4 and applied to the estimation of nitrogen. The filtrate which was deemed to contain the non-protein nitrogenous substance was directly analyzed by VAN SLYKE's method without hydrolysis.

The results are shown in table 40.

From the results of the experiment, it is discovered that diamino acid-N and total-N of monoamino acid are contained as a large part of the nitrogenous substance in Koji-Diastase, and that the content of humin-N is generally small. In the enzyme substance prepared from highly polished rice Moto-Koji, the quantity of humin-N is especially small, while reversely much diamino acid-N is contained. In the Koji-Diastase No. 2–No. 6, humin-N and total-N of monoamino acid are contained in a comparatively large amount compared with that of diamino acid-N. Also it is true that, when the diffused enzyme substance is compared with the dialyzed enzyme, a comparatively larger quantity of total-N of monoamino acid than diamino acid-N is contained in the former. No ammonia-N and amide-N are found in any cases.

4. On the content of nitrogenous substance in every enzyme substance separated by the dialysis

From the results of the dialysis made by three kinds of membranes as previous, it is deemed that the Koji-Diastase has the nature to permeate comparatively easily through the semipermeable membranes. The state of distribution of the nitrogenous substance in every dialyzate and residual enzyme substance is as shown in the following table. For the quantitative analysis of nitrogenous substance, the Micro-KJELDAHL method was used.

(a) Five hundred fifty three-thousandths grm. of the Koji-Diastase which contained 6.028 % of nitrogen was dissolved in the 10 % alcohol solution, put in the shark's air bladder and dialyzed against 10 % alcohol solution. After 50 times renewal of the outer solution during 8 months of the dialysis, 0.4974 grm. of the enzyme substance was diffused out, and 0.0561 grm. was still remaining in the membrane, and the nitrogen content in the former was 5.055 %, and that of the latter was 14.911 %. Therefore, it seems reasonable to conclude that the substance remaining in the membrane is mainly protein.

(b) The rate of the nitrogenous substance in every enzyme substance separated by dialysis in the case of "H" in chapter "II", and its rate in other samples used as control, as well as the rate of the other constituents such as non-nitrogenous substance and ash ingredient were investigated.

The results are shown in table 41.

According to this table the content of the nitrogenous substances in the diffused enzyme substance is smaller as compared with that of the

original enzyme substance of Koji-Diastase, and, reversely in the residual substance it is larger in comparison with the original. For instance in the original Koji-Diastase No. 2–No. 6 the average content of nitrogenous matter is 37.725 %; but it averages 31.200 % in the diffused enzyme substance in I-period, 26.319 % in II-period, and 15.281 % in III-period; and 44.107 % in the dialyzed enzyme substance. Next in I-period of the dialyzate the ash content increases to 15.950 % from 11.050 % in the original enzyme substance, this increase being 4.900 %. At the same time the decrease of carbohydrate is shown in this case. Therefore, it is evident that the diffusion ratio of nitrogenous substance is decreased in each case after II-period of dialysis. The velocity of the diffusion of Koji-Diastase is different according to the difference of the membrane used, and at the same time the diffusion velocity of the constituents differs each from the other. When the component ratio in dialyzate is suited to the enzymic constituents, the power is considered to be strong; and when that is not suited, its activity is considered to be weak, showing sometimes abnormal reaction. And also it is possible that there exists some kind of non-nitrogenous substance which has no reducing character when the analytical sum of the nitrogenous substance, carbohydrate and ash ingredinet differs from 100 % as seen in the table. And now it is recognized that the quantity of the nitrogenous substance in the constituents of Koji-Diastase is about 30 % and is always less than the carbohydrate.

C. The carbohydrate in "Koji-Diastase"

The purified enzyme substance produced by any method shows evidently the reaction of carbohydrate. From the results of many experiments up to this time, it may be considered that Koji-Diastase contains some kind of carbohydrates in its constituents. So to prove this point the following experiments were made.

1. Reactions of carbohydrate in "Koji-Diastase"

The qualitative reaction of the carbohydrate in Koji-Diastase and its diffused and dialyzed substances were examined. At the same time the experiments with the enzyme substances of the malt-Diastase, germinated unhulled rice-Diastase and artificial complex of protein-like substance, carbohydrate and mineral matter were performed also for reference. Here the dialyzate I+II in the case of "H" in chapter "II" and the dialyzed substance in the same case (this dialyzed substance is obtained after the

25th renewal of the outer solution) were used. And the results are shown in table 42.

According to this experiment, the existence of aldohexose is supposed in Koji-Diastase, but there is no ketose. Also there are the reactions of pentose and methyl pentose. Glucose and maltose are present a little in the enzyme substance, but they can not be recognized as the principal constituents. It is reasonable to consider that the carbohydate as the constituent of the enzyme substance is some kind of substance which is able at least to diffuse through semipermeable membranes, and able to produce the glucose, other reducing sugar, or some other decomposed substance.

2. On the content of carbohydrate in every enzyme substance of "Koji-Diastase" separated by dialysis

The composition of the enzyme substance separated by the dialysis is different from the original enzyme substance. And the ratio of ash and protein differs according to the kind of semipermeable membrane used, the nature of the enzyme solution and the period of the renewal of the outer solution. Also this difference may be due to the nature or condition of the constituents of the enzyme substance. So, the content of carbohydrate is next investigated as follows: One gram of the enzymic material was dissolved in 80 c.c. of distilled water, and 8 c.c. of 1.125 % HCl was added and hydrolyzed by heating in the water bath for 3 hours with a condenser, and it was used for the estimation of reducing sugar.

The results are shown in table 43.

The rate of diffusion of carbohydrate in Koji-Diastase is slow as compared with the other nitrogenous substance and ash in the beginning of the dialysis; and also the rate of its diffusion is different according to the kind of semipermeable membranes used its velocity being in the order of air-bladder, intestine mebmrane and pig's bladder membrane. But it is possible to believe that the diffusion rate of all the constituents of the enzyme substance is almost constant in the middle and later stages of dialysis. The content of the material that has the reducing power as carbohydrate in every separated enzyme substance is comparatively small. But the greater part of the carbohydrate is to be changed into reducing sugar by the process of hydrolysis. But there is rather a large amount of reducing sugar contained in every preserved inner residual solution, especially more is contained in the water solution in comparison with that in the 10 % alcohol solution which can be considered as the result of the diastatic hydrolysis going on in itself during the dialysis experiment

or the preservation of the inner residual solution for a long time. Furthermore, it is not difficult to see from the result of this experiment and the investigation of artificial complex in chapter IV, that when the existence of the carbohydrate is necessary as one of the constituents of the diastatic enzyme, some kind of aldohexose or some of its united matter (or united with other material) may serve for this purpose. Therefore, from the many reasons above given, it will be recognized that some kind of substance situated between simple sugar and dextrine is to be cited as a constituent of carbohydrate in Koji-Diastase.

D. Elemental composition of "Koji-Diastase"

The principal substances which are recognized as constituents of Koji-Diastase have been described already. So, the following experiment regarding the elemental analysis of the original enzyme substances and the enzyme substances separated by dialysis was performed in order to contribute to a research into the real nature of the diastatic enzyme substance.

1. Elemental composition of purified "Koji-Diastase"

The results are shown in table 44.

As is evident in the table, the rate of the content of ash, nitrogen, carbon and hydrogen differs between the market Koji and Moto-Koji, and especially it shows great difference in the ash content; and doubtless these differences are due to the nature of the rice materials, the degree of its polishing and the method of Koji-making. Therefore it is to be considered that the difference of the enzymic power occurs on account of the difference of Koji made from different materials.

2. Chemical composition of the substance in the outer and inner solution

(a) On the dialyzate in the case of "B-G" in chapter II

The next table shows the result of the enzyme substance diffused through the shark's air bladder in 10 % alcohol solution of purified Koji-Diastase.

C (%)	H (%)	N (%)	O (%)	Ash (%)
42.125	7.065	5.055	32.023	13.731

(b) On the enzyme substances separated by dialysis in the case of "H" in chapter II

The results are shown in table 45.

Koji-Diastase as already described seems to be exceedingly different in its quality and nature according to the difference of the raw materials used, the method of its preparation or purification, and the method of Koji-making. Therefore, there occurs the difference in the ratio of the constituents. The elemental composition of the classified substances of Koji-Diastase shows a great variation according to the chemical natures in the enzymes themselves. But it is considered that about 5 % of ash ingredient is contained at least as a constituent of Koji-Diastase.

The average elemental component of Koji-Diastase No. 2–No. 6 (crude ash, 11.050 %) is C, 42.189 %; N, 6.036 %; H, 7.420 %; and O, 33.305 %.

3. On the assumed form of "Koji-Diastase" based on the "poly-constituent theory"

From the results of the experiments described already and of the various studies carried on by many investigators about enzymes, especially on diastase, the "poly-constituent theory" will naturally be propounded in order to explain the true nature of Koji-Diastase. According to this theory, it is considered that the enzyme substance is constituted of ash ingredient, protein-like substance, carbohydrate, etc. A matter situated between amino acid and peptone is recognized as the protein-like substance. As to its form there may be many, such as a free form, a form weakly associated through $-NH_2$ or -COOH radical, a kind of salt in which H of -COOH is substituted by K or Na, or a combined form with PO_4 radical or other amino acids making a salt of Mg or Ca. As the carbohydrate, the substance situated between simple sugar and dextrine is to be considered; and as to its form, such forms as a free or associated, a phosphoric ester of sugars or other carbohydrates can be supposed. Also as to the ash ingredient, K, Na, Ca, Mg and PO_4, etc. are enumerated as sorts of constituents of diastase.

Therefore, for trial, glycocoll, alanine, tyrosine, tryptophane and histidine are preferred for convenience sake as the protein-like substance, and glucose as the carbohydrate. And if the rate of molecular weight of both these series were preferred, and as ash ingredient every one equivalent of Mg + K and PO_4 were preferred, it would be about 37.4, 48.3 and 14.2 in

percentage of the three kinds of constituents respectively. And this percentage is almost similar to the average values 37.725 %, 49.225 % and 11.050 % respectively in the total analysis of Koji-Diastase No. 2–No. 6. But the ash is scanty in the latter as compared with the oxide of the former. From this fact, the existence of a pretty good amount of the protein-like substances, carbohydrates made free from the mineral matter or some other associated body can be inferred; and at the same time, there can be considered in those series the existence of some amount of the higher materials which are more polymerized than amino acid or glucose already mentioned. The elemental composition of the mixture is Mg, 2.210 %; K, 3.527 %; P, 2.796 %; C, 38.430 %; H, 5.592 %; N, 6.314 %; and O, 41.521 %. It is added here that about 5.5 % in 41.121 % of the oxygen may exist as the form which can compose PO_4; and comparing with the case of ash analysis, 41.121 % oxygen shows the value which contains the oxygen which can be combined with Mg and K. Concerning the results of the elemental composition of the mixture, the values of the component parts are lower as compared respectively with the average value of Koji-Diastase No. 2–No. 6, excepting the ash, possibly the oxygen and the nitrogen. But, if in both cases the unnecessary ash is excluded altogether and the values are corrected into the same condition, the approximate value can be obtained, and it is good data to consider the form of the Koji-Diastase from the standpoint of the "poly-constituent theory".

For the experimental proof of the "poly-constituent theory", it is necessary to perform an advanced experiment to determine whether the reaction of starch decomposition is induced or not by the "mixed chemicals" which are combined artificially.

SUMMARY

According to the results of the purification and classification of Koji-Diastase made by several methods, and from the qualitative and quantitative analysis of the ash, protein-like substance and carbohydrate, furthermore, from the elemental analysis of the enzyme substances, it is to be assumed that the enzyme consists of three sorts of substances. Firstly, Mg, Ca, K, Na, PO_4, SO_4, etc. may be cited mainly as ash ingredient. Secondly, peptone, diamino acid, monoamino acid, etc. as protein-like substance. There are many reactions of the substance which is related to tyrosin, histidine, tryptophane, etc., and the existence of these nitrogenous

substances was examined also quantitatively. Thirdly, the existence of some kind of substances which are lying between simple sugar and dextrine can be proved, and they are considered to belong principally to the aldohexose series. Now referring from the results of many experiments, the diastase of Koji is found to be, not only a special single substance, nor the mixture of many enzymes, as has been considered up to this time, but it rather has the nature of a kind of associating complex composed of several kinds of ingredients.

IV. On the Enzymic Action of "Mixed Chemicals"

On the nature and action of diastase, BIEDERMANN[10] mentioned in his investigation "The nature of diastatic enzymes" that the action of starch decomposition is due to albumoses, and he[11] also recognized in his investigation "The diastatic action of albumoses and amino acids" the existence of the diastatic action through albumin, polypeptide, glycinin, leucine, alanine etc. Of course the problem of starch decomposition through amino acids was already mentioned by EFFRONT (Compt. Rend. Soc. Biol. 57, 234, 1904) or TERROINE and WEIL (J. Physiol. Path. Gen. 14, 437, 1912).

But KÖCHLING[12] described in his "The preparation of an artificial enzyme for glucolysis" that none of these combinations such as SCHLATTER's combination "peptone, glucose and bicarbonate", WORDEN's combination "Na-oleate+fibrin+glucose+buffer", and R. von SHROEDER's combination "fibrin or fibrinogen+glucose+buffer" has the enzymic action without the presence of microorganisms. On the other had HAEHN and BERENTZEN[13] mentioned in "Starch decomposition through the system, neutral salt+amino acid+peptone, II plasmophilic reaction", that by means of a combination of neutural salt+amino acid+peptone under low temperature the catalytic saccharification of soluble starch occurs and there will be produced reducing and fermentive sugar. And TAKANE[14] in "The assumed hydrolysis of starch by salts and protein cleavage products" published an opinion that is opposite to BIEDERMANN's opinion. Therefore, this subject has not yet been definitely determined, and further research will be necessary.

10) W. BIEDERMANN:- Münch. med. Wochenschr. 68, 692-693, 1921; C. A. 17, (564).
11) W. BIEDERMANN;- Arch. ue land-hysiol. 7, 151-156, 1922; C. A. 17, (564).
12) H. KÖCHLING:- Chem. Zelle u. Gewebe, 12, 247-285, 1925; C. A. 20, (1998).
13) H. HAEHN and H. BERENTZEN:- Chem. Zelle u. Gewebe, 12, 286-316, 1925; C. A. 20, (1993).
14) K. TAKANE:- Biochem. Zeitschr. 175, 241-251, 1926.

Considering the results set forth in earlier literature and the investigations, especially in "II and III" of this study on Koji-Diastase, as has already been described, it is considered that the Koji-Diastase will be an associating substance related to the amino acid and peptides which are lower than peptone, carbohydrate, of the aldohexose series which is lower than dextrine, and ash ingredients such as Mg, K, PO_4, etc. With such an assumption, an attempt was made to perform the following experiment with the mixture, acting like enzyme. Besides, the point with which this experiment is concerned is the subject of the effect of the pH value upon the starch-liquefying and saccharifying actions. According to the results of the investigations of MASLOW and DAVISON[15] such as "The effect of the hydrogen ion concentration upon the starch liquefying activity of the amylase of *Aspergillus oryzae*" and "The effect of the hydrogen ion concentration upon the dextrine liquefying activity of the dextrinase of *Aspergillus oryzae*", the optimum pH value in starch liquefaction of diastase is from 3 to 4 and in the saccharification it is 4, and at pH=2.0 the enzymic activity seems to be injured. Therefore, regarding the pH value, these results are used as a reference in this experiment.

A. The relation of the ash, carbohydrate and protein-like substance to the starch-liquefying and saccharifying actions

(1) Preparation of the sample of the mixture used

1. 0.924 grm. of $Mg_3(PO_4)_2$ was dissolved in distilled water by adding a small quantity of acetic acid, then diluted to 175 c.c. (about 2/100 M. solution).

2. 0.156 grm. of $CaH_4(PO_4)_2$ and 0.223 grm. of $Na_2HPO_4 . 12H_2O$ were dissolved in distilled water by adding a small quantity of acetic acid, and thus 50 c.c. of the solution was obtained in which the acidity and the ratio of PO_4 are respectively equal to the case of "1" (2/150 M. sol.).

3. 0.204 grm. of $MgCl_2.6H_2O$ was dissolved in distilled water by adding the small quantity of acetic acid in the same proportion as in the previous cases, and 50 c.c. of this solution was obtained. And comparing this with "1" and "2", the ratio of PO_4 and Cl is 1 : 1, and the ratio of Mg is 1/3 (2/100 M. sol).

4. 5.04 grms. of glucose were dissolved in distilled water, and made up to 100 c.c. (28/100 M. sol.).

15) H. L. MASLOW and W. C. DAVISON:– Jour. Biol. Chem. 68, 83-93; 95-99, 1926.

5. 0.36 grm. of xylose and 0.72 grm. of arabinose were dissolved in distilled water and filled up to 25 c.c. (4.32 % sol.).

6. 0.202 grm. of glucose, 0.25 gram. of xylose and 0.25 grm. of arabinose were dissolved in distilled water and filled up to 50 c.c. (5.04 % sol., and the concentration of this solution is the same as "4".).

7. 1.575 grms. of glycocoll were dissolved in distilled water and there were obtained 150 c.c. of 1.05 % solution (14/100 M. sol.).

8. 2.10 grms. of peptone were dissolved in distilled water and there were obtained 200 c.c. of 1.05 % solution.

9. 0.348 grm. of glycocoll, 0.352 grm. of asparagin and 0.352 grm. of α-alanine were dissolved with distilled water and there were obtained 100 c.c. of 1.05 % solution.

(2) Combination of the above solutions (c. c.)

	(1) Mg PO_4	(2) Ca, Na PO_4	(3) Mg Cl	(4) Glucose	(5) Xylose Arabinose	(6) Glucose Xylose Arabinose	(7) Glycocoll	(8) Peptone	(9) Glycocoll Asparagin α-alanine	H_2O added	pH	Revised pH (by CH_3COOH or NH_4OH)
a)	25	—	—	25	—	—	50	—	—	—	4.80	—
b)	25	—	—	25	—	—	25	25	—	—	4.95	—
c)	25	—	—	—	25	—	25	25	—	—	4.95	—
d)	—	25	—	—	—	25	—	25	25	—	4.50	4.95
e)	—	—	25	—	—	25	—	25	25	—	4.40	5.00
f)	—	—	—	—	—	—	—	—	50	50	4.80	—
g)	—	—	—	—	—	—	—	50	—	50	5.60	4.90
h)	25	—	—	—	—	—	—	—	—	75	4.70	—
i)	—	25	—	—	—	—	—	—	—	75	3.60	4.75
j)	—	—	25	—	—	—	—	—	—	75	3.40	4.95
k)	25	—	—	25	—	—	—	—	—	50	4.70	—
l)	25	—	—	—	—	—	50	—	—	25	4.90	—
m)	25	—	—	—	—	—	—	50	—	25	5.00	—
n)	—	—	—	25	—	—	—	—	—	75	6.25	5.00

Each solution 1–9 above mentioned was heated for a short time and cooled at room temperature. In marking these 14 kinds of solutions from "a" to "n", peptone and amino acids solutions were mixed at about 65°C. with other solutions. Every mixture was poured into an ERLENMEYER'S flask pasteurized in boiling water, and preserved by the addition of toluene.

100 c.c. of the mixture thus obtained was held at 36.5°C. for 15 hours, and after being held at room temperature for 5 hours it was used for this experiment. Besides, each 2 % solution of the substrates had the following pH value, namely, starch paste, 6.50; soluble starch solution, 6.10; maltose solution, 6.15; sucrose solution, 6.30; and glucose solution, 6.25 respectively.

The iodine reaction of these solutions was shown in conformity to the MULIKEN's color standard sheet as previously mentioned.

(3) The enzymic action of "mixed chemicals".

To 25 c.c. of 2 % solution of each substrate were added 5 c.c. of "mixed chemicals" solution and 5 c.c. of toluene. After the digestion at 38°C. for 15 hours, 5 c.c. of each solution was taken for the examination of their saccharification. For the color reaction by iodine 5 c.c. of the starch paste or 2 c.c. of the soluble starch, and 1/20 normal iodine solution were used.

The results are shown in table 46.

The results indicate that not all kinds of "mixed chemicals" "a"-"n" have power of decomposition upon the soluble starch, maltose or sucrose, and seem rather to decrease the reducing power of these mixtures. While the mixtures "a", "b" and "c" which seem to be favorably mixed with each ingredient, are plainly enough seen to have the power of starch decomposition. And among them "b" (pH=4.95) is most powerful, and Mg, PO_4, glucose, glycocoll and peptone are contained in this mixture, and in "a" (pH=4.80) Mg, PO_4, glucose and glycocoll are contained, and "c" (pH=4.95) contains Mg, PO_4, xylose, arabinose, glycocoll and peptone. From the fact that "b" is stronger than "a" it will be conjectured that the presence of peptone, not of amino acids only is more fitting for starch decomposition; and from the fact that "b" is stronger than "c" the advantage of hexose compared with pentose is to be considered. As the mixture "b" produced the reducing sugar (as glucose) of 40 % for starch used during 15 hours in this experiment, it must be considered to be fairly powerful.

The ash ingredient as Mg and PO_4 seems most useful for the starch-liquefying action. The fact that these saccharifying actions by these mixtures occured in starch paste and not in soluble starch solution is the most interesting point for the mechanism of this reaction.

The case of saccharification for 106 hours is shown in table 47.

In this case, the effect of Mg, PO_4, glucose, amino acids or peptone for starch decomposition is the same in manner as the case above des-

cribed. Also according to the result of "h" the ash ingredient seems to have a slight tendency toward liquefying starch by itself, while it has no saccharifying activity. Besides, according to the result of "g," although it seems as if the starch had been saccharified to some extent only by peptone *(Witte)*, yet it is supposed to be due to a small quantity of mineral matter and some other impurities contained in the peptone.

Now in this experiment it is evident that the substrate absolutely did not suffer from the microorganismic effect, because in the case of soluble starch no saccharification occured, and it is the same in the case of the other substrate. Also in these cases the reducing power of the solution is decreased conversely.

In this experiment, the starch-saccharifying action seems to occur as a correlative phenomenon of the "mixed chemicals". But it seems not to occur only because of hydrogen ion of the mixture, because its hydrogen ion concentration and temperature were not so high.

B. The negative case for producing enzymic power by means of "mixed chemicals"

It is evident from the literature of the negative assertion of enzyme-like action of artificial mixture that the enzymic activity is not always distinguished only by mixing the so-called diastatic ingredients. And so having the various experimental results which deny on one hand and affirm on the other the production of enzyme-like activity, it is necessary here to mention the various examples.

(1) Sample "A" used in this experiment is the mixture (400 c. c.) of each 50 c.c. of "a", "c", "g", "h", "k", "l", "m" and "n" with "a"-"n" of experiment (A), and each of them was preserved for 21 days at room temperature with the addition of toluene after experiment (A). During this preservation the propagation of microorganism and its action did not occur. Sample "B" is the mixture of 75 c.c. of sample "A" and 25 c.c. of 1 % egg-albumin solution, and sample "C" is the mixture of 75 c.c. of sample "A" and 25 c.c. of edestine solution. It was affirmed that the solution of albumin or edestine does not contain any substance which possesses reducing power.

Starches used here are those of cassava (1), arrow-root (2), indian corn (3), common rice (4), glutinous-rice (5), sorghum (6), and their soluble starches.

To 25 c.c. of 2 % solution of the substrate 5 c.c. of "mixed chemi-

cals" solution and 2 c.c. of toluene were added, the mixture reacted at 58°C. for 17.5 hrs.; and 5 c.c. of the mixture was taken for the experiment. For the iodine reaction, 1/20 normal iodine solution was used.

The results are shown in table 48.

Sample "A" is inactive in this experiment, although it was active in experiment (A). In this case, the concentration and the time for preservation are different from the former experiment. And samples "B" and "C" are both inactive and no influences of egg-albumin or edestine were observed in this case. Although the cause of such a result cannot be made clear immediately, yet that the diastatic reaction has a most important relation to the nature between the enzymic ingredients and substrate probably can be understood.

(2) The "mixed chemicals" solution which was made in experiment (A) was active at that time, but it was almost inactive in experiment (1) of (B) in which its concentration, starch and preserving days were changed. Therefore it is necessary to examine the relation of the process of combination and its preserving time, using the various samples and starches.

Sample A is the mixture of a) 15 c.c., b) 15 c.c. and c) 5 c.c. which are the same as in the case of experiment (A). It was preserved already for 45 days after its combination, using 5 c.c. of toluene as an antiseptic.

Sample B. The solutions 1), 4), 7), 8) are newly prepared in the same way as in experiment (A), and 25 c.c. of each of them are mixed together at room temperature.

Sample C. The solutions 4), 7), 8), 9) are newly prepared in the same way as in the experiment (A). And a solution 1') is also made in the following way: To 0.528 grm. of $Mg_3(PO_4)_2$ are added distilled water and a small quantity of H_3PO_4 for dissolving the sample, and the solution is filled up to 100 c.c. Then they are mixed with each other as follows: 1') 25 c.c.+4) 25 c.c.+7) 25 c.c.+8) 15 c.c.+9) 10 c.c.

Starches: 1. Potato (mixture of three kinds of potato starches produced in Sapporo, Yakumo and Iwamizawa in Hokkaido) 2. Potato (Sapporo, old) 3. Potato (Yakumo, old) 4. Potato (Iwamizawa) 5. Potato (Sapporo, new) 6. Cassava (Formosa) 7. Arrow root (Formosa) 8. Bracken (Hokkaido) 9. Common rice (Akita) 10. Glutinous rice (Akita) 11. Deccan-grass (Sapporo) 12. Sorghum (Sapporo).

Soluble starches: (2) Potato (Sapporo, old) (6) Cassava (Formosa) (9) Common rice (Akita) (12) Sorghum (Sapporo).

The experiment was made at once in the same way as stated in

experiment (1).

The results are shown in table 49.

According to the results it is observed that the saccharifying action did not occur in most cases above described. But a pretty good degree of liquefying or dextrinizing action seems to have occured, and the degree of its activity is not always equal owing to the difference of the mixture and substrate. Therefore, it is considered that only by mixing the ingredients is there a very slight probability of producing the enzymic activity.

C. Effects of dialysis and weak electric current upon the formation of the "artificial complex" which has an enzymic action

For investigation the nature of enzyme, it is convenient to know the mechanism of its production. And it has an intimate relation directly or indirectly to many physical and chemical phenomena in the general biological kingdom. It is already understood from the results of experiments (A) and (B) that it is not always possible to get the formation of the enzymic activity only by mixing the ingredients, but the mechanism of producing enzymic activity is not yet explained completely. Now to make sure the fact, experiments on the effect of dialysis or permeation of enzymic ingredients as well as of flowing a weak direct electric current into the mixture are made. In this experiment toluene was used always as an antiseptic.

(1) Sample: 1 and 1' are made in the same way as in the former experiment. The solution of 4' is 4.58 grms. of glucose and 0.5 grm. of dextrine made up to 100 c.c. 7 and 8 are also made in the same way as in the former experiment. As for the other samples, to 1.05 grms. of "Eukirin" (Anhydroxy methylene diphospho calcium magnesium) were added distilled water and a very small quantity of acetic acid to obtain 100 c.c. of its solution; to 0.525 grm. of asparagin distilled water was added to obtain 50 c.c. of its solution; and to 0.525 grm. of tyrosine distilled water and a small quantity of NH_4OH were added to obtain 50 c.c. of its solution.

Combination: A).....1) 15 c.c.+4') 15 c.c.+7) 15 c.c.+8) 15 c.c.= 60 c.c. (Mixed at room temperature, preserved for 24 hrs.)

Combination: B).....1) 15 c.c.+4') 15 c.c.+7) 15 c.c.+8) 15 c.c.= 60 c.c. (1, 4', and 7 were mixed at room temperature, and 8 (at 55°C.)

was added to the mixture, and preserved in thermostat at 38°C. for 24 hrs.)

Combination: C).....1) 15 c.c.+4) 5 c.c.
(In air bladder (+))
+4) 5 c.c.+7) 10 c.c.+8) 10 c.c.=45 c.c.
(Outside of air bladder (−))

(OSTWALD's electric liquid resistant apparatus was used, and the solutions of the inner and outer sides of the air bladder were connected with two electrodes of a weak direct current (0.1 millivolt × 0.05 milliampere) for 24 hrs. at 38°C.)

Combination: D).....Eukirin 15 c.c.+4') 15 c.c.+7) 15 c.c.+8) 15 c.c.=60 c.c. (mixed in the same manner as above, and preserved in thermostat for 24 hrs. at 38°C.)

Combination: E).....1) 10 c.c.+1') 10 c.c.+Eukirin 10 c.c.
(In air bladder (+))
+4') 20 c.c.+Tyrosine 10 c.c.+α-alanine 10 c.c.+Asparagin 10 c.c.+8) 20 c.c. =100 c.c.
(Outside of air bladder (−))

(It was examined in the way as above described in the case of C), 3.5 millivolt × 5 milliampere, for 24 hrs., at room temperature.)

Substrate: 1. Potato starch (mixture of three kinds of potato starches produced in Hokkaido), 2. potato starch (Yakumo), 3. Common rice starch (Akita), 4. Glutinous rice starch (Akita), 5. Potato soluble starch (Sapporo), 6. Common rice soluble starch (Akita), 7. Glutinous rice soluble starch (Akita).

To 25 c.c. of 2 % solution of substrate 5 c.c. of "mixed chemicals" solution and 5 c.c. of toluene were added, and reacted at 38°C. for 20 hrs.; 5 c.c. was taken for the determination of the saccharifying power.

The results are shown in table 50.

According to the results of this experiment, the "mixed chemicals" solutions "A" and "B" show the starch-liquefying activity, but no saccharifying power. "C" indicates a slight tendency towards the starch-liquefying and saccharifying actions. In "D" also there is recognized the liquefying power, but no saccharifying power can be seen. However, "E" shows the starch-liquefying and saccharifying powers evidently. For example, about 47.8 % of starch is saccharified as glucose in the case of 140 hours reaction at 38°C., using potato starch (2) without action of microorganism. This sample "E" is used after the flowing of a weak electric current for 24 hours at room temperature. On the contrary, in spite of

"C" being treated as above at 38°C., it does not show the saccharifying power at all. From these facts, therefore, although there is a little difference between them in ingredients and strength of electric current in both these solutions, it seems to be considered that the principal cause of this difference is due to the influence of the temperature in bringing about some changes among the ingredients of the "mixed chemicals".

(2) Combination of sample: The solutions of every substance used as the enzymic ingredient were made in the same way as in the case of the former experiment. Toluene was used also as an antiseptic in this experiment.

A).....1) 15 c.c.+4') 15 c.c.+7) 15 c.c.+8) 15 c.c. (cold)=60 c.c. (This mixture was used after its preservation for 17 days at room temperature.)

B).....1) 15 c.c.+4') 15 c.c.+7) 15 c.c.+8) 15 c.c. (hot)=60 c.c. (This mixture was used after its preservation for 17 days at 38°C.)

D).....Eukirin 10 c.c.+4') 20 c.c.+Tyrosine 10 c.c.+α-alanine 10 c.c. +Asparagin 10 c.c.+8) 20 c.c. (hot)=80 c.c. (This mixture was used after its preservation for 17 days at 38°C.)

E).....This was the same as "E" in the case (1), and used after its preservation for 17 days at 38°C.

F).....1) 20 c.c. (each 10 c.c. of the solution was poured into both air bladders at the right and left sides respectively)+Eukirin 10 c.c. (into the air bladder at the left side)+7) 10 c.c. (into the air bladder at the left side)+α-alanine 10 c.c. (into the air bladder at the right side)+α-alanine 10 c.c. (outside of the air bladder at both sides)+4') 20 c.c. (outside of the air bladder at both sides)+8) 20 c.c. (outside of the air bladder at both sides).

For F), using OSTWALD's electric resistant apparatus, a positive electrode was put into the solution on the outside of the air bladder at the left side, and a negative electrode was put into the solution on the outside of the air bladder at the right side. Then the weak direct current (3.5 millivolt × 5 milliampere) was passed through it for 17 hours at room temperature. And then the solution was separated into the following parts: (F+−) is the mixture of solutions in both sides; (F+) is the solution in the positive side; (F−) is the solution in the negtive side. The solutions above described were made respectively by mixing both solutions in and out of the air bladder.

F').....After the flowing of the weak electric current into solution "F" for 14 days at room temperature, the solutions in both sides of the

apparatus were mixed with each other, and again the electric current was passed through for about 3 days.

G).....1) 10 c.c.+1') 10 c.c.+Eukirin 10 c.c.+
(In air bladder, left)
4') 10 c.c.+7) 5 c.c.+Asparagin 5 c.c.+α-alanine 5 c.c.
(In air bladder, right)
+4') 10 c.c.+α-alinine 5 c.c.+Asparagin 5 c.c.+Tyrosine 5 c.c.+8) 20 c.c.
(Outside of air bladder)

The solution mixed in this manner was kept in the OSTWALD's electric resistant apparatus; both poles were put into the solution outside of air bladders, and after the flowing of the weak electic current for 3 days, the solution was used for the experiment.

Substrate: 1. Potato starch (mixture of three kinds of starch produced in Hokkaido), 2. Common rice starch (Akita), (1) Potato soluble starch (Sapporo).

Reaction: To 25 c.c. of 2 % solution of substrate 5 c.c. of "artificial complex" and 5 c.c. of toluene were added, digested at 38°C. for 24 hrs.

The results are shown in table 51.

From the results of the experimet, the existence of the liquefying power is evidently recognized in every case. It is possible to consider that there is the dextrinizing power of starch in many cases as seen by its iodine reaction, though there are many differences according to the kind of starches. The starch-saccharifying activity seems to be evidently recognized in the "mixed chemicals" only in case its ingredients existed in the mutually harmonized state. So, there are many cases where the saccharification is not plainly recognized in a short time of reaction perhaps on account of their discordance. The phenomena such as osmosis or dialysis of "mixed chemicals" solution and the flowing of a weak electric current into the solution seem to have very intimate relation in bringing about their mutually harmonious state. And, observing the result of the flowing of the electric current, it can be proved that the liquefying activity is more powerful in the cathode solution than in the anode, and that the starch-splitting activity is in general most powerful in the mixed solution of the anode and cathode, next most powerful in the cathode solution alone and least powerful in the anode solution. Moreover, considering the saccharifying action of the "mixed chemicals" in the former experiment, "E" is the most powerful. For instance, in the case of reaction for 400 hrs., from 83 mgs. of the potato soluble starch 63 mgs. of reducing sugar (as glucose) was produced, showing about 75 % for the starch.

Considering these points, it will be understood that this "mixed chemicals" is like the diastase of Koji in its starch-splitting activity.

D. Comparison of liquefying power between the "mixed chemicals" and natural enzyme

As is evident in the former experiments, the starch-splitting activity of "mixed chemicals" is always inferior to natural diastase, and especially so in the saccharifying power, though its liquefying power is quite vigorous. Therefore, it is intended to compare the liquefying power of the "mixed chemicals" with those of the enzymes of polished rice Koji (0.1143 %), red rice Koij (0.1143 %) and germinated unhulled rice (0.4 %). These liquefying powers are estimated by the following method: To 5 c.c. of potato starch paste 1 c.c. of each enzyme solution is added, mixed well, and at once 5 c.c. of this mixed solution is poured into the OSTWALD's viscosimeter which is set in water bath at 38°C. to allow for starch liquefaction.

Control 1. Starch paste 5 c.c. + Distilled water 1 c.c., viscosity 10.095

Control 2. Distilled water 5 c.c., viscosity 1.000

The results of the experiment are shown in table 52.

According to the results of the experiment, the liquefying activity of the "mixed chemicals" is remarkably powerful, and, though it is inferior to that of polished rice Koji, it is almost similar to germinated unhulled rice, and is rather stronger than that of red rice Koji made in the province of "Fukieng", China, although it will not be always so, owing to the difference of concentrations. So it is surely understood that the liquefying activity of the enzymic substance is obtained very easily by mixing various ingredients.

E. On the constitution of the "mixed chemicals" and the precipitation phenomena of its ingredients by alcohol

Considering the constituent of Koji-Diastase in various cases of purification or separation, and also from the results of the elemental analysis, some kind of "mixed chemicals" was made by the combination of several ingredients as already described. And then the constitution of each fraction separated from the "mixed chemicals" with different concentrations

of alcohol was examined in order to know the manner of precipitation of its ingredients. And so, the nature of the "mixed chemicals" obtained was compared with that of the natural Koji-Diastase.

1. Elemental constitution of "mixed-chemicals" "E"

The ingredients of the "mixed chemicals" "E" are as follows.

	Substance	Solution(%)	Solution(c.c.)	Substance (g.)		Remarks
Mineral ingredient	$Mg_3(PO_4)_2$	0.528	10	0.0528		...Dissolved by a very small quantity of acetic acid
	$Mg_3(PO_4)_2$	c.528	10	0.0528	0.2106	...,, phosphoric acid
	Eukirin	1.050	10	0.1050		...,, acetic acid
Carbo-hydrate	Glucose	4.580	20	0.9160	1.0160	
	Dextrine	0.500		0.1000		
Protein-like substance	Tyrosine	1.050	10	0.1050	0.3150	
	α-alanine	1.050	10	0.1050		
	Asparagin	1.050	10	0.1050		
	Peptone	1.050	20	0.2100		

Elemental constitution of this "mixed chemicals" is about as follows.

	CO_2	H_2O	C	H	N	Ash	O	Remarks
"Mixed chemicals" "E"	139.920	1c8.918	38.160	6.051	4.508	11.400	39.881	calculated
Control (mean value of Koji-Diastase 1–5)	154.693	133.560	42.189	7.420	6.036	11.050	33.305	Analysed

2. Fractional precipitation of "mixed chemicals" by different concentration of alcohol

Precipitation phenomena of various chemicals by alcohol are shown in table 53.

As in the table, the minimum concentration of alcohol in which various ingredients are precipitated differs. For instance, peptone begins to precipitate at 37.07 % of alcohol in volume; the compounds of Mg and PO_4, at 45.26 %; glycocoll, at 54.05 %; dextrine, at 55.57 %; and K_3PO_4, at 61.43 %; and at this 61.43 % of alcohol the substances which are supposed to be main constituents in Koji-Diastase are precipitated. So there is no contradiction to the fact observed in the case of Koji-Diastase.

Next, the experiment of fractional precipitation of the "mixed chemicals" was made.

In the case of the combination of the solutions of various ingredients, a little of the precipitate was produced at the beginning. There was 0.364 gram of precipitate in 290 c.c. of the solution, and the greater part of this precipitate seemed to come from the samples of tyrosine and peptone, and there was also contained a small quantity of glycogen in the precipitate. So, 17.413 grams of ingredients were contained in the filtrate used in this fractional analysis.

The results are shown in table 54.

As is evident from the results of the experiment, the "mixed chemicals" produces 2.942 grams of precipitate at 63.3 % of alcohol, and from the remaining solution it produces 2.2435 grams of precipitate in total at 71.35, 80.40 and 85 % of alcohol. The amount of the former precipitate is more than the sum of the latter three cases of precipitation. The total precipitate, 5.1855 grams, is very small as compared with the substance existing in the filtrate, and it corresponds to about 1/3 of the original "mixed chemicals". But almost all the ash ingredient is precipitated – in fact, at 63.3 % of alcohol the greater part of it is precipitated.

And for the trial, observing the result of the elemental analysis of the "mixed chemicals" precipitated at 63.3 %, its constitution is C, 42.513 % ; H, 8.378 % ; N, 4.132 % , Ash, 17.600 % ; and O, 27.377 % respectively; and the ash content seems to be too large as compared with the natural purified Koji-Diastase. And according to the constitution of every one separated from the enzymic substance, it seems to be precipitated fractionally in the same order of the concentration of alcohol in each ingredient, as in the case of natural enzyme substances. It is recognized that these experimental results thus obtained supply some very important data for establishing the hypothetical "poly-constituent theory" of natural diastase.

SUMMARY

Considering the results of the experiments using "mixed chemicals" under the different combinations, treatments and other various conditions of substances, which are supposed to be ingredients of diastase, "mixed chemicals" did not always show the powerful activity of starch saccharification. But in the case of the combination of $MgHPO_4$, glucose, glycocoll and peptone, the starch-decomposing power of the "mixed chemicals"

is about 40 % after 15 hours action at 38°C. And the pH value used for this experiment is 4.85 in the enzymic solution and 6.50 in the potato starch paste. It is also recognized that no saccharifying action occurs when only one or two among the several ingredients are used separately for starch saccharification. These experiments were performed of course with special effort being made to get rid of the influence of microorganisms during the reaction.

In the course of the reaction of starch decomposition, using "mixed chemicals", the liquefying action occured comparatively easily, but the saccharification did not occur at once when its ingredients only mixed with each other were used. Its saccharifying activity seems to occur when the physico-chemical treatment applied is suitable, as described already to the "mixed chemicals" solution. For instance in "E", which is a mixture of $MgHPO_4$, "Eukirin", glucose, dextrine, tyrosine, α-alanine, asparagin and peptone, about 47.8 % of starch is saccharified by it for 140 hours at 38C., and also about 76 % for 400 hrs. Also there is reason to believe that the reducing sugar produced here is mainly glucose. Therefore, it will be considered that the action of this "mixed chemicals" is almost similar to that of Koji-Diastase. Thus, it is thought that this experiment is very influential in verification of the hypothetical "poly-constituent theory" of the Koji-Diastase.

Conclusion

Concerning the nature and action of enzyme, studies have been made by many scientists up to this time, but its real constitutional substance has not yet been proved, nor has a perfect explanation been given for its action. So, we must often have obstacles in the way of improving and developing of various investigations covering the theory and its application of enzyme chemistry from the standpoint of biology in general.

In the literature relating to enzymes, especially to diastase, the "single enzyme theory of diastase" is established which accounts for various actions of diastase that have been discussed already since the discovery of "malt-diastase" by KIRCHHOFF, PAYEN and PERSOZ, etc. at the end of the 18th century. There are also found the "two enzyme theory of diastase" by WIJSMAN (1889), POTTEVIN (1899), CHRZASZCZ (1911), EFFRONT (1922), and FRICKE and KOJA (1924), and the "three enzyme theory of diastase" by CHRZASZCZ (1917).

But, observing the attitude of OPPENHEIMER and other scientists in

these latter times with regard to starch decomposition by enzyme, this enzymic decomposition seems to be very intricate, so that it must not be considered that its hydrolytic action is based upon the character of only one or of several enzymes. Thus, there are many different opinions regarding the nature and action of diastase, but every one of them has attempted to explain these things with the idea that the substance of any kind of carbohydrate-splitting enzyme must be some single organic compound.

To prove the nature of "Koji-Diastase", many further investigations have been made by the author since 1921, and they have led finally to the conclusion that "Koji-Diastase" consists of several constituents which come from carbohydrate, protein and inorganic ingredient related to one another. Therefore, the author wishes to propound here a hypothesis,— the "poly-constituent theory of Koji-Diastase".

The principal items of the experimental results on the nature of "Koji-Diastase" are as follows:

1. The power of resistance of diastase of "Koji" against the injury of heating is very high. When the diastase in aqueous solution (Koji-extract) was heated up to 140°C. (for a moment at this temperature), it was entirely destroyed; and when the solution of the diastase purified by LINTNER's method was heated up to 137.5°C., it was also entirely destroyed. The activity of the diastase injured by being heated at a temperature lower than lethal seems to be recovered to some extent by preserving it at room temperature. And its recovering phenomenon has an important relation with the colloidal nature of the enzyme solution.

2. When the "Koji-Distase" or "Malt-Diastase" is purified fractionally by different concentrations of alcohol, the strongest fraction of its starch-liquefying power does not always coincide with that of its saccharifying power. And the range of fractions which have the liquefying power is wider than that of those which have the saccharifying power. And this is observed in the results of fractional purification of "Koji-Diastase"; for example, when the diastase was fractionated by adding different concentrations of alcohol (40, 50, 60, 70, 80, 90 %) to the "Koji"-extract, the amount of the enzymic material obtained is highest at 70 %; and the diastatic power of the fraction is in the order of 80 %, 70 % and 60 %. So, the power of the saccharification of the fraction seems to be increased according to the increase of the alcohol concentration used for its precipitation. Therefore, the enzyme substance precipitated at the higher concentration of alcohol seems to be comparatively increased in its purity.

3. The power and property of "Koji-Diastase" are different according to the materials used to prepare the enzyme.

4. The enzymic power of "Koji-Diastase" is not always proportional to its concentration, being comparatively more intensive for its unit amount in the more diluted solution.

5. From the results of the electric cataphoresis experiment on "Koji-Diastase" purified by LINTNER's method, it is certain that a more purified diastase can be obtained which does not have any enzymic powers of maltase, invertase, pepsin and trypsin on account of the difference in the nature of the cataphoresis of the coexisting substances. Moreover, it is also assured that, though the solution presents the protein reactions such as MILLON, BIURET, and Xantho-protein, etc., it has a slight colloidal nature and a strong diastatic power.

6. When the activity of maltase is not present in "Koji-Diastase", its property comes almost to resemble "malt-diastase". Diastase of "Koji" is mostly charged as a positive ion in neutral or acidic solution, while as a negative ion in basic solution. So it can be recognized that the iso-electric point of the diastase lies rather in the side of basic nature. And it is worthy of attention, that the diastatic power of the enzyme solution in each pole is not exhausted by being moved to any one pole.

7. "Koji-Diastase" in aqueous or 10 % alcohol solution contains small colloidal particles that can permeate through a shark's air-bladder.

8. The diastatic power of "Koji-Diastase" diffused through semi-permeable membrane is very strong compared with that of the original enzyme solution in the same concentration.

9. When the starch is digested by using "Koji-Diastase" diffused through membrane, the viscosity of the solution occasionally becomes higher, there being accumulated much dextrine. So, it is recognized that this is an abnormal phenomenon which occurs at this stage owing to the laxity of the progression of the saccharifyiug action. More generally, in the measurement of the starch-liquefying action by OSTWALD's viscosimeter, its value changes periodically, making a figure of wave form, with a gradually decreasing tendency.

10. The appearance of every solution of "Koji-Diastase" diffused through membrane is changed alternately from a clear to a turbid state by preserving it. The pH value of every solution is, on the one hand, changed also periodically by the preservation. And on the other, it is evident that every solution has a tendency to change gradually from a neutural to an acidic condition owing to the repetition of renewal of the outer solution

in dialysis. These phenomena certainly coincide with the phenomenon that the diastatic power is increased or decreased periodically according to the order of repetition of the renewal with a gradually decreasing tendency. So, in general, in the case of preservation of diffused enzyme solutions renewed successively, if a solution is comparatively transparent, and its hydrogen ion concentration appears to be high, i. e. the enzyme in the solution exists at a comparatively dissociated state, the enzymic power of the solution is intense, while in a contrary case it is weak. These phenomena are present likewise only in the preservation of diffused enzyme solution.

11. The content of ash ingredient in 6 samples of "Koji-Diastase" purified by LINTNER's method in 4.785–12.611 %, or 10.006 % in average. According to the results and also to those of the investigation on the ash of the diffused and dialyzed enzyme substances, about one half of the average can be considered as an excessive part, while the other half may rather be recognized as a kind of constituents of the enzyme substance. It is always recognized that PO_4, Mg, etc. exist at least as long as the diastatic power is kept in the enzyme solution. In the ash ingredient which exists in "Koji-Diastase" are contained such elements as PO_4, K, SO_4, Ca, and Na. Moreover, it has been explained qualitatively and quantitatively that there exist Al, Fe, Mn, Cl and SiO_2 accessorily in the enzyme substance.

12. When observations are made upon the protein reactions of the "Koji-Diastase" diffused through semipermeable membrane, none of the various precipitating reactions is found. There is found only a little coagulation when the solution shows a little alkaline reaction, and some precipitate is produced by boiling. But it is clear that the precipitate is based mainly upon the ash contained therein. BIURET's, Xantho-protein's, MILLON's, HOPKINS and COLE's, ADAMKIWICZ's, LIEBERMANN's, NEUBAUER's, RODE's, KNOPS's, PAULY's Diazo, and the Ninhydrin colour reactions are present, but PbS reaction is not found. Moreover, the chemical analysis of protein-like substance in "Koji-Diastase" was also tried. According to the results of these various experiments, it is known that in "Koji-Diastase" amino acids and lower polypeptides exist as protein-like substance.

13. On observing the carbohydrate reactions of "Koji-Diastase", it is found that MOLISCH and UDRANSKI's, MOLISCH's, the Naphtoresorcin, and NEUMEN's reaction exist in various samples such as that purified by LINTNER's method, or fractionated by dialysis or permeation, and that the Indigocarmine, and Picric acid reactions also exist. From these results the exis-

tence of aldohexose in the enzyme substance is recognized, but there is no ketose reaction. As regards the pentose reaction, the Phlorglucin, BIAL's Orcin, and NEUMANN's reactions are present to a small extent, while the methylpentose reaction of OSHIMA and TOLLENS rarely exists. Moreover, concerning the enzyme substance and its hydrolysis product, the quantitative analysis of its carbohydrate was also tried. Judging from the various results of these experiments, the carbohydrate ingredients relating to the enzymic action of diastase are supposed to be aldohexoses which lie between simple sugar and dextrine.

14. Considering the construction of "Koji-Diastase" from the results of its qualitative, quantitative and elmental analysis and its other various examinations, it is recognized to be a kind of soft associating material which consists of several ingredients such as ash, nitrogenous and carbohydrate substances. It is not always constant on account of the difference of its material, the method of its purification, and the method of its fractional separation.

15. The substances which are able to be ingredients of starch-splitting enzyme are all so small that they can permeate as mentioned before through the semipermeable membranes. And they also have the property of being precipitated by alcohol. Therefore, investigating the manner of precipitation of the substances which can be supposed as the ingredients of "Koji-Diastase" by using different concentrated alcohols, the following results are obtained: The inorganic compounds such as phosphates of Mg and Ca are precipitated comparatively in the lower concentration of alcohol, while the phosphates and sulphates of K and Na are precipitated in the higher concentration of alcohol, then chlorides of Mg, Ca, K, Na, etc. are hardly precipitated by alcohol. This coincides with the fact that, in these concentrations of alcohol, ash ingredients of K, Mg, PO_4 which are especially supposed to have some relation to the starch-liquefying action of "Koji-Diastase", come to precipitate with some other ingredients. It is observed concerning the nitrogenous substance (lower polypeptides) that the comparatively higher one (peptone) precipitates in a lower alcoholic concentration than the lower one. And also it is assured that the carbohydrate substance has a similar tendency in various precipitation phenomena using alcohol.

16. In order to establish the "poly-constituent theory of Koji-Diastase" anew from the results of the various investigations above described, the experiments with "mixed chemicals" were made as one of the most real and powerful illustrations. The substances which will be combined

weakly, as the ingredients of diastase, are many kinds of lower polypeptides, of lower aldohexoses between simple sugar and dextrine, and of phosphates. Trying various experiments with "mixed chemicals" which can be regarded as the diastatic ingredients under different conditions, it is recognized that diastase is not only made by mixing the ingredients above mentioned, but it is necessary to give and gain some energetic influence to combine them weakly together and to take an active form. By virtue of the results of several experiments which were tried by using a weak direct electric current and semipermeable membrane to imitate the movements of the internal substances in living things as one of the physiological-chemical phenomena, it is necessary to be considered that the enzyme-like action of the "mixed chemicals" is to be gotten finally by furnishing a specially harmonious ionized condition in each and every ingredient.

Now, according to the "poly-constituent theory", the enzyme substance of "Koji-Diastase" is recognized to be a kind of weak associating material which is made up from several ingredients, namely, one of them "A" is of ash ingredients such as PO_4, Mg and K or any substance which is able to take the place of any one of them, the other "B" is the amphoteric compounds having the character both of the amino and the carboxyl group such as some lower poly-peptides lying between amino acid and peptone, and still another "C" consists of the lower aldohexoses lying between simple sugar and dextrine. Perhaps all these materials are not so large as protein or starch in their molecular weight. Moreover, by this theory, many results concerning the various periodical phenomena which take place in the outer solution renewed in dialysis of "Koji-Diastase" can be distinctly explained. And also many results on the heat durability, on the recovery of the enzymic power after heating, and on the fractional precipitation of "Koji-Diastase" in aqueous solution by different concentrations of alcohol.

Besides, it is recognized that the difference of the action between one enzyme and the others is influenced by the difference in one or more of its constituents, and also that the enzymes resembling each other should be formed with the analogous ingredients. And, it can be considered that there are many kinds of ingredients as a group of "A", of "B" or of "C" respectively. So, it is recognized of course that there are numerous and different kinds of enzyme or many analogous kinds of enzyme produced by the soft mutual coalescence of the different kinds of enzymic ingredients.

Table 1. Preparation of purified Koji-Diastase

Koji (Koji-Diastase)	Date of manufact. of Koji	Weight of Koji (used air dry) (g.)	Alcohol sol. used for extraction (c.c.)	Filtered solution of extract (c.c.)	Concentration of alcohol for precipitation	Enzyme substance (g.)	Enzyme substance (%)
No. 1	Aug. 30. 1925	2360	20 %, 5900	3200	80	2.2100	0.221
No. 2	Sept. 22. 1926	1000	30 %, 2000	1250	82	4.6705	0.467
No. 3	Oct. 8. "	1000	"	1345	"	2.8854	0.289
No. 4	" 19. "	1000	"	1380	"	4.5113	0.451
No. 5	" 25. "	1000	"	1430	"	3.9300	0.393
No. 6	" 28. "	1000	"	1450	"	4.2880	0.429
Moto-Koji	Feb. 2. 1927	12400	20 %, 16270	12270	81	34.0875	0.275

Table 2. The renewal of the outer solution

Number of renewal	Number of days after the beginning of dialysis	Time allowed for permeation	pH
Original enzyme solution	——	——	6.90
I′	1	30	6.60
I	2	50	6.60
II	4	48	6.65
III	6	48	6.65
IV	8	49	6.65
V	9	25·	6.70
VI	11	43.5	6.70
VII	12	25	6.70
VIII	13	24	6.70
IX	14	24	6.65
X	15	26	6.65
XI	18	76.5	6.65
XII	19	24	6.55
XIII	21	43	6.55
XIV	22	24	6.55
XV	27	123	6.60
(XV′)	27	(Inner solution 10 c.c.)	6.70
XVI	29	44	6.60
XVII	33	95	6.60
XVIII	37	95	6.60
XIX	41	95	6.55
XX	46	121	6.50
XXI	50	97	6.45
XXII	54	96	6.45
(XXII′)	54	(Inner solution 10 c.c. + Water 10 c.c.)	6.55
XXIII	58	96	6.50
XXIV	62	96	6.45
XXV	66	95.5	6.40
XXVI	70	96	6.35

(XXVI′)	70	(Inner solution 10 c.c. +Water 10 c.c.)	6.40
XXVII	75	121	6.30
XXVIII	79	95	6.30
XXIX	83	96	6.30
XXX	88	120	6.30
XXXI	92	96.5	6.25
XXXII	97	119.5	6.25
XXXIII	102	120	6.20
XXXIV	106	96	6.20
XXXV	110	96	6.20
(XXXV′)	110	(Inner solution 15 c.c. + Water 10 c.c.)	6.25
XXXVI	114	96	6.20
XXXVII	118	96.5	6.20
XXXVIII	123	95.5	6.20
XXXIX	127	97.5	6.20
XL	131	96.5	6.20
XLI	135	96	6.20
XLII	142	169	6.20
XLIII	159	405	6.20
XLIV	167	190.5	6.20
XLV	173	147	6.20
XLVI	179	146	6.20
XLVII	186	163.5	6.20
XLVIII	198	293	6.20
XLIX	210	283	6.20
L	219	218.5	6.20
LI	238	459.5	6.20
LII	280	1003	6.20
LIII	298	436	6.20
(LIII′)	298	(Inner solution 30 c.c. +10 % alcohol solution 30 c.c.)	6.20
(LIII″)	298	(Insoluble matter remained after dialysis for about 10 months)	—
10 % alcohol solution	——	——	6.20

Table 3. Carbohydrate-splitting power of the outer solution

I′ (October 2. 1925)

		Digestion For 16 hours		Digestion For 58 hours
		Viscosity	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)
Starch	Control	12.000	0.10	0.10
	Digested solution	1.077	7.35 (7.25)	10.45 (10.35)
Soluble starch	Control	—	0.20	0.20
	Digested solution	1.087	8.10 (7.90)	10.85
Maltose	Control	1.040	7.75	8.20
	Digested solution	1.040	8.35 (0.60)	10.85 (2.65)
Sucrose	Control	1.036	0.55	0.60
	Digested solution	1.036	1.15 (0.60)	1.50 (0.90)

II (October 5. 1925)

		For 16 hours		For 34 hours	
		Viscosity	$KMnO^4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)
Starch	Control	14.330	0.10	11.050	0.10
	Digested solution	1.138	8.90 (8.80)	1.134	10.70 (10.60)
Soluble starch	Control	1.179	0.20	1.184	0.20
	Digested solution	1.189	9.85 (9.65)	1.189	11.50 (11.30)
Maltose	Control	1.040	7.90	1.040	7.95
	Digested solution	1.038	9.55 (1.65)	1.038	11.10 (3.15)
Sucrose	Control	1.036	0.70	1.036	0.70
	Digested solution	1.036	2.40 (1.70)	1.036	3.40 (2.70)

III (October 7. 1925)

		For 16 hours		For 35 hours
		Viscosity	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)
Starch	Control	13.875	0.10	0.10
	Digested solution	1.247	8.75 (8.65)	10.90 (10.80)
Soluble starch	Control	1.186	0.20	0.20
	Digested solution	1.276	10.05 (9.85)	11.45 (11.25)
Maltose	Control	—	7.90	0.90
	Digested solution	—	9.80 (1.90)	11.85 (3.95)
Sucrose	Control	—	0.70	0.70
	Digested solution	—	2.50 (1.80)	3.70 (3.35

IV (October 9. 1925)

		For 16 hours		For 58 hours
		Viscosity	$KMnO_4$ solution (c.c.)	$KMnO^4$ solution (c.c.)
Starch	Control	14.113	0.10	0.10
	Digested solution	1.436	9.40 (7.30)	11.10 (11.00)
Soluble starch	Control	1.185	0.20	0.20
	Digested solution	1.333	9.30 (9.10)	12.35 (12.15)
Maltose	Control	—	8.00	8.90
	Digested solution	—	9.70 (1.70)	12.90 (4.00)
Sucrose	Control	—	0.75	0.75
	Digested solution	—	2.40 (1.65)	4.10 (3.35)

V, VI (October 12. 1925)

		Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)
Starch	Control	14.082	0.15	—	0.15
	V	3.050	3.15	1′28.9″	5.55
	VI	2.599	3.50	1′15″	5.65
Soluble starch	Control	1.206	0.15	—	0.15
	V	1.231	6.35	—	8.50
	VI	1.231	6.55	—	8.55
Maltose	Control	—	7.85	—	8.00
	V	—	8.55	—	9.15
	VI	—	8.60	—	9.25
Sucrose	Control	—	0.70	—	0.65
	V	—	1.55	—	1.95
	VI	—	1.35	—	1.75

Table 4. Protein-splitting power of the outer solution

(October 7. 1925)

		40 hours digestion		
		Total weight (g)	Weighing tube + Filter paper (g)	Casein remained (g)
Pepsin	Control	17.6336	17.2466	0.3870
	I	23.9162	23.5112	0.4050
	II	23.6972	23.3066	0.3908
	III	23.4474	23.0576	0.3898
Trypsin	Control	24.0616	23.6860	0.3756
	I	21.9538	21.5624	0.3914
	II	22.8906	22.5152	0.3754
	III	23.8944	23.5176	0.3768

Table 5. Comparison of the power of carbohydrate-splitting enzyme between the outer and inner solution

(1) 16 hours digestion

(October 28. 1925)

	Starch		Soluble starch	Maltose	Sucrose
	Viscosity	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)
Control (No enzyme solution)	11.919	0.10	0.20	—	—
XV (Dialyzate)	1.610	11.10	11.50	—	—
(XV′) (Inner solution)	1.481	12.30	12.90	—	—

(November 24. 1925)

Control	—	0.10	0.20	7.90	0.50
XXII (Dialyzate)	2.974	3.20	6.45	8.45	0.95
(XXII′) (Inner solution)	1.852	10.70	12.05	14.05	11.00

(December 10. 1925)

Control	7.239	0.15	0.20	7.70	0.75
XXV (Dialyzate)	1.479	7.70	8.40	9.10	2.00
(XXV′) (Inner solution)	1.467	12.30	13.10	13.15	8.45

(January 19. 1925)

Control	—	0.20	2.25	7.25	0.75
XXXV (Dialyzate)	2.529	5.00	8.70	9.40	0.75
(XXXV′) (Inner solution)	3.061	9.85	11.85	12.95	3.60

(2) 35 hours digestion

(December 10. 1925)

Control	—	0.15	0.20	7.70	0.85
XXVI	1.436	9.35	9.85	10.05	2.75
(XXVI′)	1.477	18.20	13.50	13.50	10.10

(January 19. 1926)

Control	—	0.20	0.25	7.40	0.75
XXXV	2.338	6.80	9.50	10.90	0.75
(XXXV′)	2.593	11.55	12.10	13.65	3.90

Table 6. Changes of diastatic power of the outer solution

Enzyme solution	For 5 hours			For 20 hours			
	Starch		Soluble starch	Starch		Soluble Starch	
	Viscosity	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)
Control 1 (No enzyme, 10 % alcohol solution)	6.876	0.20	0.25	5.538	0.20	1.528	0.25
Control 2 (Steeped solution of shark's air bladder, by 10 % alcohol)	6.864	0.20	0.25	5.652	0.20	1.528	0.25
I	1.348	6.40	5.85	1.327	8.05	1.614	9.00
V+VI	3.655	3.60	3.95	—	6.60	1.569	7.00
XI	6.539	3.40	3.20	2.201	5.85	1.442	6.40
XXI+XXII	2.552	4.65	4.00	—	7.65	1.514	7.00
XXIII+XXIV	2.827	6.70	5.90	—	8.50	1.598	8.85
XXV	2.699	2.60	3.25	—	6.10	1.458	6.55
XXVI	1.735	5.30	5.25	—	8.45	1.621	8.10
XXVII	1.819	6.40	5.60	—	8.40	1.672	8.60
XXVIII	1.768	6.00	5.30	—	8.50	1.653	8.00

XXIX	1.746	5.80	4.70	—	8.70	1.618	7.80
XXX	1.635	6.75	5.35	1.348	9.60	1.538	8.85
XXXI	5.434	4.10	4.00	2.421	7.10	1.262	8.00
XXXII	8.472	3.20	3.45	—	6.10	1.231	7.65
XXXIII	8.626	5.35	6.35	—	8.60	1.255	8.80
XXXIV	10.599	2.50	4.65	7.692	4.95	1.325	7.90
XXXV	9.288	3.70	4.70	6.888	5.20	1.298	7.90
(XXVI′) (Inner residual solution)	1.292	12.00	11.55	1.280	12.35	1.510	12.30
(XXXV′) (Inner residual solution)	9.793	6.40	9.45	9.458	8.85	1.251	10.65

Table 7. Changes of carbohydrate-splitting powers of the outer solution

(March 26, 1926)

Enzyme solution	20 hours reaction					
	Starch		Soluble starch	Dextrine	Maltose	Sucrose
	Appearance	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)
Control 1 (No enzyme, 10 % alcohol solution)	Quite insoluble	0.15	—	—	—	—
Control 2 (Steeped solution of shark's air bladder prepared by 10 % alcohol)	"	—	0.25	0.85	7.25	0.50
XXXVI	Soluble	8.40	8.75	5.45	7.55	0.55
XXXVII	"	8.65	8.75	6.10	9.70	0.65
XXXVIII	Insoluble	5.35	8.90	3.50	10.45	0.70
XXXIX	Somewhat insoluble	7.05	9.20	3.35	9.80	0.55
XL	Insoluble	5.65	9.60	3.20	9.60	0.60
XLI	Soluble	10.05	9.80	5.85	9.40	0.75
XLII	"	11.15	8.70	3.60	10.05	0.80
XLIII	"	8.00	9.60	6.25	10.15	0.90
XLIV	Somewhat insoluble	6.60	8.15	5.20	9.30	0.60
XLV	Soluble	6.20	3.65	2.35	7.40	0.65

(Continued)

Enzyme solution	For 65 hours		For 105 hours				
	Starch		Starch	Soluble starch	Dextrine	Maltose	Sucrose
	Viscosity	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c)
Control 1	above 73 846	0.15	0.15	—	—	—	—
Control 2	—	—	—	0.25	0.85	7.25	0.55
XXXVI	1.165	9.85	10.00	10.00	8.60	13.90	0.80
XXXVII	1.301	9.50	9.75	9.55	7.20	14.50	0.75
XXXVIII	1.188	4.55	6.20	9.75	6 95	14.55	0.70
XXXIX	1.261	6.75	8.65	9.70	6.80	13.45	0.85
XL	2.297	4.65	7.20	10.15	6.35	13.75	0.90
XLI	1.282	10.80	10.90	10.05	6.55	14.25	0.80
XLII	1.298	12.20	11.50	8.95	6.95	14.30	0.85
XLII	1.210	10.60	10.60	10.10	7 55	14.30	1.15
XLIV	1.442	8.95	9.15	8.30	6.55	12.10	0.75
XLV	1.259	6.45	7.55	6.85	5.65	9.60	0.75

Table 8. Changes of carbohydrate-splitting powers of the outer solution

(May 27. 1926)

Enzyme solution (Time allowed for permeation, Hr.)	For 15 hours ($KMnO_4$ solution, c.c.)			For 40 hours ($KMnO_4$ solution, c.c.)		
	Soluble starch	Maltose	Sucrose	Soluble starch	Maltose	Sucrose
Control 1	0.30 (0)	7.50 (0)	0.75 (0)	0.35 (0)	7.50 (0)	0.75 (0)
Control 2	0.30 (0)	7.50 (0)	0.75 (0)	0.35 (0)	7.50 (0)	0.75 (0)
XLVI (146)	1.50 (1.20)	7.70 (0.20)	0.75 (0)	4.15 (3.80)	8.35 (0.85)	0.80 (0.05)
XLVII (163.5)	0.75 (0.45)	7.60 (0.10)	0.80 (0.05)	1.70 (1.35)	7.75 (0.25)	7.95 (0.20)
XLVIII (293)	0.85 (0.55)	7.60 (0.10)	0.85 (0.10)	1.75 (1.40)	7.80 (0.30)	0.90 (0.15)
XLIX (283)	0.75 (0.45)	7.70 (0.20)	0.85 (0.10)	1.65 (1.30)	7.80 (0.30)	0.90 (0.15)
L (218.5)	0.75 (0.45)	7.60 (0.10)	0.80 (0.05)	0.95 (0.60)	7.70 (0.20)	0.85 (0.10)
LI (459.5)	0.65 (0.35)	7.70 (0.20)	0.85 (0.10)	1.00 (0.65)	7.85 (0.35)	0.90 (0.15)

(July 26. 1926)

Enzyme solution	For 15 hours					For 40 hours			
	Starch		Soluble starch	Maltose	Sucrose	Starch	Soluble starch	Maltose	Sucrose
	Viscosity	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)
Control (Steeped solution of shark's air bladder, by 10 % alcohol)	above 73.846	0.15	0.30	7.60	0.80	0.15	0.30	7.65	0.80
LII (1003)	"	0.15	0.35	7.95	0.80	0.15	0.60	8 50	0.80
LIII (436)	"	0.15	0.35	8.15	0.80	0.15	0.50	8.50	0.80
(LIII′)	"	0.15	0.55	8.90	0.80	0.15	0.60	9.50	0.80
Original enzyme solution (10 % alcoholic)	1.094	10.05	12.50	11.40	2.95	11.00	12.55	11.40	3.95

Table 9. Enzymic powers of lipase, pepsin and trypsin of the outer and inner solution

(July 26. 1926)

Enzyme solution	Lipase (N/10-NaOH solution, c.c.)	Pepsin (Remained casein, g.)	Trypsin (Remained casein, g.)
Control (Steeped solution of shark's air bladder, by 10 % alcohol)	1.45	0.4080	0.3835
LIII (Dialyzate)	1.45	0.4075	0.3603
(LIII') (Inner residual solution)	1.45	0.4067	0.3605
Original enzyme (10 % alcoholic solution)	1.95	0.3935	0.3552

Table 10. Relation between the time of dialysis and the rate of diffusion of enzyme substance

(October 28. 1925)

Enzyme solution	Time allowed for permeation (Hr)	For 16 hours ($KMnO_4$ solution, c.c.)				Rate for one hour ($KMnO_4$ solution, c.c.)			
		Starch	Soluble starch	Maltose	Sucrose	Starch	Soluble starch	Maltose	Sucrose
Control (Steeped solution of shark's air bladder, by 10% alcohol)	—	0.15 (0)	0.20 (0)	7.90 (0)	0.55 (0)	—	—	—	—
VII	25	0.60 (0.45)	2.20 (2.00)	8.35 (0.45)	0.90 (0.35)	0.018	0.080	0.018	0.014
VIII	24	1.20 (1.05)	2.90 (2.70)	8.45 (0.55)	0.90 (0.35)	0.044	0.113	0.023	0.014
IX	24	0.65 (0.50)	2.15 (1.95)	8.15 (0.25)	0.85 (0.30)	0.021	0.081	0.011	0.013
X	26	0.85 (0.70)	2.45 (2.25)	8.25 (0.35)	0.90 (0.35)	0.027	0.086	0.014	0.013
XI	76.5	3.20 (3.05)	6.75 (6.55)	8.95 (1.05)	1.25 (0.70)	0.040	0.086	0.013	0.009
XII	24	0.90 (0.75)	2.30 (2.10)	8.30 (0.40)	0.85 (0.30)	0.031	0.086	0.019	0.013
XIII	43	0.95 (0.80)	2.20 (2.60)	8.45 (0.55)	0.85 (0.30)	0.019	0.060	0.013	0.004
XIX	24	1.15 (1.00)	2.70 (2.50)	8.00 (0.10)	0.85 (0.30)	0.040	0.104	0.004	0.013
XV	123	11.10 (10.95)	11.50 (11.30)	13.00 (5.10)	4.25 (3.70)	0.089	0.092	0.041	0.030
(XV′)	—	12.30 (12.15)	12.50 (12.70)	—	—	—	—	—	—

(Continued)

Enzyme solution	Time allowed for permeation (Hr)	For 40 hours ($KMnO_4$ solution, c.c.)				Rate for one hour ($KMnO_4$ solution, c.c.)			
		Starch	Soluble starch	Maltose	Sucrose	Starch	Soluble starch	Maltose	Sucrose
Control	—	0.15 (0)	0.20 (0)	7.95 (0)	0.55 (0)	—	—	—	—
VII	25	2.70 (2.55)	3.50 (3.30)	8.50 (0.55)	0.90 (0.35)	0.102	0.132	0.022	0.014
VIII	24	2.70 (2.55)	5.30 (5.10)	8.85 (0.90)	0.95 (0.45)	0.106	0.213	0.038	0 017
IX	24	1.80 (1.65)	2.15 (1.95)	8.40 (0.45)	0.85 (0.30)	0.078	0.081	0.019	0.013
X	26	2.10 (1.95)	3.70 (3.50)	8.60 (0.65)	0.90 (0.35)	0.075	0.135	0.025	0.013
XI	76.5	5.50 (5.35)	8.90 (8.70)	9.45 (1.50)	1.35 (0.80)	0.070	0.113	0.020	0.010
XII	24	2.20 (2.05)	4.70 (4.50)	8.50 (0.55)	0.85 (0.30)	0.085	0.188	0.023	0.013
XIII	43	3.10 (2.95)	4.80 (4.60)	8.55 (0.60)	0.85 (0.30)	0.069	0.107	0.014	0.007
XIV	24	1.50 (1.35)	4.00 (3.80)	8.30 (0.35)	0.85 (0.30)	0.056	0.158	0.015	0.013
XV	123	11.30 (11.15)	11.70 (11.50)	13.60 (5.65)	6.10 (5.55)	0.091	0.093	0.046	0.045
(XV′)	—	12.50 (12.35)	13.00 (12.80)	—	—	—	—	—	—

Table 11. Increasing phenomena of viscosity

(November 24. 1925)

Enzyme solution	For 16 hours				For 32 hours				For 73 hours			
	Starch		Soluble starch		Starch		Soluble starch		Starch		Soluble starch	
	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)
Control (Steeped solution of shark's air bladder, by 10% alcohol)	6.578	0.15	1.520	0.20	5.036	—	1.575	—	5.030	0.15	1.559	0.20
XX	2.098	5.30	3.313	6.70	2.066	5.50	2.948	7.35	1.758	6.75	2.667	8.45
XXI	2.194	5.30	3.054	6.70	2.072	5.40	3.128	7.25	1.799	6.75	2.878	8.45
XXII	2.513	3.20	2.790	6.45	2.211	3.20	3.237	7.10	1.819	4.30	3.069	8.15
Original enzyme substance	7.323	0.15	1.662	0.20	7.325	0.15	1.662	0.20	7.325	—	1.662	—

(December 10. 1925)

Control	7.239	0.15	1.524	0.20	5.036	—	1.577	—	5.030	0.15	1.559	0.20
XXIII	1.446	6.75	1.251	7.35	1.436	—	1.288	—	1.465	8.35	1.502	9.05
XXIV	1.467	7.85	1.600	8.45	1.436	—	1.707	—	1.575	10.65	1.824	10.15
XXV	1.518	5.60	1.846	5.80	1.479	—	1.815	—	1.467	8.35	1.815	9.20
XXVI	1.477	7.70	1.969	8.40	1.456	—	2.437	—	1.473	11.10	1.610	11.05
Original enzyme substance	9.102	0.15	1.628	0.20	10.113	—	1.645	—	9.686	0.15	1.600	0.20

Table 12. Increasing phenomena of viscosity on starch

Enzyme solution	For 5 hours		For 63 hours					For 148 hours		
	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	N/100 IIK solution 1 drop	" 2 drops	N/10 IIK solution 1 drop	Viscosity	N/100 IIK solution 1 drop	N/10 IIK solution 1 drop
Control 1 (No enzyme, 10 % alcohol solution)	6.876	0.20	5.264	0.20	B Tint 2	B Shade 1	B Shade 2	4.010	B Shade 1	B Shade 2
Control 2 (Steeped solution of shark's air bladder, by 10 % alcohol)	6.859	0.20	5 475	0.20	B Tint 2	B Shade 1	B Shade 2	4.066	B Shade 1	B Shade 2
I	1.348	6.40	1.407	9.85	YO Tint 2	R Tint 1	R Normal tones	1.409	YO Tint 2	OR Broken tones
V+VI	3.657	3.60	—	8.30	B Tint 2	VB Tint 1	V Shade 1	1.266	B Tint 2	V Shade 2
XI	6.539	3.40	1.378	6.80	B Tint 2	VB Tint 1	V Shade 1	1 301	B Tint 1	BV Shade 2
XXI+XXII	2.552	4.65	—	9.35	B Tint 2	VB Normal tones	RV Shade 1	1.344	B Tint 2	V Shade 2
XXIII+XXIV	2.827	6.70	—	9.95	B Tint 2	VB Normal tones	RV Shade 1	1.323	B Tint 1	V Shade 2
XXV	2.699	2.60	1.339	6.60	BV Tint 2	VB Tint 1	RV Shade 1	1.305	B Tint 1	BV Shade 2
XXVI	1.735	5.30	—	10.00	BV Tint 2	BV Normal tones	VR Shade 1	1.401	B Tint 1	V Shade 2

XXVII	1.819	6.40	—	9.90	BV Tint 2	BV Normal tones	VR Shade 1	1.354	B Tint 2	V Shade 2
XXVIII	1.768	6.00	1.251	9.20	B Tint 2	V Normal tones	V Shade 1	1.331	B Tint 2	V Shade 2
XXIX	1.755	5.80	—	10.45	B Tint 2	V Normal tones	V Shade 1	1.374	B Tint 2	V Shade 2
XXX	1.635	6.75	1.301	10.70	B Tint 2	V Normal tones	V Shade 1	1.395	GB Tint 2	V Shade 1
XXXI	5.844	4.10	1.538	7.35	B Tint 2	B Shade 1	V Shade 1	1.350	GB Tint 2	V Shade 1
XXXII	8.472	3.20	—	6.65	B Shade 1	B Shade 1	V Shade 1	1.491	B Shade 1	VB Shade 2
XXXIII	8.718	5.35	—	9.95	B Tint 2	B Shade 1	V Shade 1	1.344	GB Tint 2	VB Shade 2
XXXIV	10.599	2.50	4.324	6.65	B Tint 2	B Shade 1	VB Shade 1	2.002	B Shade 1	B Shade 2
XXXV	9.391	3.70	5.717	5.70	B Tint 2	B Shade 1	VB Shade 1	2.851	B Tint 1	B Shade 2
(XXVI′) (Inner residual solution)	1.303	1.200	1.391	12.65	YO Tint 2	R Tint 1	R Normal tones	1.370	YO Tint 2	OY Tint 1
(XXXV′) (Inner residual solution)	9.793	6.40	4.369	10.20	B Tint 2	B Shade 1	BV Shade 1	1.405	B Tint 1	B Shade 2

Table 13. Increasing phenomena of viscosity on soluble starch

Enzyme solution	For 20 hours		For 63 hours				For 148 hours		
	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	N/100 IIK solution 1 drop	N/10 IIK solution 1 drop	Viscosity	N/100 IIK solution 1 drop	N/10 IIK solution 1 drop
Control 1.	1.530	0.25	1.518	0.25	B Tint 2	B Shade 2	1.532	B Shade 1	B Shade 2
Control 2.	1.530	0.25	1.518	0.25	B Tint 2	B Shade 2	1.508	B Shade 1	B Shade 2
I	1.614	9.00	1.504	11.20	No color	YO Tint 1	1.389	No color	YO Shade 1
V+VI	1.571	7.00	1.680	8.70	R Tint 1	R Shade 2	1.707	R Tint 2	R Shade 1
XI	1.442	6.40	1.664	7.70	R Tiut 1	OR Shade 2	1.836	R Tint 2	R Shade 1
XXI+XXII	1.514	7.00	1.828	7 95	R Tint 1	R Shade 2	1.651	R Tint 2	R Shade 1
XXIII+XXIV	1.598	8.85	1.869	7.70	R Tint 2	R Shade 2	1.713	R Tint 2	R Shade 1
XXV	1.458	6.65	1.272	7.85	VR Tint 1	OR Shade 2	1.645	R Tint 2	R Shade 1
XXVI	1.621	8.10	1.701	9 65	R Tint 1	R Shade 2	1.668	R Tint 2	R Shade 1
XXVII	1.672	8.60	1.811	10.30	R Tint 1	R Shade 2	1.719	R Tint 2	R Shade 1

XXVIII	1.653	8.00	1.713	9.60	R Tint 1	R Shade 2	1.692	R Tint 2	R Shade 1
XXIX	1.618	7.80	1.803	8.80	R Tint 1	R Shade 2	1.750	R Tint 2	R Shade 1
XXX	1.262	8.85	1.688	9.60	R Tint 1	R Shade 2	1 676	V Tint 2	R Shade 1
XXXI	1.262	8.00	1.266	8.60	RV Tint 2	RV Shade 2	1.286	V Tint 1	V Shade 2
XXXII	1.231	7.65	1.237	8.25	B Tint 2	V Shade 2	1.251	VB Shade 1	BV Shade 2
XXXIII	1.255	8.80	1.276	9.65	RV Tint 2	RV Shade 2	1.282	V Tint 2	BV Shade 2
XXXIV	1.325	7.90	1.259	8.55	B Tint 2	V Shade 2	1.268	VB Shade 1	BV Shade 2
XXXV	1.298	7.90	1.367	8.25	B Tint 2	V Shade 2	1.305	VB Shade 1	BV Shade 2
(XXVI′)	1.508	12.30	1.582	12.80	R Tint 2	OR Broken tones	1.370	No color	V Shade 2
(XXXV′)	1.251	10.65	1.278	11.15	B Tint 1	V Shade 2	1.282	No color	VB Shade 2

Table 14. Change of the enzymic power caused by the difference of preserving days

(October 28. 1925)

Enzyme solution	Preserving days	Time allowed for permeation (Hr)	For 16 hours			For 49 hours		
			Starch		Soluble starch	Starch		Soluble starch
			Viscosity	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)
Control (Steeped solution of shark's air bladder, by 10 % alcohol)	—	—	11.099	0.15	0.20	10.974	0.15	0.20
VII	15	25	17.108	0.60	2.20	3.409	2.70	3.50
VIII	14	24	11.413	1.20	2.90	1.703	2.70	6.30
IX	13	24	—	0.65	2.15	4.716	1.80	2.15
X	12	26	—	0.85	2.45	5.487	2.10	3.70
XII	8	24	—	0.90	2.30	4.154	2.20	4.70
XIV	5	24	—	1.15	2.70	9.253	1.50	4.00

(Continued)

(November 12. 1925)

Enzyme solution	Preserving days	Time allowed for permeation (Hr)	For 16 hours					For 33 hours				
			Starch		Soluble starch	Maltose	Sucrose	Starch		Soluble starch	Maltose	Sucrose
			Vis-cosity	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	Vis-cosity	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c)	$KMnO_4$ solution (c.c.)
Control	—	—	4 759	0.15	0 20	7.90	0.50	3.883	0.15	0.20	7.90	0.50
XVI	13	44	1.286	8.20	9 70	9.65	0.90	1.130	9.30	10.60	10.55	1.05
XVII	9	95	1.175	9.30	10.05	9.95	1.30	1.089	10.25	11.10	11.35	1.60
XVIII	5	95	1.783	5.60	7.65	7.60	0.55	1.364	7.05	9.20	9.00	0.90
XLX	1	95	1.163	9.10	9 95	9.85	1.30	1.114	10.25	11.20	11.20	1.40

(Continued)

(December 10. 1925)

Enzyme solution	Preserving days	Time allowed for permeation (Hr)	For 16 hours						For 35 hours					
			Starch		Soluble starch		Maltose	Sucrose	Starch		Soluble starch		Maltose	Sucrose
			Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)
Control	—	—	7.239	0.15	1.524	0.20	7.70	0.75	4.831	0.15	1.575	0.20	7.70	0.85
XXIII	12	96	1.446	6.75	1.251	7.35	9.25	1.20	1.436	7.75	1.288	8.35	10.20	1.25
XXIV	8	96	1.467	7.85	1.600	8.45	9.20	1.10	1.436	8.95	1.707	9.30	10.20	1.20
XXV	4	95.5	1.518	5.60	1.846	5.80	8.40	1.10	1.477	7.00	1.815	8.00	9.15	1.20
XXVI	0	96	1.477	7.70	1.969	8.40	9.10	2.00	1.456	9.35	1.822	9.85	10.05	2.75

(Continued)

(January 19. 1926)

Enzyme solution	Preserving days	Time allowed for permeation (Hr)	For 15.5 hours						For 35 hours					
			Starch		Soluble starch		Maltose	Sucrose	Starch		Soluble starch		Maltose	Sucrose
			Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)
Original substrate only	—	—	5.692	0.20	1.522	0.25	—	—	5.487	0.20	1.522	0.25	—	—
Control	—	—	5.030	0.20	1.502	0.25	7.45	0.75	5.908	0.20	1.485	0.25	7.40	0.75
XXVII	35	121	1.182	8.20	1.182	9.00	9.05	1.05	1.173	9.40	1.272	10.40	10.80	1.25
XXVIII	31	95	1.194	8.95	1.190	8.65	8.75	0.80	1.198	10.85	1.251	9.85	—	0.90
XXIX	27	96	1.178	8.50	1.190	8.25	8.45	0.75	1.151	9.95	1.276	9.50	8.90	0.75
XXX	22	120	1.194	9.10	1.196	9.00	9.30	0.75	1.151	11.10	1.262	10.50	10.95	0.75
XXXI	18	96.5	1.957	6.35	1.132	8.35	8.90	0.75	1.569	8.05	1.190	10.10	9.95	0.75
XXXII	13	119.5	5.456	4.60	1.132	8.65	8.60	0.75	3.497	5.85	1.169	9.80	9.90	0.75
XXXIII	8	120	1.861	8.35	1.130	10.15	9.65	1.00	1.436	10.60	1.171	11.75	11.10	1.25
XXXIV	4	96	5.471	4.90	1.198	8.60	9.30	0.75	3.889	6.70	1.179	9.40	10.55	0.75
XXXV	0	96	3.758	5.00	1.200	8.70	9.40	0.75	3.569	6.80	1.196	9.50	10.90	0.75

Table 15. Appearance of the reserved outer solution

Knzyme solution	Date of renewal	Time allowed for permeation	Date of observation							
			Oct. 20. 1925	Oct. 26. 1925	Nov. 10. 1925	Nov. 27. 1925	Dec. 10. 1925	Jan. 19. 1926	Feb. 10. 1026	Feb. 17. 1926
I	Oct. 3. 1925	50	Slightly white turbid	Very slightly white turbid	Transparent	Transparent	—	—	—	—
II	Oct. 5. 1925	48	"	"	"	"	—	—	—	—
III	Oct. 7. 1925	48	"	Slightly white turbid	Nearly Vransparent	Very slightly white turbid	—	—	—	—
IV	Oct. 9. 1925	49	"	"	Transparent	Transparent	—	—	—	—
V	Oct. 10. 1925	25	"	"	"	"	—	—	—	—
VI	Oct. 12. 1925	43.5	"	"	"	"	—	—	—	—
VII	Oct. 13. 1925	25	"	"	"	Very slightly white turbid	—	—	—	—
VIII	Oct. 14. 1925	24	Very slightly white turbid	"	"	"	—	—	—	—
IX	Oct. 15. 1925	24	Transparent	"	"	Transparent	—	—	—	—

X	Oct. 16. 1925	26	"	"	Nearly trans-parent	Very slightly white turbid	—	—	—	—
XI	Oct. 19. 1925	76.5	"	"	Trans-parent	"	—	—	—	—
XII	Oct. 20. 1925	24	"	Trans-parent	"	"	—	—	—	—
XIII	Oct. 22. 1925	43	—	"	"	Trans-parent	—	—	—	—
XIV	Oct. 23. 1925	24	—	"	"	Very slightly white turbid	—	—	—	—
XV	Oct. 28. 1925	123	—	—	Nearly Trans-parent	"	—	—	—	—
XVI	Oct. 30. 1925	44	—	—	"	"	—	—	—	—
XVII	Nov. 3. 1925	95	—	—	"	Trans-rarent	—	—	—	—
XVIII	Nov. 7. 1925	95	—	—	Trans-parent	Very slightly white turbid	—	—	—	—
XIX	Nov. 11 1925	95	—	—	Slightly white turbid	—	—	—	—	—
XX	Nov. 16 1925	121	—	—	"	—	—	—	—	—

XXI	Nov. 20. 1925	97	—	—	"	—	—	—	—	—
XXII	Nov. 24. 1925	96	—	—	"	—	—	—	—	—
XXIII	Nov. 28. 1925	96	—	—	—	—	Trans-parent	—	—	—
XXIV	Dec. 2. 1925	96	—	—	—	—	"	—	—	—
XXV	Dec. 6. 1925	95.5	—	—	—	—	Slightly white turbid	—	—	—
XXVI	Dec. 10. 1925	96	—	—	—	—	Trans-parent	—	—	—
XXVII	Dec. 15. 1925	121	—	—	—	—	—	Very slightly white turbid	—	—
XXVIII	Dec. 19. 1925	95	—	—	—	—	—	Trans-parent	—	—
XXIX	Dec. 23. 1925	96	—	—	—	—	—	"	—	—
XXX	Dec. 28. 1925	120	—	—	—	—	—	"	—	—
XXXI	Jan. 1. 1926	96 5	—	—	—	—	—	Very slightly white turbid	—	—

XXXII	Jan. 6. 1926	119.5	—	—	—	—	—	Transparent	—	—
XXXIII	Jan. 11. 1926	120	—	—	—	—	—	"	—	—
XXXIV	Jan. 15. 1926	96	—	—	—	—	—	Very slightly white turbid	—	—
XXXV	Jan. 19. 1926	96	—	—	—	—	—	Transparent	—	—
XXXVI	Jan. 23. 1926	96	—	—	—	—	—	—	Transparent	Transparent
XXXVII	Jan. 27. 1926	96.5	—	—	—	—	—	—	"	"
XXXVIII	Feb. 1. 1926	95.5	—	—	—	—	—	—	"	"
XXXIX	Feb. 5. 1926	97.5	—	—	—	—	—	—	Slightly white turbid	Almost transparent
XL	Feb. 9. 1926	96.5	—	—	—	—	—	—	"	Slightly white turbid

Table 16. Relation between the appearance and the diastatic power of the reserved outer and inner solution in dialysis of "Koji-Diastase"

(16 hours digestion)

Enzyme solution	Days allowed for standing after renewal of the outer solution	Starch			Soluble starch		Maltose	Sucrose
		Appearance	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)
Original substrate, only	—	—	7.871	0.15	1.608	0.20	—	—
Control (Steeped solution of shark's air bladder, by 10 % alcohol)	—	—	6.213	0.15	1.524	0.20	7.70	0.75
XXIII (96)	12	Transparent	1.446	6.75	1.251	7.35	9.25	1.20
XXIV (96)	8	"	1.467	7.85	1.600	8.45	9.20	1.10
XXV (95.5)	4	Slightly white turbid	1.518	5.90	1.846	5.80	8.40	1.10
XXVI (96)	0	Transparent	1.479	7.70	1.969	8.40	9.10	2.00
(XXVI') (Inner residual solution)	—	"	1.467	13.30	1.487	13.10	13.15	8.45

Table 17. pH value of the outer solution

(February 4. 1926)

Sample	Time allowed for permeation (Hr)	pH value	Difference compared with the value of the steeped solution of shark's air bladder prepared by 10% alcohol	Difference compared with the value of the original enzyme solution of purified "Koji-Diastase"
Distilled water	—	6.20	—	—
10% alcohol solution	—	6.20	—	—
Steeped solution of shark's air bladder prepared by 10% alcohol	48	6.10	—	—
Original enzyme solution of purified "Koji-Diastase"	—	6.90	+ 0.80	—
I	50	6.55	+ 0.45	- 0.35
II	48	6.65	+ 0.55	- 0.25
III	48	6.70	+ 0.60	- 0.20
IV	48	6.65	+ 0.55	- 0.25
V+VI	33.5	6.75	+ 0.65	- 0.15
VII+VIII	24.5	6.65	+ 0.55	- 0.25
IX+X	25	6.55	+ 0.45	- 0.35
XI	76.5	6.50	+ 0.40	- 0.40
XII	24	6.45	+ 0.35	- 0.45
XIII	43	6.50	+ 0.40	- 0.40
XIV	24	6.40	+ 0.30	- 0.50
XV	12.3	6.50	+ 0.40	- 0.40
XVI	44	6.65	+ 0.55	- 0.25
XVII	95	6.65	+ 0.55	- 0.25
XVIII	95	6.65	+ 0.55	- 0.25
XIX	95	6.50	+ 0.40	- 0.40
XX	12.1	6.30	+ 0.20	- 0.60
XXV	95.5	6.25	+ 0.15	- 0.65
XVI	96	6.20	+ 0.10	- 0.70
(XXVI′) (Inner residual solution)	—	6.50	+ 0.40	- 0.40

Table 18. Change of pH value of the preserved outer solution diluted at the same rate of diffusing hour

	pH value		
	Original outer solution	When the previous sample was diluted with distilled water at the rate of 19.2 hours permeation	Difference as compared with the value of the control
(Date of measurement)	(February 4. 1926)	(February 9. 1926)	—
Control (Steeped solution of shark's air bladder, by 10% alcohol)	6.10	6.15	—
I	6.55	6.65	+0.50
II	6.65	6.70	+0.55
III	6.70	6.65	+0.50
IV	6.65	6.60	+0.45
V+VI	6.75	6.70	+0.55
VII+VIII	6.65	6.75	+0.60
IX+X	6.55	6.70	+0.55
XI	6.50	6.40	+0.25
XII	6.45	6.40	+0.25
XIII	6.50	6.50	+0.35
XIV	6.40	6.50	+0.35
XV	6.50	6.30	+0.15
XVI	6.65	6.55	+0.40
XVII	6.65	6.60	+0.45
XVIII	6.65	6.65	+0.50
XIX	6.50	6.45	+0.30
XX	6.30	6.20	+0.05
XXI	6.35	6.20	+0.05
XXII	6.40	6.30	+0.15
XXIII	6.55	6.40	+0.25
XXIV	6.45	6.40	+0.25
XXV	6.25	6.30	+0.15
XXVI	6.20	6.20	+0.05

Table 19. Change of pH value during the outer solution is preserved

Date of measurement	Feb. 4. 1926	Feb. 9. 1926	Feb. 13. 1926	Feb. 17. 1926	Mar. 9. 1926	Mar. 16. 1926
Control	6.10	6.10	6.10	6.10	6.10	6.10
I	6.55	6.65	6.65	9.75	6.50	6.50
II	6.65	6.70	6.75	6.80	6.80	6.75
III	6.70	6.65	6.60	6.80	6.65	6.50
IV	6.65	6.60	6.60	6.75	6.60	6.45
V+VI	6.75	6.70	6.70	6.85	6.80	6.70
VII+VIII	6.65	6.75	6.75	6.85	6.90	6.95
IX+X	6.55	6.70	6.70	6.80	6.85	6.90
XI	6.50	6.40	6.30	6.60	6.65	6.70
XII	6.45	6.40	6.40	6.70	6.85	6.90
XIII	6.50	6.50	6.50	6.75	6.85	6.90
XIV	6.40	6.50	6.60	6.75	6.85	6.90
XV	6.50	6.30	6.20	6.60	6.65	6.75
XVI	6.65	6.55	6.50	6.70	6.85	6.90
XVII	6.65	6.60	6.50	6.70	6.80	6.85
XVIII	6.65	6.65	6.60	6.70	6.80	6.85
XIX	6.50	6.45	6.40	6.60	6.70	6.75
XX	6.30	6.25	6.25	6.35	6.40	6.45

Table 20. Optimum pH value for several enzymes

Name of enzyme	Raw material of the enzyme	Investigator	Optimum pH value	Remark
Amylase	Koji	SHERMAN	4.8	—
"	Koji	OSHIMA and ITAYA	4.5—5.2	—
"	Malt	SHERMAN	4.5	—
"	Malt	EULER	5.0	—
Maltase	Ferment	MICHAELIS	6.1—6.8	—
Invertase	Ferment	SÖRENSEN	4.4—4.6	—
"	Ferment	MICHAELIS	4.2	—
Pepsin	—	MICHAELIS	1.4	—
Trypsin	—	MICHAELIS	9.7	—
Protein splitting enzyme	*Aspergillus orizae*	OKADA	5.07	—

Table 21. pH value of 2 % solution of substrate used in this experiment

Substrate	pH value	Remarks (Toluene was added as an antiseptic)
Starch (potato)	6.40—7.30	There seems to be an inclination of decrease of pH value of starch paste as a consequence of its preservation
Soluble starch (potato)	6.10—6.00	There seems to be almost no decrease in the pH value of soluble starch as a consequence of its preservation
Dextrine	6.40—6.30	There seems to be no change in the pH value of the aqueous solution by its preservation
Maltose	6.20—6.00	"
Sucrose	6.30—6.20	"
Distilled water	6.20	——

Table 22. Relation between the enzymic power and the pH value changing. 1.
(16 houra action)

(February 9. 1926)

Enzyme solution	pH value of outer solution (Rate of 19.2 hrs. permeation)	Starch				Soluble starch			
		Vis-cosity	N/100 IIK solution 1 drop, for 2 c.c.	N/10 IIK solution 1 drop, for 2 c c.	$KMnO_4$ solution (Value deducted ½ maltase power) (c.c.)	Vis-cosity	N/100 IIK solution, 1 drop, for 2 c.c.	N/10 IIK solution, 1 drop, for 2 c.c.	$KMnO_4$ solution (Value deducted ½ maltase power) (c.c.)
Control	6.15	5.823	B Shade 1	B Shade 2	0.15 (—)	1.477	B Shade 1	B Shade 2	0.25 (—)
I	6.65	1.508	B Tint 1	VB Shade 2	6.00 (5.50)	1.210	VO Tint 2	V Shade 2	6.15 (5.55)
II	**6.70**	2.501	"	B Shade 2	6.15 (5.30)	1.284	No color	OR Shade 1	7.40 (6.45)
III	6.65	3.287	B Shade 1	"	6.35 (5.30)	1.268	"	"	7.50 (6.35)
IV	6.60	4.490	"	"	5.25 (4.45)	1.251	"	R Shade 2	7.25 (6.35)
V+VI	6.70	4.129	"	"	3.85 (3.45)	1.196	"	"	4.40 (3.90)
VII+VIII	**6.75**	3.483	"	"	2.30 (1.90)	1.225	V Tint 1	RV Shade 2	2.60 (2.10)
IX+X	6.70	3.943	B Tint 2	"	1.90 (1.65)	1.223	"	VB Shade 2	1.90 (1.55)

XI	6.40	4.332	"	"	2.00 (1.75)	1.229	V Tint 2	"	2.00 (1.65)
XII	6.40	3.442	B Shade 1	"	1.90 (1.50)	1.225	"	V Shade 2	2.10 (1.60)
XIII	**6.50**	3.356	"	"	1.70 (1.35)	1.253	V Tint 1	"	1.75 (1.30)
XIV	6.50	3.535	"	"	1.75 (1.35)	1.184	"	RV Shade 2	1.80 (1.30)
XV	6.30	4.010	B Tint 2	"	5.45 (4.55)	1.190	V Tint 2	OR Shade 2	7.80 (6.80)
XVI	6.55	3.411	B Shade 1	"	4.95 (4.20)	1.179	"	VR Shade 2	7.15 (6.30)
XVII	6.60	4.010	B Tint 2	"	3.20 (2.70)	1.204	VB Tint 2	"	4.20 (3.60)
XVIII	**6.65**	3.587	B Shade 1	"	1.70 (1.40)	1.239	V Tint 1	RV Shade 2	3.00 (2.60)
XIX	6.45	3.411	"	"	3.10 (2.65)	1.188	B Tint 2	V Shade 2	4.60 (4.05)
XX	6.20	3.403	"	"	1.75 (1.55)	1.237	B Tint 1	BV Shade 1	2.85 (2.55)
XXI+XXII	6.25	3.643	"	"	1.75 (1.40)	1.214	B Shade 1	VB Shade 1	2.30 (1.95)

Table 23. Relation between the enzymic power and the pH value changing. 2.

Enzyme solution	pH value	Soluble starch + Dextrine				Dextrine	Maltose	Sucrose
		Viscosity	N/100 IIK solution, 1 drop	N/10 IIK solution, 1 drop	$KMnO_4$ solution (Value deducted $\frac{1}{2}$ maltase power) (c.c.)	$KMnO_4$ solution (Value deducted $\frac{1}{2}$ maltase power) (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)
Control	6.15	1.477	B Shade 1	B Shade 2	0.45 (—)	0.70 (—)	7.20 (0)	0.50 (0)
I	6.65	—	—	—	—	4.65 (3.60)	7.90 (0.70)	0.80 (0.30)
II	**6.70**	1.227	No color	VR Tint 2	6.35 (5.25)	5.50 (4.15)	8.55 (1.35)	—
III	6.65	—	—	—	—	5.70 (4.15)	8.95 (4.15)	0.70 (0.20)
IV	6.60	1.178	No color	VR Tint 2	6.85 (5.75)	5.30 (3.95)	8.50 (1.30)	—
V+VI	6.70	—	—	—	—	4.40 (3.45)	7.75 (0.55)	0.60 (0.10)
VII+VIII	**6.75**	1.108	No color	V Shade 1	3.75 (3.05)	3.75 (2.80)	7.75 (0.55)	—
IX+X	6.70	—	—	—	—	3.10 (2.30)	7.45 (0.25)	0.55 (0.05)
XI	6.40	1.149	No color	V Shade 1	4.00 (3.45)	3.25 (2.45)	7.40 (0.20)	—
XII	6.40	—	—	—	—	3.15 (2.20)	7.70 (0.50)	0.55 (0.05)
XIII	**6.50**	1.147	No color	V Shade 1	3.15 (2.50)	2.30 (1.40)	7.60 (0.40)	—
XIV	6.50	—	—	—	—	2.80 (1.85)	7.70 (0.50)	0.50 (0)
XV	6.30	1.149	No color	VR Tint 2	7.00 (5.80)	5.50 (4.05)	8.70 (1.50)	—
XVI	6.55	—	—	—	—	5.20 (3.90)	8.40 (1.20)	0.55 (0.05)
XVII	6.60	1.179	No color	VR Shade 2	5.50 (4.70)	4.30 (3.25)	7.90 (0.70)	—
XVIII	**6.65**	—	—	—	—	2.80 (1.95)	7.55 (0.35)	0.50 (0)
XIX	6.45	1.186	No color	VR Shade 2	4.85 (4.10)	4.20 (3.20)	7.85 (0.65)	—
XX	6.20	—	—	—	—	2.80 (2.05)	7.30 (0.10)	0.85 (0.35)
XXI+XXII	6.25	1.145	B Tint 2	V Shade 2	4.10 (3.45)	3.15 (1.25)	7.60 (0.40)	—

Table 24. Relation between the enzymic power and changes of the pH value in enzyme solution

(February 6. 1926)

Enzyme solution	pH value of dialyzate (Rate of 19.2 hours permeation)	Starch						Soluble starch		
		pH value before action	pH value after action	Viscosity after action	N/100IIK solution, 1 drop	N/10 IIK solution, 1 drop	$KMnO_4$ solution (c.c.)	pH value before action	pH value after action	$KMnO_4$ solution (c.c.)
Control	6.15	6.30	6.30	4.972	B Shade 1	B Shade 2	0.25	6.10	6.10	0.25
I	6.65	6.70	7.10	1.272	"	RV Shade 1	9.15	6.10	7.40	8.20
II	6.70	—	7.30	1.448	"	V Shade 1	9.30	—	6.00	10.30
III	6.65	—	7.00	2.146	"	"	8.45	—	6.00	10.35
IV	6.60	—	7.10	2.365	"	"	7.95	—	6.00	10.00
V+VI	6.70	—	7.10	2.845	"	"	6.80	—	6.10	8.75
VII+VIII	6.75	—	7.20	3.026	"	"	4.05	—	6.10	4.70
IX+X	6.70	6.75	7.10	2.837	"	"	3.05	6.10	6.00	4.00
XI	6.40	—	7.00	2.903	"	"	4.35	—	6.00	4.60
XII	6.40	—	6.90	2.835	"	"	3.75	—	6.00	4.30
XIII	6.50	—	7.20	3.327	"	"	3.25	—	6.10	3.45
XIV	6.50	—	7.00	2.884	"	"	2.55	—	0.10	3.40
XV	6.30	—	6.90	3.499	"	"	6.80	—	6.40	10.20
XVI	6.55	—	6.80	3.282	"	"	7.60	—	6.60	9.90
XVII	6.60	—	6.90	3.054	"	"	5.80	—	6.10	8.00
XVIII	6.65	—	7.00	2.976	"	"	4.30	—	7.30	5.20
XIX	6.45	—	6.90	2.825	"	"	5.35	—	7.40	6.80
XX	6.20	—	6.90	2.919	"	"	3.30	—	7.30	4.90
XXI+XXII	6.25	6.40	6.80	3.007	"	"	4.55	6.10	6.00	4.65

(Continued)

Enzyme solution	pH value of outer solution	Dextrine			Maltose			Sucrose		
		pH value before action	pH value after action	$KMnO_4$ solution (c.c.)	pH value before action	pH value after action	$KMnO_4$ solution (c.c.)	pH value before action	pH value after action	$KMnO_4$ solution (c.c.)
Control	6.15	6.00	7.20	0.85	6.00	6.10	7.75	6.00	7.30	0.55
I	6.65	6.45	7.30	4.25	6.35	6.60	9.05	6.60	6.90	0.65
II	6.70	—	7.00	7.50	—	6.70	10.35	—	—	—
III	6.65	—	6.75	7.25	—	6.50	10.85	—	7.30	1.10
IV	6.60	—	7.20	6.05	—	6.90	9.75	—	—	—
V+VI	6.70	—	6.85	5.45	—	7.00	8.25	—	7.10	0.80
VII+VIII	6.75	—	6.50	5.45	—	6.25	8.30	—	—	—
IX+X	6.70	6.50	6.55	5.15	6.40	6.40	8.35	6.70	6.90	0.75
XI	6.40	—	6.50	5.55	—	6.60	8.40	—	—	—
XII	6.40	—	6.40	5.20	—	6.70	8.25	—	6.85	0.80
XIII	6.50	—	7.40	3.45	—	6.40	8.35	—	—	—
XIV	6.50	—	6.50	4.35	—	6.50	8.40	—	7.35	0.55
XV	6.30	—	6.80	6.25	—	6.45	10.70	—	—	—
XVI	6.55	—	6.30	7.05	—	6.20	10.25	—	7.60	2.30
XVII	6.60	—	6.45	6.25	—	6.50	9.10	—	—	—
XVIII	6.65	—	7.00	4.75	—	6.30	8.25	—	7.35	0.55
XIX	6.45	—	6.40	6.50	—	6.20	9.05	—	—	—
XX	6.20	—	6.40	5.35	—	6.20	8.25	—	7.40	0.65
XXI+XXII	6.25	6.10	6.40	4.50	6.10	6.20	8.45	6.10	—	—

Table 25. Comparison of the enzymic power between the permeated and the original enzyme solution

Enzyme solution	For 15 hours					For 40 hours			
	Starch		Soluble starch	Maltose	Sucrose	Starch	Soluble starch	Maltose	Sucrose
	Viscosity	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)
a	1.819	4.80	8.25	8.05	0.95	7.70	9.95	8.90	1.45
b	13.716	1.75	4.40	7.80	0.80	4.30	7.90	8.40	0.80
c (Control)	Above 70.846	0.15	0.20	7.45	0.80	0.15	0.20	7.45	0.80

Table 26. Method of dialysis

Membrane	"Koji-Diastase" enzyme substance (g.)	Solvent (c.c.)	Toluene (c.c.)	Dialysis area of membrane (cm^2)	Number of articles
1) Air bladder	0.5	Distilled water (50)	2	58.38	3
2) "	0.5	10 % alcohol (50)	0	58.38	3
3) Intestine membrane	1.0	Distilled water (100)	3	93.92	1
4) "	1.0	10 % alcohol (100)	0	93.92	2
5) Bladder	1.5	Distilled water (150)	4	138.23	1
6) "	1.5	10 % alcohol (150)	0	138.23	1

Table 27. Renewal of the outer solution

Period	I	II	III
Number of renewal	1 2 3 4 5	6 7 8 9 10 11 12 13 14 15	16 17 18 19 20 21 22 23 24 25
Number of days allowed for dialysis	2 4 7 10 13	16 18 22 25 28 31 34 37 41 45	49 53 57 63 73 95 102 109 116 126
Hours of diffusion	48 48 72 72 72	72 48 96 72 72 72 72 72 96 96	96 96 96 144 240 328 168 168 168 240

Table 28. Change of volume of the inner solution

	Volume of enzyme sol. (Jan. 18. 1927, c.c.)	Total days of dialysis (Volume increase of inner residual solution ceased almost)	Volume of inner residual sol. (c.c.)	Total increase of volume of inner residual solution (c.c.)	Mean value of increase per 100 square cm. of dialysis membrane in one day (c.c.)
1) Air-bladder (Dist. water)	150	28	223 } 440	73 } 140	1.490 } 2.615
2) Air-bladder (10 % alco. sol.)	150	34	217	67	1.125
3) Intestine-memb. (Dist. water)	100	25	113 } 446	13 } 146	0.554 } 2.637
4) Intestine-memb. (10 % alco. sol.)	200	34	333	133	2.083
5) Bladder (Dist. water)	150	149 (not ceased yet)	895 } 1520	745 } 1220	3.617 } 5.923
6) Bladder (10 % alco. sol.)	150	149 (not ceased yet)	625	475	2.306

Table 29. Power of carbohydrate-splitting enzyme in the outer solution

Part of the 2nd time renewal (20 hours reaction; $KMnO_4$ solution, c.c.)

Outer solution / Substrate (Kind of enzyme)	Air bladder		Intestine membrance		Bladder		Control		
	1 (Distilled water)	2 (10 % alcohol solution)	3 (Distilled water)	4 (10 % alcohol solution)	5 (Distilled water)	6 (10 % alcohol solution)	Distilled water	10 % alcohol solution	Original enzyme solution
Potato starch (Diastase)	10.90	9.15	10.20	2.30	7.50	1.90	0.15	0.15	—
Maltose (Maltase)	11.10	11.00	11.05	9.00	9.50	9.10	8.50	8.50	—
Sucrose (Invertase)	2.60	2.60	2.60	1.40	1.75	1.45	1.50	1.50	—
pH value (Brom-thymolblue)	6.60	6.95	6.85	6.80	6.85	6.75	6.00	6.60	7.15

(Continued)

Part of the 8th time renewal (45 hours reaction)

Outer solution / Substrate	Air bladder				Intestine membrane				Bladder				Control (Distilled water)	
	1		2		3		4		5		6			
	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)
Potato starch	1.062	13.70	2.315	12.10	2.274	10.80	4.467	0.85	3.838	2.30	5.543	0.50	—	0.20
Maltose	—	13.40	—	12.10	—	12.50	—	7.50	—	13.60	—	6.65	—	7.60
Sucrose	—	5.55	—	2.70	—	2.35	—	1.60	—	3.30	—	1.70	—	1.40

(Continued)

Part of the 12th time renewal (21.5 hours reaction, 20 c.c. of 2 % solution of substrate, 5 c.c. of enzyme solution and 2 c.c. of toluene were mixed)

Outer solution / sub-strate	Air bladder				Intestine membrane				Bladder				Control (Distilled water)		Original substrate
	1		2		3		4		5		6				
	Vis-cosity	$KMnO_4$ solution (c.c.)	Vis-cosity	$KMnO_4$ solution (c.c.)	Vis-cosity	$KMnO_4$ solution (c.c.)	Vis-cosity	$KMnO_4$ solution (c.c.)	Vis-cosity	$KMnO_4$ solution (c.c.)	Vis-cosity	$KMnO_4$ solution (c.c.)	Vis-cosity	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)
Potato starch	1.127	11.65	1.170	10.85	1.131	10.65	1.198	6.55	1.470	1.90	1.482	1.45	—	0.25	0.25
Maltose	—	10.00	—	11.05	—	8.60	—	8.45	—	7.95	—	7.90	—	6.80	—
Sucrose	—	3.35	—	2.35	—	2.90	—	1.20	—	0.85	—	0.80	—	0.80	—
Glucose	—	2.65 (?)	—	2.70 (?)	—	2.40 (?)	—	3.00 (?)	—	2.45 (?)	—	2.60 (?)	—	14.20	14.15
pH value	(6.15)		(6.50)		(6.30)		(6.40)		(6.20)		(6.45)		(6.00)		—

76 hours reaction

Outer solution / Substrate	Air bladder		Intestine membrane		Bladder		Control (Distilled water)	Original substrate
	1	2	3	4	5	6		
	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)
Potato starch	12.00	12.30	10.50	8.30	3.25	2.95	0.25	—
Maltose	12.20	12.70	10.70	8.60	8.10	7.90	7.50	—
Sucrose	4.90	3.40	5.10	1.30	0.80	0.80	0.80	—
Glucose	14.35	14.60	14.20	13.75	13.60	14.10	13.80	14.15

(Continued)

Part of the 17th time renewal (95 hours reaction, 25 c.c. of 2 % solution of substrate, 5 c.c. of enzyme solution and 2 c.c. of toluene were mixed)

Outer solution / Substrate	Air bladder				Intestine membrane				Bladder				Control (Distilled water)	
	1		2		3		4		5		6			
	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)
Potato starch	1.235	6.50	1.291	10.40	1.618	8.30	1.883	6.45	7.492	1.90	14.071	1.35	>73.096	0.15

(Continued)

Part of the 25th time renewal (25 c.c. of 2 % solution of substrate, 5 c.c. of enzyme solution and 2 c.c. of toluene were mixed, time used for reaction was 20 hours for starch liquefaction, 45 hrs. for soluble starch, maltose and sucrose decomposition, and 67 hrs. for starch saccharification)

Outer solution / Substrate	Air bladder				Intestine membrane				Bladder				Control (Distilled water)	
	1		2		3		4		5		6			
	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)
Potato starch	19.472	7.05	21.970	6.95	12.102	7.40	11.249	9.30	9.773	7.40	12.193	1.25	10.172	0.15
Soluble starch	—	10.00	—	11.20	—	11.10	—	10.50	—	10.60	—	0.94	—	0.30
Maltose	—	10.25	—	11.20	—	10.30	—	9.80	—	9.60	—	8.00	—	7.70
Sucrose	—	1.55	—	1.20	—	2.40	—	1.35	—	2.50	—	1.50	—	0.80

On inner residual solution

Inner solution / Substrate	Air bladder				Intestine membrane				Bladder				Control (Distilled water)	
	1		2		3		4		5		6			
	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)	Viscosity	$KMnO_4$ solution (c.c.)
Potato starch	2.453	13.70	4.798	13.50	1.101	14.30	1.127	13.90	1.147	13.20	1.185	12.00	—	0.15
Soluble starch	—	13.80	—	13.30	—	14.15	—	14.65	—	13.30	—	14.35	—	0.30
Maltose	—	12.25	—	13.30	—	12.30	—	13.15	—	13.30	—	13.80	—	7.70
Sucrose	—	9.60	—	7.20	—	13.50	—	9.40	—	14.20	—	9.40	—	0.80

Comparison of starch liquefaction. (Viscosity)

	Air bladder		Intestine membrane		Bladder	
	Distilled water solution	10 % alcoholic water solution	Distilled water solution	10 % alcoholic water solution	Distilled water solution	10 % alcoholic water solution
Outer solution	1.980	2.396	1.401	2.051	1.340	3.015
Inner residual solution	1.065	1.086	1.056	1.078	1.086	1.127

Table 30. Power of protease in the outer solution

(Casein remained, g.)

		Air bladder		Intestine membrane		Bladder	
		Distilled water solution	10 % alcoholic water solution	Distilled water solution	10 % alcoholic water solution	Distilled water solution	10 % alcoholic water solution
Pepsin	Outer solution	0.3990	0.3985	0.3980	0.3960	0.4005	0.3950
	Inner residual solution	0.3935	0.3870	0.3565	0.3565	0.3710	0.3785
Trypsin	Outer solution	0.3795	0.3680	0.3740	0.3740	0.4085	0.3730
	Inner residual solution	0.3690	0.3655	0.3645	0.3580	0.3625	0.3615

Table 31. Power of lipase in the outer solution

(1/10 normal NaOH solution, c.c.)

	Air bladder		Intestine membrane		Bladder		
	Distilled water solution	10 % alcoholic water solution	Distilled water solution	10 % alcoholic water solution	Distilled water solution	10 % alcoholic water solution	Control
Outer solution	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Inner residual solution	0.80	0.90	0.80	0.95	0.80	0.85	0.75

Table 32. Change of pH value of the outer and inner solution

Change of pH value of control solution

	Distlled water	10 % alcoholic water solution	Original enzyme solution	Time of the measurement
pH	6.00	6.00	7.25	Jan. 22. 1927– May 2. 1927

Change of pH value of the outer solution

Repeated number of renewal of the outer solution	Date of measurement	Air bladder		Intestine membrane		Bladder	
		1 (Distilled water solution)	2 (10% alcoholic water solution)	3 (Distilled water solution)	4 (10% alcoholic water solution)	5 (Distilled water solution)	6 (10% alcoholic water solution)
2nd	Jan. 22. 1927	7.00	6.95	6.95	6.90	6.95	6.85
9th	Feb. 12. "	6.65	6.70	6.70	6.50	6.45	6.55
"	Feb. 21. "	6.85	6.90	6.90	6.60	6.55	6.70
12th	" " "	6.25	6.60	6.40	6.50	6.30	6.55
"	" 24. "	6.35	6.75	6.55	6.65	6.40	6.70
13th	" " "	6.25	6.60	6.25	6.50	6.25	6.55
"	" 28. "	6.50	6.75	6.50	6.60	6.45	6.65
14th	" " "	6.20	6.60	6.20	6.50	6.15	6.40
"	Mar. 4. "	6.30	6.70	6.40	6.60	6.20	6.55
15th	" " "	6.10	6.55	6.15	6.50	6.10	6.30
"	" 8. "	6.20	6.65	6.25	6.60	6.20	6.45
16th	" " "	6.10	6.40	6.15	6.40	6.10	6.25
"	" 12. "	6.20	6.55	6.25	6.50	9.20	6.35
17th	" " "	6.10	6.35	6.10	6.40	6.10	6.25
"	" 16. "	6.15	6.50	6.20	6.50	6.20	6.35
18th	" " "	6.10	6.25	6.10	6.40	6.10	6.25
"	" 22. "	6.10	6.35	6.15	6.45	6.15	6.35
19th	" " "	6.10	6.20	6.10	6.40	6.10	6.20
"	Apr. 1. "	6.10	6.30	6.25	6.55	6.20	6.35
20th	" " "	6.10	6.20	6.10	6.40	6.10	6.20
"	" 23. "	6.10	6.25	6.15	6.55	6.10	6.20
21th	" " "	6.10	6.20	6.10	6.40	6.10	6.20
22th	" 30. "	6.10	6.20	6.10	6.40	6.10	6.20
"	May. 7. "	6.10	6.25	6.20	6.55	6.20	6.30
23th	" " "	6.20	6.15	6.20	6.35	6.15	6.20
24th	" 14. "	6.15	6.10	6.15	6.35	6.15	6.20
25th	" 24. "	6.10	6.10	6.15	6.30	6.20	6.15

Change of pH value of the inner solution

	Air bladder		Intestine membrane		Bladder	
	1	2	3	4	5	6
Original enzyme solution	7.25	7.25	7.25	7.25	7.25	7.25
Inner solution (Afther 25 renewals of the outer solution)	6.15	6.10	6.40	6.45	6.25	6.30

Table 33. Percentage of ash in purified "Koji-Diastase"

"Koji-Diastase"	No. 2	No. 3	No. 4	No. 5	No. 6	"Moto-Koji" (KATAOKA)	Mean value of No. 2—No. 6	Total mean value
Ash (%)	11.387	11.532	10.811	8.857	12.611	4.785	11.050	10.006

Table 34. Distribution of ash ingredient in the outer and inner solutions

	Air bladder		Intestine membrane		Bladder		(Sum) and mean	
	1 (Distilled water)	2 (10 % alcoholic water solution)	3 (Distilled water)	4 (10 % alcoholic water solution)	5 (Distilled water)	6 (10 % alcoholic water solution)	(Distilled water)	(10 % alcoholic water solution)
Original "Koji-Diastase" enzyme substance	(g.) 0.16575	(g.) 0.16575	(g.) 0.11050	(g.) 0.22100	(g.) 0.16575	(g.) 0.16575	(g.) 0.44200	(g.) 0.35250
I (5 renewals, wanted 13 days)	0.20685		0.21454		0.22351		(0.64490)...0.21497	
II (10 renewals, wanted 29 days)	0.07026		0.05146		0.03864		(0.16036)...0.05812	
III (10 renewals, wanted 81 days)	0.04185		0.03423		0.02146		(0.09740)...0.03247	
Residue of "Koji-Diastase" enzyme substance, after the dialysis	0.01307		0.02830		0.04408		(0.08447)...0.02816	

Table 35. Relation between the ash content and the diastase enzymic power in the inner solution

Inner residual solution	Air bladder		Intestine membrane		Bladder		Control
	1. (Distilled water)	2. (10 % alcoholic water solution)	3. (Distilled water)	4. (10 % alcoholic water solution)	5. (Distilled water)	6. (10 % alcoholic water solution)	
Ash (g.)	0.00649	0.00658	0.00468	0.02362	0.01945	0.02463	—
Starch Viscosity	1.924	1.503	1.310	1.005	1.036	0.995	21.015
Starch $KMnO_4$ solution (c.c.)	7.35	9.30	9.40	13.90	14.00	15.15	0.15
Soluble starch ($KMnO_4$ solution, c.c.)	10.40	12.15	12.30	14.50	13.90	15.25	0.35

(Continued)

(Starch liquefying action, viscosity)

Time allowed for reaction (Sec.) / Inner residual solution	0 (Control)	1	15	30	45	60	75	90	1440
1 (Distilled water sol.)	15.821	12.990	14.619	15.025	14.928	13.909	13.198	11.817	1.924
2 (10 % alcoholic water sol.)	15.821	11.188	4.005	1.807	1.330	1.264	1.294	1.320	1.503
3 (Distilled water sol.)	15.821	17.503	18.416	—	15.868	12.761	10.771	9.131	1.310
4 (10 % alcoholic water sol.)	15.821	17.162	8.568	4.102	2.701	2.061	1.594	1.289	1.005
5 (Distilled water sol.)	15.821	15.381	5.929	2.791	1.827	1.574	1.350	1.228	1.036
6 (10 % alcoholic water sol.)	15.821	11.756	1.888	1.269	1.218	1.223	1.041	0.986	0.995

Table 36. Quantitative analysis of the main constituents of ash in "Koji-Diastase"

Mineral matter (g.) / Enzyme substance	Ash	Fe_2O_3	Al_2O_3	MnO	CaO	MgO	SO_3	P_2O_5	K_2O	Na_2O	SiO_2	Cl
Original enzyme substance	0.1590	0.0120	0.0115	0.0043	0.0351	0.0172	0.0126	0.0340	0.0210	0.0045	0.005	0.0059
Dialyzate I (5 renewals, wanted 13 days)	0.0993	0.0025	0.0022	0.0025	0.0221	0.0119	0.0087	0.0246	0.0153	0.0033	0.0003	0.0047
Dialyzate II (10 renewals, wanted 29 days)	0.0347	0.0015	0.0018	0.0013	0.0059	0.0038	0.0042	0.0069	0.0058	0.0014	0.0001	0.0015
Inner residual solution (after 25 renewals, wanted 123 days)	0.0510	0.0141	0.0136	0.0014	0.0128	0.0029	0.0006	0.0048	0.0005	0	0.0002	0

(Continued) percentage

Mineral matter / Enzyme substance	Ash	Fe_2O_3	Al_2O_3	MnO	CaO	MgO	SO_3	P_2O_5	K_2O	Na_2O	SiO_2	Cl
Original enzyme substance	100	7.547	7.233	2.704	22.075	10.841	7.994	21.384	13.208	2.830	0.314	3.711
Dialyzate I	100	2.518	2.216	2.518	22.256	11.984	8.761	24.773	15.408	3.323	0.302	4.532
Dialyzate II	100	4.323	5.187	3.746	17.003	10.951	12.104	19.885	16.715	4.035	0.288	4.323
Inner residual solution	100	27.647	26.667	2.745	25.098	5.686	1.176	9.412	0.980	0	0.392	0

Table 37. Influence of mineral matter upon the carbohydrate-splitting power of dialyzate

	Starch		Soluble starch	Maltose	Sucrose
	Viscosity	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)
Control 1	>73.096	0.15	0.20	7.50	0.80
a)	28.426	0.20	0.40	7.80	0.80
b)	1.352	0.75	0.50	7.75	0.80
Control 2	34.248	0.15	0.20	7.70	0.80
c)	28.305	0.20	0.90	7.60	0.80
d)	1.706	0.55	0.95	7.85	0.80
Control 3	44.284	0.15	0.75	7.75	0.80
Control 4	2.805	0.15	0.40	7.50	0.80
Control (Substrate)	>73.096	0.15	0.20	7.50	0.80

(Continued)

In case of digestion for 40 hours

	Starch	Soluble starch	Maltose	Sucrose
	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)	$KMnO_4$ solution (c.c.)
Control 1	0.15	0.20	7.50	0.80
a)	0.15	0.45	8.00	0.75
b)	0.90	0.65	7.85	0.75
Control 2	0.15	0.45	7.95	0.80
c)	0.20	2.00	7.90	0.80
d)	1.85	1.55	8.05	0.80
Control 3	0.15	1.35	8.05	0.85
Control 4	0.15	0.40	7.65	0.80
Control	0.15	0.20	7.50	0.80

Table 38. Protein reaction of the purified original "Koji-Diastase" and other enzyme substances

Kind of reaction \ Enzyme substance	"Koji-Diastase" (No. 2-No. 6)	"Koji-Diastase" (KATAOKA's "Moto-Koji")	"Malt-Diastase" (Beer)	"Germinated unhulled rice-Diastase"	"Taka-Diastase"	"Pepsinum"	Artificial, "mixed chemicals"
BIURET	+	+	+	+	+	+	+
Xanthoprotein	+	+	+	+	+	+	+
MILLON	+	(+)	+	+	+	+	(+)
ADAMKIEWICZ	+	+	+	+	+	+	+
NEUBAUER and ROHDE	+	+	+	+	+	+	+
PbS	−	−	−	−	−	−	−
MOLISCH	+	+	+	+	+	+	+
LIEBERMANN (and COLE)	+	(+)	(+)	+	+	+	+
HOPKINS and COLE	+	+	...	+	...	...	(−)
REICHER	(+)	...	...	+	...	...	(+)
PAULY's Diazo	+	+	+	+	+	+	(+)
Ninhydrin	+	+	+	+	+	+	+
PIRIA	(+)	...	...	...	...	...	(+)
MÖRNER	(+)	...	...	...	...	...	(+)
KNOPS	+	+	+	+	+	+	(−)

Table 39. Protein reaction

(a) Precipitation reaction

Kind of reaction	Dialyzate of the enzyme substance No. 1	Dialyzate of the enzyme substance No. 2	Inner solution (Dialyzed enzyme substance)	Remarks
Boiling reaction (in neutral)	−	−	−	——
Boiling reaction (in acidic)	−	−	+	——
Boiling reaction (in basic)	+	+	+	Perhaps the precipitation of dialyzate seems to depend upon its ash ingredient
NaCl or Na_2SO_4	−	−	(+)	The degree of clarity in the outer solution seems to be accelerated by boiling
HELLER's test	−	−	−	——
Acetic acid and $K_2Fe(CN)_6$	−	−	(+)	The degree of clarity in the outer solution seems to be accelerated by boiling
Picric acid	−	−	(+)	"
Phosphotungstic acid	(+)	(+)	(+)	In this case the reaction is not clear, as this observation is performed for a short time
Basic lead acetate	+	+	+	——
Sulpho-salitylic acid	(−)	(−)	(+)	——

(Continued)

(b) Colour reaction

Kind of reaction	Dialyzate of the enzyme substance No. 1	Dialyzate of the enzyme substance No. 2	Inner solution (Dialyzed enzyme substance)
BIURET	+	+	+
Xanthoprotein	+	+	+
MILLON	+	+	(+)
ADAMKIEWICZ	+	+	(+)
NEUBAUER and ROHDE	+	+	+
PbS	−	−	−
MOLISCH	+	+	+
LIEBERMANN (and COLE)	+	+	+
HOPKINS and COLE	(+)	(+)	+
REICHER	(+)	(+)	...
PAULY's Diazo	+	+	+
Ninhydrin	+	+	+
PIRIA	(+)	(+)	(+)
MÖRNER	(+)	(+)	(+)
KNOPS	+	+	(+)

Table 40. Classification of various nitrogenous substances in "Koji-Diastase" and in its hydrolysis products

Kind of nitrogen	Percentage of nitrogen for enzyme substance				Percentage of nitrogen for total nitrogen of enzyme substance			
	(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)
Total-N	4.670	4.407	6.736	4.670	100	100	100	100
Protein-N	—	—	—	0.438	—	—	—	9.358
Ammonia-N	0	0	0	0	0	0	0	0
Total Amide-N	0	0	0	0	0	0	0	0
Humin-N	0.355	0.560	1.171	0.167	7.602	12.707	17.384	3 576
Diamino acid-N	2.401	1.401	2.232	2.918	51.413	31.790	33.135	62.484
Total-N of mono-amino acid	1.721	2.400	3.068	1.145	36.852	54.459	45.548	24.518
Amino-N of mono-amino acid	1.508	1.679	1.892	—	32.291	38.098	28.088	—

Table 41. Total nitrogenous substance in every separated enzyme substance

"Koji-Diastase" enzyme substance (day)	Total nitrogen (%)	Nitrogenous substance (%)	Non-nitrogenous substance (glucose, %)	Ash (%)	Total (%)	Remarks
"Koji-Diastase", original (No. 2–No. 6)	6.036	37.725	49.225	11.050	98.000	——
"Koji-Diastase", original (KATAOKA's "Moto-Koji")	4.670	29.188	58.750	4.785	92.723	——
Artificial, "mixed chemicals" (2 of E in study IV)	4.132	25.823	50.890	17.600	93.313	Precipitate in the case of 63.3% of alcoholic concentration
Air bladder dialyzate I (water solution + 10 % alcoholic solution)	4.815	30.094	45.330	14.990	90.414	"Koji-Diastase" substance (No. 2–No. 6)
Intestine membrane dialyzate I (")	4.737	29.606	43.117	15.670	88.343	"
Bladder dialyzate I (")	5.424	33.900	31.810	17.190	72.900	"
Air bladder dialyzate II (")	4.013	25.081	62.500	7.821	95.402	"
Intestine membrane dialyzate II (")	3.651	22.819	59.667	8.143	90.629	"
Bladder dialyzate II (")	4.969	31.056	53.430	8.495	92.981	"
Water dialyzate III (Air bladder, Intestine membrane, Bladder)	2.353	14.706	67.488	8.157	90.351	"

10 % alcoholic dialyzate III (")	2.537	15.856	67.389	8.413	91.530	"
Inner residual solution 1 (Air bladder, water solution)	7.719	48.244	45.043	6.728	100.015	"
Inner residual solution 2 (Air bladder, 10 % alcoholic solution)	8.080	50.499	43.589	5.911	99.999	"
Inner residual solution 3 (Intestine membrane, water solution)	6.476	40.497	53.217	6.289	100.003	"
Inner residual solution 4 (Intestine membrane, 10 % alcoholic solution)	6.442	40.265	55.042	4.694	100.001	"
Inner residual solution 5 Bladder, water)	7.321	45.759	50.121	4.122	100.002	"
Inner residual solution 6 (Bladder, 10 % alcoholic solution)	6.298	39.365	55.888	4.735	100.388	"

Table 42. Carbohydrate reaction in "Koji-Diastase" and other diastase enzyme substance

Kind of reaction \ Enzyme substance	"Koji-Diastase" (No. 2–No. 6)	"Koji-Diastase" (KATAOKA's "Moto-Koji")	"Malt-Diastase"	"Germinated unhulled rice-Diastase"	Artificial, "mixed chemicals"	Dialyzate of enzyme substance	Inner solution (Dialyed enzyme substrnce)	Remarks
MOLISCH	+	+	+	+	+	+	+	Carbohydrate
MOLISCH and UDRANSKI	+	+	+	+	+	+	+	Aldohexose
Naphtoresorcin	+	+	...	...	...	...	+	"
NEUMAN	+	+	+	+	...	+	...	"
Indigo-Carmin	(+)	+	...	...	...	...	...	"
Picric acid	(+)	(+)	...	...	...	...	...	"
SELIWANOFF	−	−	−	(+)	...	−	−	Ketose
PINOFF	−	−	(+)	(+)	(+)	−	−	"
BORCHARDT	−	−	...	...	...	−	−	"
Phloroglucin	(+)	(+)	(−)	+	...	...	...	Pentose
BIAL's Orcin	+	+	+	+	...	+	(−)	"
Furfurol	+	+	+	+	...	+	(+)	"
TOLLENS and OSHIMA	+	+	+	+	...	...	...	Methyl pentose
Diazo-benzosulphonic acid	(+)	(+)	−	...	(+)	+	+	Glucose
UDRANSKI and BAUMANN	(−)	(−)	−	...	(+)	+	+	"
Maltosazone	(−)	(−)	(+)	...	−	...	...	Maltose

Table 43. Content of carbohydrate in every separated enzyme substance of "Koji-Diastase"

"Koji-Diastase" enzyme substance (dry)	Carbohydrate (after hydrolysis, as glucose,%)	Reducing sugar (before hydrolysis, as glucose, %)	Non reducing substance beside carbohydrate, substance of protein-like & ash (difference, %)	Remarks
"Koji-Diastase" enzyme substance, original (No.2–No. 6)	49.225	1.040	2.000	—
"Koji-Diastase" enzyme substance, (KATAOKA's "Moto-Koji")	58.750	1.300	7.277	—
Artificial, "mixed chemicals" (2 of E in study IV) A	50.890	0.500	6.687	Precipitate in the case of 63.3 % of alcoholic water sol.
Artificial, "mixed chemicals" (2 of E in study IV) B	42.220	1.230	—	" in the case of 71.25 % "
Artificial, "mixed chemicals" (2 of E in study IV) C	35.160	0.500	—	" in the case of 80.40 % "
(Air bladder) Dialyzate of enzyme substance I	45.330	2.600	9 586	"Koji-Diastase" No. 2–No. 6
(Intestine membrane) Dialyzate of enzyme substance I	43.117	2.080	11.657	"
(Bladder membrane) Dialyzate of enzyme substance I	31.810	6.425	17.100	"
(Air bladder) Dialyzate of enzyme substance II	62.500	2.900	4.598	"
(Intestine membrane) Dialyzate of enzyme substance II	59.667	7.000	9.371	"
(Bladder membrane) Dialyzate of enzyme substance II	53.430	9.310	7.019	"

(Water) Dialyzate of enzyme substance III	67.488	1.250	9.649	"
(10% alcohol solution) Dialyzate of enzyme substance III	67.389	1.761	8.470	"
Inner solution (Dialyed enzyme substance) 1	45.043	14.576	0	"
" 2	43.589	7.200	0	"
" 3	53.217	17.038	0	"
" 4	55.042	9.899	0	"
" 5	50.121	24.976	0	"
" 6	55.888	7.360	0	"

Table 44. Elemental composition of purified "Koji-Diastase"

Enzyme substance (dry)	(CO_2,%)	C (%)	(H_2O,%)	H (%)	N (%)	O (%)	Ash(%)	Remarks
"Koji-Diastase" No. 2 (WATANABE)	——	——	115.758	6.431	——	——	11.384	Polished rice "Koji" made from 3rd class rice of "Asahigawa"
" No. 3 (")	156.630	42.725	120.600	6.700	5.505	32.538	11.532	"
" No. 4 (")	149.660	40.824	159.948	8.886	5.736	33.743	10.811	"
" No. 5 (")	152.546	41.611	150.750	8.375	7.127	33.730	8.857	"
" No. 6 (")	158.716	43.294	120.708	6.706	5.775	31.564	12.661	"
" No. 2–No. 6 (")	154.665	42.189	133.560	7.420	6 036	33.305	11.050	"
"Moto-Koji-Diastase" (KATAOKA)	183.458	50.043	169.974	9.443	4.670	31.059	4.785	"Moto-Koji" for Japanese "Sake" brewing made from 3rd class rice of "Banshu"

Table 45. Elemental composition of enzyme substances

	Dry	Air bladder			Intestine	
		(1) Distilled water		(2) 10 % alcoholic water solution	(3) Distilled water	
Enzyme substance (gram)	Original enzyme substance	1.5	—	1.5	1.0	—
	Dialyzate I	—	(1.3798)	—	—	(1.3691)
	" II	—	(0.8983)	—	—	(0.6319)
	" III	—	(0.5141)	—	—	(0.42133)
	Inner residual solution	0.09653	—	0.11129	0.07445	—
	"Koji" extract *	—	—	—	—	—
Ash (%)	Original enzyme substance	11.050	—	11.050	11.050	—
	Dialyzate I	—	—	—	—	—
	" II	—	—	—	—	—
	" III	—	—	—	—	—
	Inner residual solution	—	—	—	—	—
	"Koji" extract	—	0.357	—	—	0.357
C (%)	Original enzyme substance	42.189	—	42.189	42.189	—
	Dialyzate I	—	(39.647)	—	—	(38.527)
	" II	—	(43.577)	—	—	(40.548)
	" III	—	—	—	—	—
	Inner residual solution	49.321	(49.805)	50.288	47.330	(47.660)
	"Koji" extract	48.162	—	48.162	48.162	—
H (%)	Original enzyme substance	7.420	—	7.420	7.420	—
	Dialyzate I	—	(7.665)	—	—	(6.201)
	" II	—	(7.348)	—	—	(8.791)
	" III	—	—	—	—	—
	Inner residual solution	6.807	(6.754)	6.701	6.895	(6.952)
	"Koji" extract	8.917	—	8.917	8.917	—
N (%)	Original enzyme substance	6.036	—	6.036	6.036	—
	Dialyzate I	—	(4.815)	—	—	(4.737)
	" II	—	(4.013)	—	—	(3.651)
	" III	—	—	—	—	—
	Inner residual solution	7.719	(7.900)	8.080	6.476	(6.459)
	"Koji" extract	0.622	—	0.622	0.622	—
O (%)	Original enzyme substance	33.305	—	33.305	33.305	—
	Dialyzate I	—	(32.883)	—	—	(34.865)
	" II	—	(37.241)	—	—	(38.867)
	" III	—	—	—	—	—
	Inner residual solution	29.425	(29.223)	29.020	33.010	(33.438)
	"Koji" extract	41.942	—	41.942	41.942	—

* To 765 grams of polished rice was added 1500 c.c. of 20 % alcoholic water solution; 1150 c.c. of filtrate were obtained after 3 days of steeping; and 152 grams (air dry) of "Koji" extract was obtained.

separated from "Koji-Diastase" by means of dialysis

membrane	Bladder			Total, average		
(4) 10 % alcoholic water solution	(5) Distilled water		(6) 10 % alcoholic water solution	Distilled water		10 % alcoholic water solution
2.0	1.5	—	1.5	4.0	—	5.0
—	—	(1.2996)	—	—	(4.0485)	—
—	—	(0.4548)	—	—	(1.9850)	—
—	—	(0.25323)	—	0.43168	(1.18866)	0.75698
0.50322	0.47180	—	0.52055	0.64278	(1.77784)	1.13506
—	—	—	—	—	—	—
11.050	11.050	—	11.050	11.050	—	11.050
—	—	—	—	—	(15.927)	—
—	—	—	—	—	(8.078)	—
—	—	—	—	—	(8.275)	—
—	—	—	—	—	(4.800)	—
—	—	0.357	—	—	0.357	—
42.189	42.189	—	42.189	42.189	—	42.189
—	—	(37.906)	—	—	(38.693)	—
—	—	(40.920)	—	—	(41.682)	—
—	—	—	—	40.480	(40.620)	40.760
47.990	49.780	(49.136)	44.398	48.813	(48.185)	47.557
48.162	48.162	—	48.162	48.162	—	48.162
7.420	7.420	—	7.420	7.420	—	7.420
—	—	(7.916)	—	—	(7.261)	—
—	—	(8.599)	—	—	(8.246)	—
—	—	—	—	6.110	(6.150)	6.190
7.009	7.008	(7.065)	7.121	6.903	(6.923)	6.944
8.917	8.917	—	8.917	8.917	—	8.917
6.036	6.036	—	6.036	6.036	—	6.036
—	—	(5.424)	—	—	(4.992)	—
—	—	(4.969)	—	—	(4.211)	—
—	—	—	—	2.353	(2.445)	2.537
6.442	7.321	(6.810)	6.298	7.172	(7.056)	6.940
0.622	0.622	—	0.622	0.622	—	0.622
33.305	33.305	—	33.305	33.305	—	33.305
—	—	(31.564)	—	—	(33.104)	—
—	—	(37.017)	—	—	(37.708)	—
—	—	—	—	42.882	(42.555)	42.228
33.865	31.769	(35.519)	39.268	31.401	(32.726)	34.051
41.942	41.942	—	41.942	41.942	—	41.942

Table 46. Enzyme-like action of the "mixed chemicals"

	Starch						
	Iodine reaction				Appearance	Viscosity	c.c. of $KMnO_4$ solution used for titration
	1 drop	2 drops	5 drops	10 drops			
a)	YO Tint 2	YO Shade 1	O Shade 1	OR Shade 2	Transparent	1.067	5.25 (2.70)
b)	No color	Y Tint 2	Y Normal tones	OY Normal tones	Transparent	1.071	7.95 (5.60)
c)	"	OR Tint 2	OR Shade 1	OR Shade 2	Transparent	1.087	3.85 (1.90)
d)	B Shade 1	B Shade 2	B Shade 2	B Shade 2	—	1.194	2.60 (0.35)
e)	"	"	"	"	—	1.210	2.50 (0.25)
f)	B Shade 2	"	"	"	Transparent	1.323	0.60 (0.30)
g)	B Shade 1	"	"	"	—	1.344	0.20 (−0.05)
h)	B Shade 2	"	"	"	—	2.591	0.20 (−0.05)
i)	"	"	"	"	—	1.272	0.50 (0.25)
j)	"	"	"	"	—	1.231	0.40 (0.15)
k)	"	"	"	"	—	1.347	2.05 (−0.30)
l)	"	"	"	"	—	2.550	0.20 (−0.15)
m)	BV Tint 2	"	"	"	—	1.450	0.20 (−0.10)
n)	B Shade 2	"	"	"	Opaque	3.368	2.05 (−0.15)
Control (Substrate)	"	"	"	"	—	>73.846	0.20 (0)
Control (Ferric sulphate)	—	—	—	—	—	—	(0.10)

(Continued)

	Soluble starch		Maltose	Sucrose	Control
	Iodine reaction (1 drop)	c.c. of $KMnO_4$ solution used for titration	c.c. of $KMnO_4$ solution used for titration	c.c. of $KMnO_4$ solution used for titration	"Mixed chemicals", only
a)	B Shade 2	2.10 (−0.50)	10.05 (0)	3.00 (−0.15)	2.45 (0)
b)	"	2.35 (−0.50)	9.40 (−0.45)	2.70 (−0.25)	2.35 (0)
c)	"	2.05 (0.05)	9.35 (−0.10)	2.55 (0)	1.85 (0)
d)	"	2.10 (−0.20)	9.50 (−0.25)	2.75 (−0.10)	2.15 (0)
e)	"	2.10 (−0.20)	9.40 (−0.35)	2.65 (−0.20)	2.15 (0)
f)	"	0.25 (−0.10)	7.65 (−0.15)	0.90 (0)	0.20 (0)
g)	"	0.20 (−0.10)	7.75 (0)	0.80 (−0.05)	0.15 (0)
h)	"	0.25 (−0.05)	7.45 (−0.30)	0.80 (−0.05)	0.15 (0)
i)	"	0.25 (−0.05)	7.65 (−0.10)	0.80 (−0.05)	0.15 (0)
j)	"	0.30 (0)	7.75 (0)	0.85 (0)	0.15 (0)
k)	"	2.10 (−0.30)	9.55 (−0.30)	2.70 (−0.25)	2.25 (0)
l)	"	0.25 (−0.15)	7.70 (−0.15)	0.90 (−0.05)	0.25 (0)
m)	"	0.25 (−0.10)	7.60 (−0.20)	0.95 (0)	0.20 (0)
n)	"	2.10 (−0.15)	9.70 (0)	2.70 (−0.10)	2.10 (0)
Control (Substrate)	"	0.25 (0)	7.70 (0)	0.80 (0)	—
Control (Ferric sulphate)	—	(0.10)	(0.10)	(0.10)	(0.10)

Table 47. Enzyme-like action of the "mixed chemicals"

	c.c. of $KMnO_4$ solution used for titration				
	Starch	Soluble starch	Maltose	Sucrose	Control ("Mixed chemicals", only)
a)	7.60 (5.00)	2.20 (−0.50)	9.40 (−0.50)	2.85 (−0.35)	2.50 (0)
b)	8.00 (5.65)	2.40 (−0.05)	9.60 (−0.25)	2.85 (−0.10)	2.25 (0)
c)	7.35 (5.40)	2.15 (0.10)	9.45 (0)	2.55 (0)	1.85 (0)
d)	5.40 (3.15)	2.25 (−0.10)	9.55 (−0.20)	2.75 (−0.10)	2.15 (0)
e)	5.10 (2.85)	2.25 (−0.10)	9.60 (−0.15)	2.65 (−0.20)	2.15 (0)
f)	1.90 (1.60)	0.25 (0.15)	7.80 (0)	0.90 (0)	0.20 (0)
g)	2.85 (2.60)	0.20 (−0.15)	7.75 (0)	0.80 (−0.05)	0.15 (0)
h)	0.20 (−0.05)	0.25 (−0.10)	7.55 (−0.20)	0.80 (−0.05)	0.15 (0)
i)	0.70 (0.45)	0.30 (−0.05)	7.75 (0)	0.80 (−0.05)	0.15 (0)
j)	0.70 (0.45)	0.30 (−0.05)	7.75 (0)	0.85 (0)	0.15 (0)
k)	2.75 (0.50)	2.20 (−0.15)	9.75 (0)	2.70 (−0.15)	2.15 (0)
l)	0.20 (−0.15)	0.30 (−0.15)	7.70 (−0.15)	0.95 (0)	0.25 (0)
m)	1.55 (1.25)	0.30 (−0.10)	7.80 (0)	0.95 (0)	0.20 (0)
n)	2.05 (−0.20)	2.20 (−0.15)	9.75 (0)	2.70 (−0.15)	2.25 (0)
Control (Substrate)	0.20 (0)	0.30 (0)	7.70 (0)	0.80 (0)	—
Control (Ferric sulphate)	(0.10)	(0.10)	(0.10)	(0.10)	(0.10)

Table 48. Negative results for producing enzymic power by means of the "mixed chemicals"

		A				B			
		Iodine reaction		Viscosity	c.c. of the $KMnO_4$ sol. used for titration	Iodine reaction		Viscosity (Minute, second)	c.c. of the $KMnO_4$ sol. used for titration
		one drop	4 drops			one drop	4 drops		
Starch	(1)	B Shade 1	B Shade 2	8.739	1.20	B Shade 1	B Shade 2	8.835	1.05
	(2)	"	"	6.476	1.20	"	"	7.979	1.10
	(3)	VB Shade 1	"	2.667	1.30	BV Shade 1	"	3.194	1.20
	(4)	"	"	5.827	1.50	"	"	8.558	1.40
	(5)	B Tint 1	V Shade 2	8.702	1.05	B Tint 1	"	7.754	1.00
	(6)	"	"	10.691	10.5	"	"	7.559	0.95
Soluble starch	(1)	B Shade 1	B Shade 1	—	1.85	B Shade 1	B Shade 2	—	1.35
	(2)	"	"	—	1.70	"	"	—	1.45
	(3)	BV Shade 1	B Shade 2	—	1.50	BV Shade 1	BV Shade 2	—	1.30
	(4)	V Shade 1	"	—	1.40	V Shade 1	"	—	1.40
	(5)	B Shade 1	"	—	1.50	B Shade 1	"	—	1.40
	(6)	V Shade 1	V Shade 2	—	1.50	V Shade 1	V Shade 2	—	1.30
Control 1 ("Mixed chemicals", distill. water)		—	—	1.009	1.20	—	—	1.026	1.05
Control 2 (Eggalbumin, distill. water)		—	—	—	—	—	—	—	0.25
Control 3 (Edestine, distill. water)		—	—	—	—	—	—	—	—

(Continued)

		C				Control (Starch, only)			
		Iodine reaction		Viscosity	c.c. of the $KMnO_4$ sol. used for titration	Iodine reaction		Viscosity	c.c. of the $KMnO_4$ sol. used for titration
		one drop	4 drops			one drop	4 drops		
Starch	(1)	B Shade 1	B Shade 2	8.053	1.00	B Shade 1	B Shade 2	7.873	0.30
	(2)	"	"	8.583	1.10	"	"	5.348	0.25
	(3)	BV Shade 1	"	5.559	1.20	"	"	2.923	0.25
	(4)	"	"	8.656	1.40	"	"	5.200	0.20
	(5)	B Tint 1	"	8.375	1.00	"	V Shade 2	4.103	0.25
	(6)	"	"	8.174	0.90	BV Shade 1	"	11.241	0.25
Soluble starch	(1)	B Shape 1	B Shade 2	—	1.35	B Shade 1	B Shade 2	—	0.35
	(2)	"	"	—	1.45	"	"	—	0.30
	(3)	BV Shade 1	BV Shade 2	—	1.30	BV Shade 1	BV Shade 2	—	0.25
	(4)	V Shade 1	"	—	1.15	V Shade 1	"	—	0.20
	(5)	B Shade 1	"	—	1.35	B Shade 1	"	—	0.40
	(1)	V Shade 1	V Shade 2	—	1.15	V Shade 1	V Shade 2	—	0.30
Control 1 ("Mixed chemicals", distill. water)		—	—	1.015	1.05	—	—	—	—
Control 2 (Eggalbumin, dislill. water)		—	—	—	—	—	—	—	—
Control 3 (Edenstine, distill. water)		—	—	—	0.25	—	—	—	—

Table 49. Negative results for producing enzymic power by means of the "mixed chemicals"

	A			B			C			Starch only	
	Iodine reaction	Viscosity	c.c. of the $KMnO_4$ solution (Difference for control)	Iodine reaction	Viscosity	c.c. of the $KMnO_4$ solution (Difference for control)	Iodine reaction	Viscosity	c.c. of the $KMnO_4$ solution (Difference for control)	Iodine reaction	Viscosity
1	VB Normal tone	4.103	0.35	VB Normal tone	4.646	-0.10	VB Normal tone	4.437	0.25	B Normal tone	31.897
2	"	2.981	0.15	"	2.944	0.15	"	3.258	-0.30	"	31.842
3	"	4.312	0.35	"	4.578	0.05	"	4.361	-0.25	"	38.585
4	"	2.831	0.05	"	2.839	-0.05	"	3.179	-0.10	"	23.344
5	"	4.636	-0.05	"	4.628	-0.20	"	5.169	-0.10	"	10.092
6	B Shade 1	5.231	-0.25	B Shade 1	5.682	-0.30	B Shade 1	4.691	-0.15	B Shade 1	8.355
7	"	2.667	-0.10	"	3.270	-0.30	BV Shade 1	2.461	0	"	4.267
8	"	1.405	0.05	"	1.448	-0.15	B Shade 1	1.473	0.15	"	1.491
9	BV Shade 1	4.297	0	BV Shade 1	4.472	-0.30	BV Shade 1	5.393	0.20	"	7.122
10	"	4.070	0.05	"	5.280	-0.30	V Shade 1	2.708	0.50	BV Shade 1	9.655
11	"	9.155	-0.25	"	11.206	-0.35	BV Shade 1	7.046	0.40	B Shade 1	17.022
12	B Shade 1	5.206	-0.25	B Shade 1	5.846	-0.25	RV Shade 1	4.950	0.25	"	12.193
(2)	"	1.296	-0.20	"	1.323	-0.30	B Shade 1	1.307	-0.20	"	1.450
(6)	"	1.286	-0.05	"	1.286	-0.10	"	1.286	-0.15	"	1.216
(9)	V Shade 1	1.344	-0.20	V Shade 1	1.346	-0.15	V Shade 1	1.346	0	V Shade 1	1.274
(12)	BV Shade 1	1.221	0	BV Shade 1	1.223	0	BV Shade 1	1.216	0.05	BV Shade 1	1.218

Table 50. Starch saccharification of the "mixed chemicals"

The case of reaction for 20 hrs. (c. c. of the $KMnO_4$ solution)

	A	B	C	D	E	Control (Starch only)
1	2.15	2.30	2.30	2.30	2.70	0.15
2	2.35	2.30	2.50	2.30	2.60	0.15
3	2.00	2.25	—	2.35	2.40	0.15
4	2.35	2.25	2.45	2.25	2.90	0.30
5	2.35	2.20	2.45	2.35	2.65	0.20
6	2.60	2.45	—	2.50	2.95	0.30
7	2.50	2.30	2.55	2.65	2.85	0.40
Control ("Mixed chemicals", only)	2.35	2.30	2.30	2.20	1.80	—

The case of reaction for 140 hrs.

	A		B		C	
	Vis-cosity	$KMnO_4$ solution (c.c.)	Vis-cosity	$KMnO_4$ solution (c.c.)	Vis-cosity	$KMnO_4$ solution (c c.)
1	1.621	2.15	2.185	2.45	1.350	2.85
2	2.728	2.45	2.472	2.40	1.846	2.85
3	2.146	2.30	2.144	2.40	—	—
4	5.662	2.60	5.836	2.50	1.286	2.60
(5)	1.345	2.45	1.337	2.45	1.342	2.45
(6)	1.557	2.50	1.573	2.70	—	—
(7)	1.255	2.60	1.225	2.70	1.143	2.85
Control ("Mixed chemicals", only)	—	2.40	—	2.35	—	2.35

(Continued)

	D		E		Control (Starch, only)	
	Vis-cosity	$KMnO_4$ solution (c c.)	Vis-cosity	$KMnO_4$ solution (c.c.)	Vis-cosity	$KMnO_4$ solution (c c.)
1	1.936	2.40	1.143	9.10	31.498	0.15
2	2.728	2.35	1.141	9.40	38.995	0.15
3	1.924	2.40	1.221	8.15	6.912	0.15
4	8.390	2.40	2.723	8.90	9.450	0.35
(5)	1.374	2.70	2.272	9.50	1.327	0.30
(6)	1.553	2.70	1.237	9.10	1.723	0.35
(7)	1.275	2.50	1.065	8.30	1.204	0.40
Control	—	2.30	—	1.80	—	—

Table 51. Diastase enzymic activity of the "mixed chemicals"

The case of reaction for 24 hrs. (c.c. of the $KMnO_4$ solution)

	A	B	D	E	F+	F−	F+−	F′	G	Control (Starch, only)
1	2.00	2.10	2.10	2.80	1.85	3.00	2.70	2.55	2.20	0.15
2	2.10	2.00	2.40	3.20	2.00	3.10	2.70	2.60	2.30	0.20
(1)	2.80	3.80	2.60	4.40	2.20	3.30	3.10	2.80	2.30	0.35
Control ("Mixed chemicals", only)	1.95	2.10	2.10	2.10	1.80	2.80	2.70	2.65	2.10	—

The case of reaction for 118 hrs.

	A		B		D		E		F+	
	Vis-cosity	c.c. of the $KMnO_4$ solution used for titration	Vis-cosity	c.c. of the $KMnO_4$ solution used for titration	Vis-cosity	c.c. of the $KMnO_4$ solution used for titration	Vis-cosity	c.c. of the $KMnO_4$ solution used for titration	Vis-cosity	c.c. of the $KMnO_4$ solution used for titration
1	5.914	2.10	4.667	2.00	4.649	2.20	1.101	7.50	3.209	1.90
2	4.927	2.20	4.155	2.40	4.602	2.60	1.338	7.10	3.880	2.10
(1)	1.398	2.90	1.396	2.80	1.398	2.80	1.333	7.20	1.376	2.80
Control ("Mixed chemicals", only)	1.000	2.00	1.009	2.15	1.006	2.15	1.014	2.15	1.008	1.80

(Continued)

	F−		F+−		F′		G		Control (Starch, only)	
	Vis-cosity	c.c. of the $KMnO_4$ solution used for titration	Vis-cosity	c.c. of the $KMnO_4$ solution used for titraiton	Vis-cosity	c.c. of the $KMnO_4$ solution used for titration	Vis-cosity	c.c. of the $KMnO_4$ solution used for titration	Vis-cosity	c.c. of the $KMnO_4$ solution used for titration
1	1.841	3.35	1.060	3.40	3.561	2.75	1.538	2.50	26.774	0.25
2	1.630	3.20	2.974	3.40	3.088	2.75	1.628	2.50	7.344	0.20
(1)	1.299	3.30	1.377	3.10	1.406	2.90	1.373	2.80	1.391	0.40
Control	1.015	2.85	1.015	2.75	1.004	2.70	1.011	2.10	—	—

The case of reaction for 400 hrs.

(For iodine reaction, sample taken 2 c.c., and used N/20 I.IK solution)

	A			B			D			E			E+		
	Iodine reaction		$KMnO_4$ solution (c.c.)	Iodine reaction		$KMnO_4$ solution (c.c.)	Iodine reaction		$KMnO_4$ solution (c.c.)	Iodine reaction		$KMnO_4$ solution (c.c.)	Iodine reaction		$KMnO_4$ solution (c.c.)
	1 drop	3 drops		1 drop	3 drops		1 drop	3 drops		1 drop	3 drops		1 drop	3 drops	
1	B Shade 2	B Shade 2	3.00	B Shade 2	B Shade 2	3.40	B Shade 2	B Shade 2	3.40	B Tint 2	O Shade 2	13.40	B Shade 2	B Shade 2	2.20
2	V Shade 2	BV Shade 2	3.30	RV Shade 1	V Shade 2	2.75	BV Shade 1	BV Shade 2	2.60	BV Shade 1	VB Shade 1	13.60	BV Shade 2	VB Shade 2	3.40
(1)	B Shade 2	B Shade 2	2.90	B Shade 1	B Shade 2	2.90	B Shade 2	B Shade 2	2.95	VB Tint 1	OR Broken tones	14.00	B Shade 2	B Shade 2	3.50
Control ("Mixed chemicals", only)	YO Normal tones	—	2.00	—	—	2.20	—	—	2.20	OY Tint 1	—	2.15	YO Shade 1	—	1.80

(Continued)

	F−			F+−			F′			G			Control (Starch, only)		
	Iodine reaction		$KMnO_4$ solution (c.c.)	Iodine reaction		$KMnO_4$ solution (c.c.)	Iodine reaction		$KMnO_4$ solution (c.c.)	Iodine reaction		$KMnO_4$ solution (c.c.)	Iodine reaction		$KMnO_4$ solution (c.c.)
	1 drop	3 drops		1 drop	3 drops		1 drop	3 drops		1 drop	3 drops		1 drop	3 drops	
1	VB Shade 1	B Shade 2	3.15	RV Shade 1	VR Shade 2	5.15	B Shade 2	B Shade 2	2.75	VB Shade 1	B Shade 2	2.75	B Shace 2	B Shade 2	0.10
2	V Shade 2	BV Shade 2	4.20	BV Shade 2	BV Shade 2	4.50	BV Shade 2	BV Shade 2	3.40	RV Shade 2	BV Shade 1	2.80	B Shade 2	B Shade 2	0.10
(1)	VB Shade 1	B Shade 2	4.35	VB Shade 1	B Shade 2	4.35	B Shade 2	B Shade 2	3.75	BV Shade 2	B Shade 2	3.20	B Shade 2	B Shade 2	0.45
Control	OY Tint 1	—	2.85	—	—	2.75	OY Shade 1	—	2.65	OY Shade 1	—	2.05	—	—	—

Table 52. Comparison of starch liquefying power between the "mixed chemicals" and the natural enzyme solution

Enzyme solution \ Time allowed for reaction (Minute)	1	5	10	15	20	25	30
Polished rice "Koji"	4.579	1.650	1.442	1.183	1.120	1.030	1.025
Red rice "Koji"	5.360	4.461	4.499	4.248	4.077	3.888	3.574
Germinated unhulled rice	3.405	3.497	3.020	2.183	1.071	1.812	2.853
Artificial, "mixed chemicals"	3.558	4.117	5.096	3.126	2.457	2.187	1.574

(Continued)

Enzyme solution \ Time allowed for reaction (Minute)	35	40	45	50	55	60
Polished rice "Koji"	1.010	1.010	1.013	1.003	1.005	1.005
Red rice "Koji"	3.308	3.054	2.792	2.581	2.335	2.002
Germinated unhulled rice	1.518	1.411	1.356	1.274	1.269	1.239
Artificial, "mixed chemicals"	1.437	1.345	1.244	1.259	1.269	1.259

Table 53. Precipitation phenomena of various chemicals by alcohol

Substance	pH (Brom thymol blve)	%	c.c.	g.		c.c. of the 95 % alcohol used for turbiding of 10 c.c. of each solution
$Mg_3(PO_4)_2$, Merck (Acetic acid)	3.8	2.049	25	0.51225		9.1 (45.26)
$Ca_3(PO_4)_2$, S.K & Co. (Acetic acid)	3.7	0.94	20	0.18838		4.9
K_3PO_4, Merck	7.6	2.12	25	0.53000	1.61963	18.3 (61.43)
Na_2SO_4, KOJIMA Co.	5.0	1.42	20	0.28400		25.2
"Eukirin", Sankyo (Acetic acid)	4.15	1.05	10	0.10500		<10.0
Dextrine, Merck	6.30	10.00	20	2.00000		14.1 (55.57)
Maltose, Merck	6.10	10.00	20	2.00000		90.0
Glycogen, Sankyo	7.00	10.00	10	1.00000	9.00000	6.5
Glucose, Merck	6.25	10.00	20	2.00000		>90.0
Sucrose, Merck	6.25	10.00	20	2.00000		>90.0
Peptone, Merck	6.60	10.00	35	3.50000		6.4 (37.07)
Glycocoll, Merck	5.90	10.00	10	2.00000		13.2 (54.05)
Asparagine, Merck	3.80	5.00	30	1.50000	7.15750	<20.0
α-alanine, Kahlbaum	5.80	10.00	10	1.00000		<20.0
Tyrosine, Merck (Ammoniumhydroxide)	8.25	1.05	15	0.15750		—

Table 54. Fractional precipitation of the "mixed chemicals" by alcohol

Concentration of alcohol used for precipitation of "mixed chemicals", fractionally (%)	Original, "mixed chemicals"	63.30	71.25	80.40	85.00	Non precipitated matter in 85 % alcohol solution of the "mixed chemicals"
"Mixed chemicals", precipitated (g)	17.413	2.9420	0.8280	0.7835	0.6320	12.2275
Ash of "mixed chemicals" (%)	9.301	17.600	—	—	—	3.592