On the Differences of the Physico-chemical Properties of the Protein, Oryzenin, as found in Glutinous and in Common Rice.

By

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Introduction.

Many authors who have studied on the chemical nature of glutinous and common rices have deduced that the differences of these rices are mainly due to the physico-chemical properties of their starches. Although there are many who have attempted to explain quantitatively, the differences between the constituents (proteins, fats, sugars or others) of the two kinds of rice, their results are not very accurate.

On the differences of the physico-chemical properties of both the starches, one of the authors[1] has studied already and reported details. From the view point of biochemistry, the authors must conclude that the main difference of starch formation is in their physiological action in the two kinds of rice plants. If so, the difference of the physiological actions is due first to the physico-chemical properties of the proteins which are the principal constituents of the protoplasma and second to the properties of oryzenin, the main protein of rices. If the oryzenin should not be a simple chemical substance, one is able to hope to find differences between the glutinous and common rices as in the case of starches of the two rices, if he adopt a very precise method of studying.

Before entering into our chemical study of the proteins of these two kinds of rice, such literature as has direct connection with the present investigation should be mentioned.

Rosenheim and Kajiura[2] reported that albumin, globulin and glutenin were present as the proteins in rice. The glutenin which made up more than one half of the contents was named oryzenin by them. Suzuki, Yoshimura and Fuji[3] find the existence of the alcohol-

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soluble protein and confirmed the amino acids as the hydrolytic products of the protein according to Ritthausen's method. The researches of Kellner[4], Takahashi and Sato[5], Sato, Masuda, Terada, Hoshino and Shikamata[6] and Tadokoro[7] were on the fermentation of rice proteins. Some went into a detailed separation of the amino acids and others studied the protein distribution between rice-bran and rice. These have no direct connection with the present investigation.

Osborne, Van Slyke, Leavenworth, and Vinograd[8] estimated the amino acids of oryzenin; Kurokawa[9] repeated the same study on the NaOH soluble protein; Suzuki, Okuda, Okimoto, and Nagasawa[10] fixed its nutritive value by feeding experiments; Yoshimura and Chin[11] isolated the amines from the putrified rice protein. Recently, Kondo[12] studied the refractive indices of the solutions of the proteins of hulled glutinous and common rices. He ascertained that in the elementary analysis, the common rice protein soluble in 0.2 % NaOH solution contains copious nitrogen and sulphur and less carbon and hydrogen than does glutinous rice protein. Kondo found that the refractive index is higher in common rice protein than in glutinous rice, so he deduces that the former is optically more dense than the latter. He also reported that the quantity of nitrogen soluble in water, 10 % NaCl solution, 70 % alcohol solution, and 0.2 % NaOH solution, is larger in glutinous as compared with common rice.

Though Kondo's study had the same purpose as the authors', when a rice was extracted with 0.2 % NaOH solution only, it must be contaminated by water, NaCl solution and alcohol soluble proteins. The authors knew from Tadokoro[7] also how the ratios of the four kinds of proteins vary according to the difference of hulling degrees of the rice, so it is possible to estimate the differences in the elementary compositions of protein, which is extracted only with alkali. Therefore, having these facts in mind, the authors proposed to prepare pure oryzenins, free from other proteins. They carried on the following experiments.

I. Estimation of Water-, NaCl-, Alcohol-, and Alkali-soluble Proteins.

Five g. of rice powder that was defatted and dried were mixed with 40 ccm of water, the mixture was shaken for 30 minutes by a shaking machine and then it was placed overnight in an ice-dox. The mixture

was then centrifuged for 20 minutes by a centrifugal machine. The nitrogen in 10 ccm of the supernatant solution was determined by Kjeldahl's method. The residue was treated with 10 % NaCl solution instead of water and nitrogen estimation was made as in the former case. Further nitrogen estimations were carried on also with the subsequent residues by means of 70 % alcohol and then 0.2 % NaOH solution. 0.5 g. of the rice was digested for determination of total nitrogen according to Kjeldahl's method.

Table I.

Distribution of nitrogen.

	Total N %	H_2O sol. N %	10 % NaCl sol. N %	70 % alcohol sol. N %	0.2 % NaOH sol. N %
Akita Common Hulled Rice	1.303	0.0439	0.1099	0.0549	0.2857
Akita Glutinous Hulled Rice	1.168	0.0549	0.1154	0.0604	0.3293
Etchu Common Hulled Rice	0.962	0.0439	0.1099	0.0769	0.2747
Etchu Glutinous Hulled Rice	1.110	0.0549	0.1389	0.0904	0.3897
Asahikawa Common Hulled Rice	1.236	0.0494	0.1209	0.0659	0.2970
Asahikawa Glutinous Hulled Rice	1.484	0.0549	0.1648	0.0714	0.4286
Hyogo Common Hulled Rice	1.211	0.0488	0.1323	0.0659	0.3956
Hyogo Glutinous Hulled Rice	1.365	0.0576	0.1586	0.0846	0.4835
Ibaragi Common Hulled Rice	1.255	0.0477	0.1088	0.0499	0.2511
Ibaragi Glutinous Hulled Rice	1.436	0.0556	0.1466	0.0623	0.4189
Saitama Common Hulled Rice	1.096	0.0488	0.1138	0.0605	0.3110
Saitama Glutinous Hulled Rice	1.353	0.0548	0.1678	0.0906	0.5246
Echigo Common Hulled Rice	1.199	0.0443	0.1055	0.0597	0.2883
Echigo Glutinous Hulled Rice	1.466	0.0599	0.1583	0.0755	0.4499

These figures are calculated on the basis of total nitrogen as 100 and tabulated as follows :—

Table II.

Distribution of nitrogen calculated on a basis of 100.

	Total N %	H_2O sol. N %	10 % NaCl sol. N %	70 % alcohol sol. N %	0.2 % NaOH sol. N %
Akita Common Hulled Rice	100.00	4.261	10.667	5.328	27.731
Akita Glutinous Hulled Rice	100.00	4.700	9.880	5.171	28.236
Etchu Common Hulled Rice	100.00	4.563	11.425	7.911	28.555
Etchu Glutinous Hulled Rice	100.00	4.991	12.627	5.332	34.963
Asahikawa Common Hulled Rice	100.00	3.997	9.781	4.811	26.675
Asahikawa Glutinous Hulled Rice	100.00	3.699	11.105	4.811	28.881
Hyogo Common Hulled Rice	100.00	4.029	10.935	5.441	25.235
Hyogo Glutinous Hulled Rice	100.00	4.218	11.615	6.195	35.481
Ibaragi Common Hulled Rice	100.00	3.799	8.667	3.975	20.003
Ibaragi Glutinous Hulled Rice	100.00	3.870	10.205	4.336	29.159
Saitama Common Hulled Rice	100.00	4.450	10.378	5.517	28.360
Saitama Glutinous Hulled Rice	100.00	4.049	12.399	6.694	38.764
Echigo Common Hulled Rice	100.00	3.692	8.793	4.975	24.029
Echigo Glutinous Hulled Rice	100.00	4.083	10.792	5.147	30.672

From the table, it is impossible to find a constant difference between the two kinds in the quantities of water soluble, 10 % NaCl solution soluble and 70 % alcohol solution soluble proteins, but the quantity of 0.2 % NaOH solution soluble protein is always greater in the glutinous than in the common rice as reported already by Kondo. Thus the following table indicates the fact clearly.

Table III.

Ratio between glutinous and common rices of .2% NaOH solution soluble protein.

0.2% NaOH solution soluble protein	Akita	Etchu	Asahi-kawa	Hyogo	Ibaragi	Saitama	Echigo
Glutinous	100	100	100	100	100	100	100
Common (ca.).	98	81	92	72	69	73	78

II. Separation and Purification of the Oryzenin.

The samples of the authors' research were collected from different parts of Japan in 1922 and 1923, i. e. in the 11th and 12th years of Taisho. There was no glutinous among the common rice, but the glutinous was adulterated with 0.1–2 % of common rice. The mixtures were removed as completely as possible and the material was washed with water till the washings became clear. It was next dried in the open air and then powdered with a stone mill. The powdered rice was extracted with ether in a Soxhlet's apparatus and again was dried in the open air.

To 250 g. of the powder was added 1500 ccm of 10 % NaCl solution and the mixture was shaken for thirty minutes by a shaking machine. After that it was placed overnight in an ice chamber. The upper liquid was decanted, the residue was filtered through a filter paper and was washed with distilled water until no chlorine was detected. Then to the residue were added a NaOH solution and water until the whole was made up to 1500 ccm and 0.2 % NaOH. The mixture was then shaken for thirty minutes by a shaking machine and placed overnight in an ice chamber. The upper liquid was obtained by a decantation and the residue was filtered. It was filtered several times until no iodine reaction to starch was observed by a Buchner's funnel which contained filter pulp of 1–2 cm thickness.

The clear filtrate thus obtained was very slightly acidified with dilute acetic acid whereupon the protein precipitated as a white amorphous mass. The upper solution was decanted off and again the precipitate was centrifuged for the sake of removing the liquid. The protein was placed in a 70 % alcohol solution, well stirred and again centrifuged. The same treatment was repeated three times.

Thus the alcohol soluble protein was all removed. The protein obtained was examined for the presence of the proteins soluble by water, 10 % NaCl solution and 70 % alcohol, and the absence of each of the three was verified. The separated protein was then dialysed by means of a dialyser for 1–2 weeks for the sake of removing mineral matter. The remaining protein was washed three times with absolute alcohol, three times with ether in a centrifugal machine and then dried in a H_2SO_4-desiccator of diminished pressure. The dried protein was powdered with an agate mortar and was again kept in a H_2SO_4-desiccator. The water and ash content of the material were determined as the following table indicates.

Table IV.

Ash and water content of oryzenins.

	Common rice Oryzenin			Glutinous rice Oryzenin		
	Water %	Ash % of dry matter	Ratio of common & glutinous	Water %	Ash % of dry matter	Ratio of common & glutinous
Akita	2.550	0.439	100.00	2.400	0.369	74.8
Etchu	3.850	0.564	100.00	4.250	0.418	74.1
Asahikawa	5.700	0.599	100.00	4.700	0.502	83.8
Hyogo	5.340	0.498	100.00	5.950	0.374	75.1
Saitama	9.269	0.318	100.00	3.432	0.291	91.4
Ibaragi	7.692	0.303	100.00	4.233	0.292	93.1
Echigo	8.807	0.373	100.00	4.422	0.314	81.2

III. Iso-electric Point of the Oryzenin.

Most proteins belong to an amphoteric electrolyte whose dissociation constant as an acid is always higher than the dissociation constant as a base. Probably this is due to the constitution of the amino acids which constitute the protein, and also to the abundance of the mono-amino acids which excel in their acidic properties. The iso-electric point of protein solution that is composed of so many amino acids and peptids with such different dissociation constants as an acid or a base, is attained when the concentration of the cation is equal

to that of the anion and the dissociation equilibrium is established. That occurs when the equation $Ka/Kb=C_H/C_{OH}$ is obtained, if Ka is a dissociation constant of an acid, Kb of a base, C_H a H-ion concentration and C_{OH} a OH-ion concentration. Therefore, it is said that the iso-electric point indicates the greatest value of the number of the electrically neutral particles or the undissociated residue.

Pauli[13], Matula[14], Loeb[15], Michaelis and Rona[16], Michaelis and Pechstein[17] and others have reported that at the iso-electric point, the protein solution shows the lowest value of osmotic pressure, viscosity, the quantity of alcohol demanded for precipitation of protein, electric conductivity and swelling. On the other hand, at the iso-electric point the protein solution shows the highest value of turbidity and surface tension. Therefore, if a difference can be discovered with respect to the iso-electric points of the oryzenins of glutinous and common rices, it can be assumed that there are quantitative or qualitative differences of the constituent amino acids and at the same time differences of condensing condition of such amino acids. The authors examined the iso-electric point of both oryzenins in the following preliminary experiments.

(1) Preliminary experiment.

(a) Change of the surface tension of the alkali protein solution in titration with HCl solution.

One-tenth g. of the oryzenin from each rice was dissolved in 10 ccm of $^1/_{50}$ normal NaOH solution. After standing for 24 hours (15° C.), 1 ccm of the protein solution was diluted with 9 ccm of pure distilled water (ca. pH 7.0). The mixture was titrated with $^1/_{100}$ normal HCl solution. The surface tension of the mixture was measured with a Nouy's apparatus[18] constructed on the principle of a torsion balance which torsion angle is proportional to the surface tension of the liquid. The following numbers are dyne per sq. cm. ($H_2O=75$) and are the mean of two experiments.

Table V.

Change of the surface tension of alkali protein solution.

HCl ccm (20°C.)		1.40	1.50	1.55	1.60	1.65	1.70	1.75	1.80	1.85	1.90	1.95	2.00
		0.1 g./10 ccm											
Hyogo	Common	61.5	62.3	64.6	63.0	62.7	62.1	61.7					
	Glutin.	61.5	61.3	62.1	63.1	64.0	62.0	61.4					
Ibaragi	Common	65.9	65.9	61.2	61.2	60.1	59.8	59.8	59.8	61.8	60.1	60.1	59.8
	Glutin.	76.6	66.6	64.6	63.9	63.2	61.8	60.5	59.8	60.5	60.5	61.8	59.8
Echigo	Common	64.6	64.6	60.5	60.5	59.8	59.8	59.8	59.8	61.8	59.9	59.1	—
	Glutin.	66.6	66.6	63.2	61.8	60.1	59.8	59.8	59.1	59.1	59.1	60.1	61.2
Saitama	Common	64.6	64.6	61.2	60.5	59.8	59.8	59.8	59.1	60.5	61.8	60.1	59.8
	Glutin.	66.6	66.6	63.9	60.5	59.8	59.8	59.8	59.8	59.1	59.8	60.1	61.2
Shonai	Common	64.6	64.6	60.5	59.8	59.8	59.8	59.1	59.1	61.2	60.5	59.8	—
	Glutin.	65.9	65.9	61.2	60.5	59.8	59.8	59.8	59.8	59.1	59.1	61.2	—
		0.07 g./10 ccm											
Mie	Common		63.2	—	61.2	—	59.8	—	61.2	—	62.7	—	61.2
	Glutin.		64.2	—	61.2	—	59.8	—	59.8	—	61.2	—	62.7
Shiga	Common		63.2	—	61.2	—	59.8	—	59.8	—	63.9	—	61.2
	Glutin.		64.2	—	61.2	—	59.8	—	59.8	—	60.5	—	62.7

In the above table, the maximum point of surface tension shows a marked difference between the two kinds of oryzenins, i. e. glutinous rice protein solution reached the maximum surface tension at a point of greater addition of HCl than did the common rice protein.

(b) Change of the turbidity of the alkali protein solution in titration with HCl solution.

As in the above experiments, 0.07–0.1 g. of the oryzenin was dissolved in 10 ccm of $^1/_{50}$ normal NaOH solution and 1 ccm of the protein solution was diluted with 9 ccm of pure distilled water. The mixture was titrated with a $^1/_{100}$ normal HCl solution and the point of maximum turbidity was determined, using a Dubosque's Nephelometer. These figures show the turbidity, i. e. the large number shows the stronger turbidity. They are the mean of two experiments.

Table VI.

Change of the turbidity of the alkali protein solution.

(20°C.) HCl ccm		1.40	1.50	1.55	1.60	1.65	1.70	1.75	1.80	1.85	1.90	1.95	2.00
		0.1 g./10 ccm											
Hyogo	Common	1.00	18.5	39.7	30.2	13.5	7.0						
	Glutin.	1.00	4.0	8.8	14.7	29.5	12.5						
		0.07 g./10 ccm											
Saitama	Common		1.0	2.3	4.2	9.5	10.5	15.3	20.0	28.2	37.1	25.8	20.2
	Glutin.		1.0	2.0	3.2	5.8	8.2	12.2	16.5	20.2	22.5	23.4	28.7
Ibaragi	Common		1.0	2.0	3.8	7.8	11.5	14.0	19.2	29.5	33.5	27.0	18.7
	Glutin.		1.0	1.8	2.0	4.5	7.5	8.9	12.5	19.5	20.2	22.0	24.8
Echigo	Common		1.0	2.9	4.2	6.7	10.5	17.5	28.2	34.2	25.2	20.7	17.8
	Glutin.		1.0	1.5	2.8	3.7	4.2	5.8	12.2	18.5	22.1	24.3	20.0
Shonai	Common		1.0	2.3	3.9	7.0	8.5	18.0	22.0	29.3	20.1	19.3	—
	Glutin.		1.0	1.7	2.5	4.2	5.0	7.9	13.5	18.5	21.5	27.5	22.0
Shiga	Common		1.0	4.2	7.8	10.0	13.2	18.7	23.5	26.7	30.2	26.7	20.5
	Glutin.		1.0	2.1	3.4	7.5	9.0	12.5	15.0	19.2	22.5	24.6	26.5
Mie	Common		1.0	3.7	4.2	8.5	14.5	17.7	20.5	24.0	33.5	27.0	19.9
	Glutin.		1.0	2.5	3.5	5.8	9.0	12.5	17.2	20.0	22.3	24.0	28.5

According to the table, to obtain the maximum turbidity of glutinous rice protein solution a large quantity of acid is needed, while in the case of common rice protein solution the reverse is true.

(2) Estimation of the iso-electric point of the protein solution.

From the results of the preliminary experiments, it was to be supposed that there must be a difference between the iso-electric points of the common and the glutinous rice oryzenins. The authors, therefore, attempted the following more accurate method to compare the iso-electric points. 0.07 g. of the protein was dissolved in 10 ccm of $^1/_{10}$ normal NaOH solution, and 0.5 ccm of the solution was measured in a cleanly washed and dried hard glass test tube. Then the solution was brought to a known pH value mixing to it Sörensen's K-phthalate, NaOH mixture[19]. The surface tension of the turbid fluid thus obtained was measured by Nouy's apparatus and the viscosity by Ostwald's viscosimeter.

Table VII.

Change of surface tension of oryzenins dissolved in alkali.

(pH value colorimetric method)		4.6	4.8	5.0	5.2	5.4	5.6
Electrical method (m. volt) 18° C.		522	550	565	571	581	590
pH calculated from above m.v.		4.67	5.18	5.43	5.51	5.70	5.85
Surface tension (16° C.)		dy/cm	dy/cm	dy/cm	dy/cm	dy/cm	dy/cm
Saitama	Common	61.2	62.5	62.5	64.2	62.5	60.5
	Glutinous	61.2	63.9	62.5	63.2	63.2	60.5
Echigo	Common	61.8	61.2	61.8	61.8	63.2	61.8
	Glutinous	61.2	62.2	61.8	61.8	61.8	60.5
Ibaragi	Common	61.2	61.8	60.5	64.2	63.9	63.2
	Glutinous	61.8	63.2	61.2	61.2	61.2	60.5
Asahikawa	Common	62.5	61.9	61.2	60.5	64.2	63.3
	Glutinous	62.5	61.8	63.9	62.5	61.9	61.9
Shonai	Common	62.5	61.8	61.8	62.5	63.2	61.2
	Glutinous	61.2	62.5	61.2	61.2	61.2	60.5
Etchu	Common	62.5	62.5	61.9	62.5	62.2	64.2
	Glutinous	63.2	63.2	64.2	63.2	61.9	62.5
Akita	Common	63.9	63.2	62.5	63.2	64.2	63.9
	Gluitnous	63.9	63.2	64.2	63.2	62.5	61.9
Hyogo	Common	61.9	62.5	62.5	63.2	64.2	62.5
	Glutinous	62.5	61.5	63.2	62.5	61.9	62.5

Change of viscosity of oryzenins dissolved in alkali.

Viscosity (flowing time 15° C.)							
Saitama	Common	1'21.8''	1'21.8''	1'21.8''	1'21.0''	1'22.0''	1'22.8''
	Glutin.	1'21.8''	1'19.8''	1'20.0''	1'20.4''	1'21.2''	1'20.8''
Echigo	Common	1'16.8''	1'17.2''	1'17.2''	1'16.8''	1'16.2''	1.17.2''
	Glutin.	1'17.0''	1'15.8''	1'16.8''	1'16.8''	1'16.8''	1'17.0''
Ibaragi	Common	1'21.8''	1'21.2''	1'21.4''	1'20.2''	1'21.6''	1'22.0''
	Glutin.	1'21.8''	1'20.8''	1'21.0''	1'21.2''	1'21.8''	1'22.0''
Shonai	Common	1'16.8''	1'17.4''	1'16.8''	1'17.4''	1'16.4''	1'17.8''
	Glutin.	1'16.8''	1'15.8''	1'15.0''	1'15.8''	1'16.8''	1'16.8''
Etchu	Common	1'24.2''	1'23.6''	1'24.2''	1'23.8''	1'23.6''	1'22.8''
	Glutin.	1'23.4''	1'23.8''	1'23.2''	1'24.8''	1'25.0''	1'25.4''
Akita	Common	1'26.6''	1'25.8''	1'25.4''	1'25.2''	1'24.2''	1'26.8''
	Glutin.	1'26.4''	1'25.8''	1'24.4''	1'25.4''	1'24.8''	1'25.2''
Asahikawa	Common	1'21.6''	1'21.6''	1'21.4''	1'22.0''	1'21.0''	1'22.2''
	Glutin.	1'20.8''	1'20.8''	1'20.0''	1'21.2''	1'21.2''	1'21.2''

The table shows that in the same kind of rice protein solution the pH value of the maximum surface tension and minimum viscosity show the same tendency though the rice was produced in different provinces. The difference of pH values of the iso-electric points in the two kinds of oryzenin is 0.07–0.52 and those of all glutinous rice proteins are more acidic than those of common rice proteins. However, slight differences are observed among the iso-electric points of common rice proteins, produced in different places. The fact shows that the iso-electric point of the common rice protein is found nearer to the alkaline side or on the higher pH value than the glutinous. Therefore, the protein of the former contains a larger proportion of the amino group than of the carboxyl group.

IV. Other Physico-chemical Properties.

The physico-chemical properties such as solubility, refractive index and rotatory power have intimate relation to the molecular structure of organic compounds and specially to that of protein. The authors intending to investigate these properties in both kinds of oryzenin solutions performed the following experiments.

(a) The solubility of oryzenin in alkali solution.

Robertson[20] once discussed the solubility of protein and stated that it is concerned with its polypeptide structure. Equal quantities of both kinds of oryzenin were taken and dissolved in equal quantities of dilute alkali solution. After standing for a fortnight in an ice-box, the oryzenin solution of common rice was more turbid than that of glutinous rice. It was then proposed to examine the solubility of oryzenin by the following two different methods.

(1) One-tenth g. of sifted (0.5 mm sieve) oryzenin powder was added to 25 ccm of $^1/_{100}$ normal NaOH solution. The whole was shaken well, and placed for 24 hours in an ice-box. Then the solution was filtered by a dried filter paper and the nitrogen of the residue was determined. The following numbers are calculated on the basis of 100 ccm of filtrate.

Table VIII.

Ratio of solubility in alkali of various oryzenins.

Kind of rice	Akita		Etchu		Asahikawa	
	Common	Glutinous	Common	Glutinous	Common	Glutinous
Nitrogen	0.01511	0.01167	0.02406	0.01099	0.01648	0.01374
Ratio	100.0	76.5	100.0	45.6	100.0	83.3

From the above table, it is to be expected that the common rice oryzenins are more difficult to dissolve in alkali solution than the glutinous rice oryzenins.

(2) One-tenth g. of the oryzenin was dissolved in 10 ccm of $^1/_{50}$ normal NaOH solution and 1 ccm of the protein solution was diluted with 9 ccm of pure distilled water. The mixture was titrated with a $^1/_{100}$ normal HCl solution and the resultant turbid liquid dissolved by further addition of HCl solution. Thus the authors compared the solubility of common rice oryzenin to that of glutinous. The following figures show the turbidities of the protein solutions.

Table IX.

Turbidity of various oryzenins dissolved in alkali.

HCl quantity		1.70 ccm	1.80 ccm	2.00 ccm	2.10 ccm	2.20 ccm
Mie	Common	1.00	2.00	7.50	12.00	2.73
	Glutinous	1.30	1.69	10.95	7.80	1.56
Ibaragi	Common	1.00	2.00	8.40	7.90	3.30
	Glutinous	1.10	2.86	7.32	2.06	1.45
Shiga	Common	1.00	5.00	16.00	15.00	7.50
	Glutinous	2.00	5.00	14.80	10.90	2.00
Saitama	Common	1.00	3.32	20.02	15.20	6.50
	Glutinous	1.20	1.98	12.00	7.98	2.06
Echigo	Common	1.00	2.61	7.50	6.65	2.14
	Glutinous	1.00	1.88	7.50	4.62	1.73

The table shows that common rice oryzenin solution becomes turbid by an addition of a small quantity of HCl and holds its turbid condition longer in the presence of a large quantity of HCl solution, while glutinous rice oryzenin solution becomes turbid with greater difficulty and is easy to re-dissolve by an addition of large quantities of HCl solution.

(b) The refractive index of oryzenin alkali solution.

Kondo[12] has examined the refractive indices of protein extracted from hulled rice by NaOH solution and has deduced that the common rice protein is constituted optically more dense than that of the glutinous owing to the higher refractive index of the former. The authors, however, examined the same index using a pure oryzenin freed from any trace of water soluble, 10 % NaCl solution soluble or 70 % alcohol solution soluble proteins.

The oryzenin was dissolved in $^1/_{10}$ normal NaOH solution to make the solution contain 7.5 % oryzenin. The refractive index of the solution was examined by means of Ferry's refractometer at 25–26°C.

Table X.

The refractive indices of various oryzenins in alkali.

Kind of rice	Toyama		Niigata		Mie	
	Common	Glutinous	Common	Glutinous	Common	Glutinous
Refractive index	1.3460	1.3432	1.3448	1.3435	1.3465	1.3443
Its difference	0.0028		0.0013		0.0022	

The table shows that the refractive index of the common is always higher than that of the glutinous.

(c) The specific rotatory power of oryzenin alkali solution.

The rotatory power of an organic compound is caused by the presence of asymmetric carbon atoms in its molecule and it is increased by the complexity of molecule, by the saturation in molecular structure and by the special atomic groups[21]. Therefore, it has great meaning for this investigation to examine the rotatory power of the alkali solution of oryzenin. One-tenth g. of each kind of oryzenin was dissolved in 15 ccm of $^1/_{10}$ or $^1/_{25}$ normal NaOH solution and the solution was examined by a Haensch-Schmidt half shadow polariscope. After portions of each solution had been illuminated 20 and 50 minutes respectively under a Quartz lamp at a height of 1 foot, their rotatory power was determined in the same way.

Table XI.

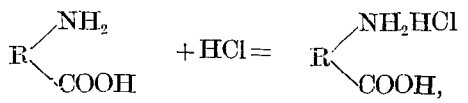
Rotatory power of various oryzenins dissolved in alkali.

Sp. rotatory power		0.1 g. N/25 NaOH	0.1 g. N/10 NaOH		
			Illumination $[\alpha]_D^{20°}$		
		$[\alpha]_D^{20°}$ $\alpha°$	0′	20′	50′
Saitama	Common	—64.875 (—2.5)	—77.850 (—3.0)	—70.065	—64.875
	Glutinous	—59.685 (—2.3)	—59.685 (—2.3)	—53.1975	—51.900
Ibaragi	Common	—60.982 (—2.35)	—80.445 (—3.1)	—72.660	—59.685
	Glutinous	—54.480 (—2.1)	—60.982 (—2.35)	—54.480	—50.602
Shonai	Common	—62.205 (—2.4)	—75.255 (—2.9)	—67.470	—59.685
	Glutinous	—59.685 (—2.3)	—60.982 (—2.35)	—55.792	—51.900
Echigo	Common	—63.5775 (—2.45)	—67.470 (—2.6)	—62.205	—58.387
	Glutinous	—60.9825 (—2.35)	—62.205 (—2.4)	—50.602	—54.480
Akita	Common	—62.205 (—2.4)	—76.552 (—2.95)	—66.170	—51.900
	Glutinous	—55.7925 (—2.15)	—58.387 (—2.25)	—54.480	—53.197
Etchu	Common	—62.205 (—2.4)	—75.255 (—2.9)	—54.480	—54.580
	Glutinous	—51.900 (—2.0)	—54.480 (—2.1)	—49.305	—49.305
Asahikawa	Common	—59.685 (—2.3)	—62.205 (—2.4)	—57.090	—57.090
	Glutinous	—49.305 (—1.9)	—53.197 (—2.05)	—52.205	—51.900
Hyogo	Common	—60.982 (—2.35)	—71.362 (—2.75)	—62.205	—62.205
	Glutinous	—51.900 (—2.0)	—55.792 (—2.15)	—49.305	—51.900

The rotatory power of the alkali oryzenin solution of common rice shows higher values than that of glutinous rice. In the presence of much alkali, the former increases its rotatory power at a higher ratio than that of glutinous.

V. The Determination of the Quantities of HCl Combined with the Oryzenin.

As the simplest case of the combination of the inorganic acid with a protein occurs according to the reaction



it may be said that in a given concentration of HCl, the HCl combining with the added protein is proportional to the free amino groups of the protein. However, according to Robertson[21], the -COHN- group of keto form ($H_2N{\cdot}CH_2CO{\cdot}HN{\cdot}CH_2COOH$) of polypeptide linkage

has a neutralizing effect, and enol form ($H_2N \cdot CH_2C(OH)NCH_2COOH$) changes the valence of N of the -C(OH)N- group from three to five and combines with acid according to the formula

$$—CO:N— + HCl = (COH)^{++} + :\underset{\underset{Cl}{\cdot}}{\overset{\overset{H}{\cdot}}{N}}—.$$

These considerations lead elsewhere than to the difference of the quantity of free amino nitrogen of the common and glutinous rice oryzenins. Another difference of combining power with acid must exist in both kinds of oryzenin.

Cohnheim[22] proposed a method of calculating the combination degree of HCl with protein as follows. The inversion of sucrose by pure HCl is a mono-molecular reaction and follows the formula

$$\log \frac{A}{A-X} = Kt,$$

where A is the concentration of sucrose before inversion, X the same after inversion of a given time t, and K is a constant which is a direct variable of the hydrogen ion concentration of the solution. If two inversions of sucrose by HCl with and without protein were carried out, the quantity of HCl combined with the protein may be calculated from the formula

$$\frac{C}{C'} = \frac{\log A - \log (A-X)}{\log A - \log (A-X')},$$

where C represents the hydrogen ion concentration of pure HCl, C' the same of a solution of protein in HCl, A the quantity of sucrose used, X and X' the quantities of sucrose inverted by pure acid and protein acid solution respectively. C and C' are figures proportional to the hydrogen ion concentration of the solution.

Twenty mg. of oryzenin were dissolved in 5 ccm of $^1/_{10}$ normal HCl solution and the whole was stood overnight. To the protein solution was added 25 ccm of ca. 5 % sucrose solution. The mixture was inverted for exactly 5 minutes in a boiling water bath. At the end of the time, the solution was cooled to room temperature and the quantity of inverted sugar was estimated by Bertland's method. The quantity of sucrose was calculated multiplying a factor 0.9499 to the obtained results. The quantity of HCl in free state is calculated as follows, where $^1/_{10}$ normal HCl solution contains 18.35 mg (C) of HCl in 5 ccm and the concentration of used sucrose was 1423.966 mg. in 25 ccm.

Table XII

Combining power of oryzenins with HCl.

		$KMnO_4$ (ccm)	Cu (mg)	Invert sugar (mg)	Sucrose (mg)	Sucrose in 25 ccm (mg)
HCl		35.15	352.695	195.427	185.650	1113.899
Akita	Common	31.25	313.563	171.838	163.241	979.445
	Glutinous	32.20	323.095	177.457	168.579	1011.473
Etchu	Common	30.60	303.040	167.924	159.523	957.136
	Glutinous	30.90	310.051	169.730	161.238	967.430
Asahikawa	Common	31.60	317.074	173.945	165.242	991.455
	Glutinous	33.00	331.122	182.273	173.154	1038.925
HCl		36.40	359.562	196.446	186.617	1119.701
Saitama	Common	35.25	544.417	186.910	177.559	1065.353
	Glutinous	35.60	347.927	189.257	179.788	1078.731
Ibaragi	Common	35.30	344.906	187.286	177.916	1067.497
	Glutinous	35.60	349.837	190.411	180.885	1085.307
Echigo	Common	35.40	345.882	188.119	178.707	1072.214
	Glutinous	35.80	349.791	190 386	180.861	1085.166

$$\frac{C}{C'} = \frac{\log A - \log (A-X)}{\log A - \log (A-X')}$$

$\log A = 3.15330$		$\log (A-X) = 2.491369$	$\log A - \log (A-X) = 0.661931$
Akita	Common	$\log (A-X') = 2.647891$	$\log A - \log (A-X') = 0.505409$
	Glutinous	$\log (A-X') = 2.615413$	$\log A - \log (A-X') = 0.537887$
Etchu	Common	$\log (A-X') = 2.669157$	$\log A - \log (A-X') = 0.484143$
	Glutinous	$\log (A-X') = 2.659476$	$\log A - \log (A-X') = 0.493824$
Asahikawa	Common	$\log (A-X') = 2.635901$	$\log A - \log (A-X') = 0.517399$
	Glutinous	$\log (A-X') = 2.585505$	$\log A - \log (A-X') = 0.567795$
		$\log (A-X) = 2.460678$	$\log A - \log (A-X) = 0.688093$
Saitama	Common	$\log (A-X') = 2.535500$	$\log A - \log (A-X') = 0.613221$
	Glutinous	$\log (A-X') = 2.518278$	$\log A - \log (A-X') = 0.630493$
Ibaragi	Common	$\log (A-X') = 2.532823$	$\log A - \log (A-X') = 0.615948$
	Glutinous	$\log (A-X') = 2.509533$	$\log A - \log (A-X') = 0.639238$
Echigo	Common	$\log (A-X') = 2.526742$	$\log A - \log (A-X') = 0.622029$
	Glutinous	$\log (A-X') = 2.509723$	$\log A - \log (A-X') = 0.639048$

		HCl in free state (mg)	HCl in combined state (mg)
Akita	Common	13.903	4.312
	Glutinous	14.818	3.417
Etchu	Common	13.337	4.898
	Glutinous	13.604	4.613
Asahikawa	Common	14.253	3.982
	Glutinous	15.642	2.582

		HCl in free state (mg.)	HCl in combined state (mg.)
Saitama	Common	16.251	1.984
	Glutinous	16.709	1.526
Ibaragi	Common	16.526	1.909
	Glutinous	16.940	1.295
Echigo	Common	16.470	1.765
	Glutinous	16.935	1.300

In the case of the common rice oryzenin, the quantity of HCl combined is always greater than in the case of glutinous. This is also an evidence from the opposite side that the free amino group is predominant in the former compared with the latter.

VI. The Elementary Composition of Oryzenins.

For reference, the data of Takahashi and Sato[5] obtained from two hulled rices produced in Osaka-fu are stated, as follows:

Table XIII.

Takahashi and Sato's data of the elementary composition of oryzenins.

Elements	Carbon %	Hydrogen %	Nitrogen %	Oxygen & Sulphur %
Free from prolamin I	50.15	8.8	17.6	23.45
II	49.50	8.5	15.5	26.50

The data of others are not derived from oryzenin free from albumin, globulin, and prolamin, so they are omitted here.

The authors estimate carbon and hydrogen by the ordinary method using an electric furnace. Nitrogen is estimated by Kjeldahl's method.

Table XIV.

Elementary composition of oryzenins.

Kind of rice		Sample (g.)	CO_2(g.)	H_2O(g.)	Carbon (%)	Hydrogen (%)
Akita						
Common	1	0.1008	0.1926	0.0897	52.12	9.89
	2	0.0967	0.1864	0.0812	52.59	9.33
	Average				52.35	9.61
Glutinous	1	0.1031	0.1929	0.0806	51.03	8.68
	2	0.1018	0.1902	0.0803	51.06	8.76
	Average				51.04	8.72

Kind of rice		Sample (g.)	CO_2(g.)	H_2O(g.)	Carbon (%)	Hydrogen (%)
Etchu						
Common	1	0.1032	0.1968	0.0911	52.01	9.81
	2	0.0998	0.1901	0.0880	51.59	9.80
	Average				51.98	9.80
Glutinous	1	0.1115	0.2017	0.0863	50.15	8.60
	2	0.1004	0.1932	0.0827	49.52	8.63
	Average				49.83	8.61
Asahikawa						
Common	1	0.1034	0.1976	0.0814	52.12	8.75
	2	0.1047	0.1980	0.0826	51.58	8.77
	Average				51.85	8.76
Glutinous	1	0.1040	0.1928	0.0782	50.56	8.36
	2	0.1168	0.2169	0.0879	50.62	8.36
	Average				50.59	8.35
Saitama						
Common	1	0.0575	0.1178	0.0457	55.79	8.83
	2	0.0949	0.1938	0.0760	55.68	8.83
	Average				55.74	8.83
Glutinous	1	0.0995	0.1858	0.0639	50.90	7.13
	2	0.1030	0.1886	0.0684	50.45	7.12
	Average				50.67	7.12
Ibaragi						
Common	1	0.0753	0.1383	0.0601	50.04	8.87
	2	0.0855	0.1596		50.87	
	3	0.1185		0.0917		8.60
	Average				50.45	8.73
Glutinous	1	0.0751	0.1349	0.0510	48.88	7.55
	2	0.0868	0.1530	0.0606	48.09	7.75
	Average				48.48	7.65
Echigo						
Common	1	0.1122	0.2019	0.0835	50.84	8.27
	2	0.0959	0.1797		51.08	
	3	0.1054		0.0792		8.36
	Average				50.96	8.31
Glutinous	1	0.0966	0.1527	0.0642	45.53	7.38
	2	0.1113	0.1864	0.0774	45.75	7.72
	Average				45.63	7.55

The above data are summarized and the ratio of carbon to oxygen is calculated.

Table XV.

Elementary composition of oryzenin by per cent and ratio of carbon to oxygen.

Kind of rice		Nitrogen (%)	Hydrogen (%)	Carbon (%)	Oxygen (%)	Ratio C/O
Akita	Common	18.33	9.61	52.35	18.85	2.777
	Glutinous	17.59	8.72	51.04	21.89	2.331
Etchu	Common	17.86	9.80	51.98	19.54	2.659
	Glutinous	16.86	8.62	49.83	23.99	2.077
Asahikawa	Common	17.30	8.76	51.85	21.39	2.424
	Glutinous	16.22	8.35	50.59	24.18	2.092
Hyogo	Common	18.64	8.27	53.15	19.31	2.751
	Glutinous	17.67	7.47	51.45	22.80	2.256
Saitama	Common	19.31	8.83	55.74	15.31	3.640
	Glutinous	18.38	7.12	50.67	23.06	2.197
Ibaragi	Common	19.07	8.73	50.45	20.98	2.404
	Glutinous	18.02	7.65	48.48	25.17	1.926
Echigo	Common	18.45	8.31	50.96	21.53	2.366
	Glutinous	17.50	7.55	45.63	29.67	1.537

The nitrogen content of common rice oryzenin is ca. 1 % higher than that of glutinous rice oryzenin. This is the reason why the alkalinity of the former is superior to that of the latter. The lower ratio of carbon to oxygen of the glutinous rice oryzenin as compared with that of the common rice oryzenin shows the predominance of the acidity and the carboxyl groups of the former.

Sulphur was estimated by Denis-Benedict's method and phosphorous present in the ash of oryzenin, by the ordinary method.

Table XVI.

Sulphur and phosphorus content of oryzenins and ratios existing between them in various kinds of rice.

% of dry matter / Kind of rice		Sulphur (%)	Phosphorus (%)	Ratio of S of glutinous to common	Ratio of P of glutinous to common
Akita	Common	0.892	0.166	100.0	88.4
	Glutinous	0.779	0.188	93.2	100.0
Etchu	Common	0.828	0.162	100.0	87.1
	Glutinous	0.777	0.186	87.4	100.0

% of dry matter / Kind of rice		Sulphur (%)	Phosphorus (%)	Ratio of S of glutinous to common	Ratio of P of glutinous to common
Asahikawa	Common	0.745	0.153	100.0	87.6
	Glutinous	0.695	0.175	93.9	100.0
Hyogo	Common	0.635	0.167	100.0	88.3
	Glutinous	0.596	0.189	93.8	100.0
Saitama	Common	0.809	0.103	100.0	60.3
	Glutinous	0.771	0.168	95.2	100.0
Ibaragi	Common	0.773	0.101	100.0	57.6
	Glutinous	0.681	0.174	88.0	100.0
Echigo	Common	0.749	0.095	100.0	57.6
	Glutinous	0.654	0.164	87.0	100.0

The existence of phosphorus in the ash of oryzenin was observed by the authors to be about $^1/_5$ of the amount of sulphur. There were, however strange tendencies that the higher sulphur content and the lower phosphorus content were found in the common rice oryzenin and vice versa in the glutinous rice oryzenin.

VII. The Separation and Determination of Amino Acids.

The factor which controls the iso-electric point of a protein is the dissociation constant of amino acids and poly-peptids which compose the protein molecule; thus, the protein's iso-electric point is effected according to the predominance of dissociation constants as acids or bases. In moncamino acids the dissociation constants as acids are predominant, while in diamino acids and amides which decompose to ammonia easily, the dissociation constants as bases are predominant. Accordingly, it has a very important meaning for a comparison of proteins to separate and determine the quantity and kinds of amino acids of a protein.

A difference of amino acids both in quantity and kind of the two oryzenins may be expected, from the data of elementary analyses, which show that the quantities of the nitrogen and carboxyl groups differ markedly.

The authors thus undertook the following experiments according

to Van Slyke's method[23]. To a given quantity of sample was added 20 times its weight of 20 % HCl; the mixture was boiled and hydrolyzed for 8 hours on a sand bath under a reflux condenser. Next, almost all the HCl of the solution was driven off under a diminished pressure at below 40°C. The residue was neutralized and made slightly alkaline by the addition of 10 % solution of calcium hydroxide. The ammonia nitrogen liberated was distilled into a standard sulphuric acid under a diminished pressure at below 40°C. The remaining fluid was filtered, and separated from melanin nitrogen. The melanin nitrogen was determined by Kjeldahl's method. The filtrate was acidified with HCl and evaporated under diminished pressure at below 40°C. To the concentrated filtrate were added 18 ccm of conc. HCl and 15 g. of phosphotungstic acid and the diamino acids were precipitated. After 48 hours' undisturbed standing, the precipitate was filtered by suction and treated with a mixture of ether and amyl alcohol according to Van Slyke's modified method[24]. The diamino acid nitrogen was determined separately. Using Van Slyke's micro-apparatus the free amino nitrogen was determined.

Table XVII shows the results of the experiments described and Table XVIII shows the percentages of each nitrogen to the total nitrogen of oryzenin.

Table XVII.

Kinds of nitrogen found in oryzenin.

Kind of nitrogen / Kind of rice	Total N	Ammonia N	Melanin N	Mono-amino N	Di-amino N	Argi-nin N	Lysin N	His-tidin N	Cystin N
Akita									
Common	18.330	1.784	0.409	7.640	8.500	5.616	0.978	1.737	0.179
Glutinous	17.590	1.286	0.409	7.740	8.153	4.515	0.554	2.859	0.225
Etchu									
Common	17.860	1.633	0.437	7.840	7.953	5.061	0.555	2.173	0.164
Glutinous	16.860	1.497	0.356	7.740	7.269	3.566	0.402	3.102	0.199
Asahikawa									
Common	17.300	1.700	0.343	9.050	6.209	2.836	1.790	1.369	0.212
Glutinous	16.220	1.153	0.319	8.640	6.108	2.763	1.035	1.939	0.371
Hyogo									
Common	18.640	1.697	0.459	7.777	8.711	5.117	1.159	2.265	0.169
Glutinous	17.672	1.564	0.391	7.613	8.102	3.704	0.529	3.265	0.206

Kind of nitrogen / Kind of rice	Total N	Ammonia N	Melanin N	Monoamino N	Diamino N	Arginiu N	Lysin N	Histidin N	Cystin N
Saitama									
Common	19.319	1.795	0.598	8.343	8.583	5.088	1.546	1.042	0.133
Glutinous	18.385	1.568	0.533	8.177	8.106	3.512	2.574	0.446	0.285
Ibaragi									
Common	19.048	1.764	0.588	8.212	8.412	4.988	1.461	1.115	0.116
Glutinous	18.023	1.474	0.453	8.147	7.948	3.488	2.413	0.570	0.270
Echigo									
Common	18.458	1.786	0.535	8.047	8.088	4.806	1.459	1.038	0.100
Glutinous	17.500	1.477	0.397	7.904	7.721	3.422	2.352	0.525	0.245

Table XVIII.

Nitrogen percentages.

Kind of nitrogen / Kind of rice	Total N	Ammonia N	Melanin N	Monoamino N	Diamino N	Argining N	Lysin N	Histidin N	Cystin N
Akita									
Common	100.00	9.732	2.234	41.680	46.371	30.638	5.336	9.476	0.976
Glutinous	100.00	7.311	2.319	44.002	46.350	25.668	3.149	16.253	1.279
Etchu									
Common	100.00	9.143	2.447	43.897	44.530	26.337	3.107	12.167	0.918
Glutinous	100.00	8.879	2.111	45.907	43.114	21.150	2.384	18.398	1.180
Asahikawa									
Common	100.00	9.826	1.983	52.312	35.890	16.404	10.347	7.913	1.225
Glutinous	100.00	7.108	1.962	53.167	37.657	16.868	6.381	11.954	2.287
Hyogo									
Common	100.00	9.105	2.405	41.713	46.722	27.444	6.216	12.150	0.910
Glutinous	100.00	8.851	2.213	43.084	45.850	20.959	2.482	20.742	1.165
Saitama									
Common	100.00	9.293	3.097	43.178	44.427	26.340	5.393	8.003	0.689
Glutinous	100.00	8.528	2.904	44.478	44.092	19.104	2.425	14.004	1.555
Ibaragi									
Common	100.00	9.263	3.087	43.115	44.164	26.190	5.854	7.672	0.612
Glutinous	100.00	8.178	2.516	45.205	43.544	19.355	3.162	13.387	1.497
Echigo									
Common	100.00	9.677	2.903	45.597	43.821	26.040	5.569	7.742	0.542
Glutinous	100.00	8.440	2.272	45.165	44.121	19.553	3.004	13.434	1.403

Table XIX.

Percentages of nitrogen contained in different amino acids as found in common and glutinous rices. The maximum quantity is taken as 100.

Kind of rice		Ammonia N	Monoamino N	Arginin N	Lysin N	Histidin N	Cystin N
Akita	Common	100.00	94.72	100.00	100.00	58.30	76.30
	Glutinous	75.12	100.00	84.52	59.01	100.00	100.00
Etchu	Common	100.00	95.62	100.00	100.00	66.13	77.79
	Glutinous	97.11	100.00	80.30	76.72	100.00	100.00
Asahikawa	Common	100.00	98.29	100.00	100.00	66.19	53.56
	Glutinous	72.33	100.00	? 102.82	61.67	100.00	100.00
Hyogo	Common	100.00	96.81	100.00	100.00	58.57	78.11
	Glutinous	97.29	100.00	76.37	47.98	100.00	100.00
Saitama	Common	100.00	97.07	100.00	100.00	57.14	44.30
	Glutinous	91.70	100.00	72.52	44.96	100.00	100.00
Ibaragi	Common	100.00	95.39	100.00	100.00	57.30	40.88
	Glutinous	88.29	100.00	73.90	54.01	100.00	100.00
Echigo	Common	100.00	96.52	100.00	100.00	57.62	38.64
	Glutinous	87.21	100.00	75.08	53.94	100.00	100 00

According to Table XIX, with a few exceptions, there are constant proportions as follows:

Table XX.

Constant ratios between the nitrogen containd in different amino acids formed as decomposition products of common and glutinous rice oryzenins.

	Ammonia N	Monoamino N	Arginin N	Lysin N	Histidin N	Cystin N
Rich in	Common	Glutinous	Common	Common	Glutinous	Glutinous
Maximum	97.29	97.07	84.52	76.72	66.72	78.11
Minimum	75.12	94.72	72.52	44.96	52.14	38.64
Averag e	87.00	96.33	73.11	56.89	60.17	58.51

Thus it is seen that in common rice oryzenin there is a predominance of ammonia, arginin and lysin nitrogen, while in the glutinous there is an excess of monoamino, histidin and cystin nitrogen. The quantities of ammonia nitrogen in the glutinous rice oryzenin are about

87 % of those of the common, arginin nitrogen 73 %, lysin nitrogen about 60 %, while monoamino nitrogen of the common is 96 % of the glutinous, and histidin and cystin nitrogen ca. 60 %.

The production of ammonia nitrogen of the common rice cryzenin is superior to that of the glutinous. Experiments were carried out to determine whether or not this holds under different conditions of hydrolysis, that is under changed acidity and duration of hydrolysis. The used acidities were 20 % and 10 % HCl and the time duration was 4 hours while the other treatment was the same as described above.

Table XXI.

Production of ammonia nitrogen.

Conc. of HCl	Time duration	Akita		Etchu		Asahikawa	
		Common	Glutinous	Common	Glutinous	Common	Glutinous
20 %	4 hours	1.851	1.683	1.717	1.683	1.786	1.628
10 %	4 hours	1.704	1.510	1.616	1.561	1.634	1.634

From the table, it is observed that the common rice oryzenin always produced more ammonia than the glutinous and that the greater proportion of it was produced when hydrolyzed with 10 % HCl for 4 hours.

VIII. The Determination of Tyrosin and Tryptophan.

For the determination of tyrosin of a protein, Fürth and Fleischmann[25] had proposed the colorimetric method of diazo reaction. One half gram of oryzenin was hydrolyzed with 20 % HCl under a reflux condenser for 12 hours. To the solution was added 20 % of phosphotungstic acid, the mixture was filtered and washed with a small quantity of HCl. The phosphotungstic acid of the filtrate was freed by barium hydroxide. From the filtrate from barium phosphotungstate the barium hydroxide was precipitated by a saturation of sodium carbonate. The filtrate was neutralized with HCl and was concentrated to 50 ccm. To this solution and a standard solution of tyrosin were added a reagent of sulphuric acid and sodium nitrate, and after NaOH had also been added a colorimetric estimation was carried out.

Using the method of Fürth and Nobel[26a,b], the tryptophan content was determined. 0.1 g. of oryzenin was dissolved in 20 ccm of $^1/_5$ normal NaOH solution. 2 ccm of the solution were taken in a 30 ccm colorimeteric tube. Added one drop of 25 % formaldehyde solution,

and filled up to 20 ccm with conc. HCl. With 2 ccm of 0.02 % tryptophan solution (0.002 g. tryptophan dissolved in 10 ccm of 2 % NaF solution), the same procedure was carried out. The colour nuances of the two solutions were compared by means of a Dubosque's colorimeter.

As the first experiments on the oryzenins of the common and glutinous rices produced in Etchu, Akita and Asahikawa, did not show marked differences between the common and glutinous, the experiment was not continued further.

Table XXII.

Percentage content of tryptophan and tyrosin.

	Akita		Etchu		Asahikawa	
	Common	Glutinous	Common	Glutinous	Common	Glutinous
Tryptophan (%)	1.23	1.36	1.05	1.26	1.30	1.16
Tyrosin (%)	0.79	0.83	1.20	1.05	0.92	1.02

IX. The Formation of Jodoprotein.

Recently, Blum and Straus[27] compared the degree of combination of protein with iodines using nitrogen iodide, for the reason that the iodine combines with the tyrosin group and imidazol ring of protein. It is known that there is no marked difference in the quantity of tyrosin between the two oryzenins and that the glutinous contains more histidin which possesses the imidazol ring. It is expected that the degree of combination with iodine must excel in the glutinous.

To 0.2 g. of oryzenin were added 150 ccm of 3.5 % ammonium hydroxide solution. The mixture was stirred well and stood over night. 200 ccm of $^1/_5$ normal iodine alcohol solution were added to the solution and stood over night. There followed a clear yellow solution which was filtered by a suction. When the filtrate was acidified by a dilute sulphuric acid, a precipitate of jodoprotein was formed. After a while, the upper solution was decanted off, the precipitate was suspended in water and dissolved by an addition of NaOH solution and again precipitated with sulphuric acid. The treatment was repeated twice for the purpose of removing the excess of iodine from the protein. The precipitate was washed with water using a centrifugal machine until no more ammonia was observed in the washing, then washed with alcohol, absolute alcohol and ether as usual. The jodoprotein thus obtained

was dried in a desiccator of diminished pressure over sulphuric acid. The compound was fine a slightly yellowish powder.

By means of Baubigny and Chavanne's method[28], its iodine content was determined as follows:—40 ccm conc. H_2SO_4, 0.1 g. powdered silver nitrate, and potassium bichromate were taken in an Erlenmeyer flask of 300 ccm, and shaken well. To the mixture was added the jodoprotein and the whole was heated to 150–170° C. till no more oxygen was evolved. After its cooling, 150 ccm of water were added and again 10 ccm of alcohol. The fluid was reduced by a saturated solution of sodium sulphite and precipitates of silver iodide were obtained. The silver iodide was filtered, washed and weighed as usual by means of a gooch crucible. The results are tabulated in Table XXIII.

Table XXIII.

Iodine content of jodoprotein.

	Akita		Etchu		Asahikawa	
	Common	Glutinous	Common	Glutinous	Common	Glutinous
Sample (g.)	0.1304	0.1132	0.1264	0.1492	0.1626	0.1085
Silver iodide (g.)	0.0221	0.0199	0.0234	0.0292	0.0316	0.0236
Iodine (mg.)	11.9463	10.7627	12.6750	15.8042	17.0905	12.7638
Percentage	9.161	9.507	10.027	10.657	10.511	11.764

From the table, it is evident that the glutinous rice oryzenin combines with more iodine than the common. If Blum and Straus' opinion that the iodine combination value is due to the presence of tyrosin group and imidazol ring in the protein molecule were accepted, the difference of the iodine combination value is due to histidin which possesses the imidazol ring, as the quantity of tyrosin is not different between the two oryzenins. The results of the experiment coincide very well with the determination of amino acids of the former paragraph.

X. The Determination of Free Amino Nitrogen.

It is a widely accepted assumption that the substances giving a biuret reaction, i.e. those combining more than two amino groups of –CONH–, –CSNH–, –C(NH)NH–, –CHNH–, contain small quantities of free amino nitrogen. At the same time, Van Slyke and Birchard[34] estimated the quantities of free amino nitrogen of all kinds of proteins and proposed that it is proportional to lysin nitrogen of protein. The authors know the lysin nitrogen is predominant in the common rice oryzenin by a ratio to glutinous of 100 : 57 (see Table XX). Accepting Van

Slyke and Birchard's suggestion, it may be considered that the quantity of free amino nitrogen of common is superior to that of the glutinous.

As Wilson[29] has confirmed that Sörensen's method[30] excelled Van Slyke's method for the determination of free amino nitrogen, the authors adopted the former for the purpose.

Two-tenths g. of oryzenin was dissolved in 25 ccm of $^1/_5$ normal NaOH solution. To 20 ccm of the solution were added 10 ccm neutral formalin (50 ccm of formalin of purchase was titrated with $^1/_5$ normal NaOH solution using 1 ccm of 1 % phenolphthalein as indicator until the solution was coloured slight pink). The solution was titrated with $^1/_5$ normal HCl solution until it became slightly pink in colour. With 20 ccm of water, 5 ccm of $^1/_5$ normal NaOH solution and 10 ccm of neutral formalin, a control experiment was carried out and necessary corrections were made. 1 ccm of $^1/_5$ normal NaOH solution corresponds to 0.028 mg. of free amino nitrogen.

Table XXIV.

Content of free amino nitrogen in various oryzenins.

	Common rice oryzenin			Glutinous rice oryzenin		
	N/5 NaOH (ccm)	Nitrogen (g.)	% of total N	N/5 NaOH (ccm)	Nitrogen (g.)	% of total N
Akita	0.55	0.00154	10.50	0.50	0.00140	9.95
Etchu	0.65	0.00182	12.92	0.55	0.09154	11.42
Asahikawa	0.50	0.00140	10.12	0.45	0.00126	9.71
Hyogo	0.61	0.00171	9.07	0.42	0.00117	6.60
Saitama	0.62	0.00175	12.23	0.52	0.00147	11.54
Ibaragi	0.60	0.00168	11.94	0.55	0.00154	11.15
Echigo	0.55	0.00154	11.43	0.50	0.00140	10.46

According to the table, the content of free amino nitrogen of the common rice oryzenin is always superior to that of glutinous. This coincides well with the case of lysin nitrogen. Therefore, the figures of free amino nitrogen after Sörensen's method must be studied further.

In case the polypeptide linkage of the protein molecule is destroyed, the quantity of free amino nitrogen is increased. The authors, therefore, determined the free amino nitrogen of alkali protein solution after an exposure to ultra-violet ray. The figures of Table XXV are the percentages of free amino nitrogen to the total nitrogen of alkali protein solution as found by Sörensen's method of exposure to a German quartz mercury lamp at a distance of 1 foot.

Table XXV.

Ratio of free amino nitrogen to the total nitrogen of the various alkali protein solutions.

Illumination	30 minutes		50 minutes		90 minutes	
Kind of rice	Common	Glutinous	Common	Glutinous	Common	Glutinous
Akita	23.873	27.850	25.783	29.840	44.882	49.732
Etchu	22.857	29.065	29.810	33.217	56.747	62.282
Asahikawa	26.304	23.741 (?)	28.319	33.454	59.688	65.828
Saitama	23.961	26.505	26.113	30.004	46.384	49.230
Ibaragi	23.652	27.784	25.889	31.634	48.263	55.883
Echigo	23.854	27.986	26.023	31.986	53.631	61.731

It can be seen from the table that the quantity of free amino nitrogen of oryzenin increases rapidly by the action of ultra-violet ray. In the glutinous the ratio of increase is more notable. The alkali protein solution decomposes by the action of the ultra-violet ray and the high ratio of increase of the glutinous indicates that it is more easy to decompose than the common. The authors, to study the change of the protein in the process, examined the change of turbidities and the quantity of precipitable proteins.

Seven-hundredths g. oryzenin was dissolved in 25 ccm of $^1/_{75}$ normal NaOH solution, a half of the protein solution was exposed to the ultra-violet ray and its turbidity was compared with the other half. For the comparison of the turbidity, 1 ccm of the solution was diluted

Table XXVI.

Comparative turbidities.

HCl (ccm)	Illumination (minutes)	Akita		Etchu		Asahikawa	
		Common	Glutinous	Common	Glutinous	Common	Glutinous
		unexposed / exposed	unexposed / exposed	unexposed / exposed	unexposed / exposed	unexposed / exposed	unexposed / exposed
0.3 ccm	15	1—2.5	1—7.0	1—8.5	1—7.5	1—10.0	1—9.2
0.4 ccm	15	1—4.8	1—2.7	1—5.0	1—7.1	1—15.0	1—18.0
0.5 ccm	15	1—2.5	1—3.3	1—10.0	1—12.0	1—10.0	1—17.0
0.6 ccm	15	1—1.2	1—1.2	1—2.0	1—10.0	1—4.0	1—6.0
0.3 ccm	10	1—1.0	1—1.2	1—1.0	1—1.1	1—1.3	1—2.7
0.4 ccm	10	1—1.0	1—1.2	1—1.0	1—2.7	1—1.7	1—4.6
0.5 ccm	10	1—1.6	1—1.7	1—4.0	1—6.0	1—7.5	1—10.0
0.6 ccm	10	1—0.8	1—1.4	1—10.9	1—15.0	1—1.0	1—11.0

with 9 ccm of pure water, the mixture was titrated with $^1/_{50}$ normal HCl and compared as follows.

The figures were obtained using a Dubosque's colorimetric nephelometer. The increase of turbidity of the glutiuous is greater than that of the common and the turbidity increases with the duration of exposure to the ray.

After $1\,^1/_2$ hours' exposure, the alkali protein solution was acidified with acetic acid, filtered and the nitrogen of the filtrate and precipitate was estimated. (The figures are the percentages of the total nitrogen of the protein).

Table XXVII.

Percentages of the nitrogen in the filtrate and precipitate derived from alkali protein solutions, when treated with acetie acid.

	Common rice		Glutinous rice	
	N of precipitate (%)	N of filtrate (%)	N of precipitate (%)	N of filtrate (%)
Akita	81.067	18.933	83.920	16.079
Etchu	71.980	23.020	77.213	22.787
Asahikawa	75.234	24.776	85.184	14.816
Saitama	88.383	11.616	90.191	9.808
Ibaragi	88.438	11.561	90.169	9.830
Echigo	87.903	12.096	89.844	10.155

XI. The Decomposition of Oryzenin by Pancreatin.

A difference was observed in the degree of decomposition of oryzenins when they were exposed to an ultra-violet ray as described in a former paragraph (p. 156). The authors, therefore, proposed to examine the difference of the degree of decomposition by treatment with an enzyme. The following experiments were carried out.

One g. of oryzenin was dissolved in 100 ccm of $^1/_{50}$ normal NaOH solution. 1 g. of purchased pancreatin was dissolved in 20 ccm of water and the solution was filtered to obtain a clear solution of enzyme. To 25 ccm of oryzenin solution were added 2 ccm of enzyme solution and 1 ccm of toluol. The mixtures were then digested in an incubator at 40° C. for 1.5, 20, and 42 hours respectively. At the end of each period, the albumose formed was precipitated by an addition of acidic zinc sulphate solution and the nitrogen was estimated by Kjeldahl's method. To the filtrate from albumose was added phosphotungstic acid and

Table XXVIII.

Decomposition of oryzenin by pancreatin.

	N in 10 ccm (mg.)		% of total N	
	Common (Asahikawa)	Glutinous (Shonai)	Common (Asahikawa)	Glutinous (Shonai)
Total nitrogen	12.25	11.75	16.869	16.181

Digesting period	1.5 hours		20.0 hours		42.0 hours	
Kind of rice	Common (Asahikawa)	Glutinous (Shonai)	Common (Asahikawa)	Glutinous (Shonai)	Common (Asahikawa)	Glutinous (Shonai)
N of precipitate by Zn-sulphate(mg.)(I)	27.197	24.202	24.203	20.903	18.505	15.421
N of precipitate by phosphotungstic acid (mg.) (II)	6.198	10.623	6.541	10.966	9.983	10.966
% of (I) to total nitrogen	64.693	59.827	57.380	51.660	43.865	38.134
Ratio of (I)	100.000	92.47	100.000	90.03	100.000	86.93
% of (II) to total nitrogen	14.706	26.012	15.415	27.132	21.398	27.132
Ratio of (II)	56.53	100.00	56.81	100.00	78.86	100.00

the nitrogen of the precipitate was estimated in the usual way by Kjeldahl's method. The total nitrogen of the protein solution was estimated with an aliquot part of the solution. The nitrogen of each form was calculated on the basis of total nitrogen as the following table indicates.

The table shows that the glutinous rice oryzenin decomposes more rapidly than the common by the action of pancreatin, that the nitrogen precipitable with zinc sulphate predominates in the common and that, on the contrary, the nitrogen precipitable with phosphotungstic acid is more abundant in the glutinous.

The two degrees of decomposition of oryzenin by ultra-violet ray and pancreatin are a remarkable evidence of the idea that the polymerization of the protein molecule is more advanced and complicated in the common rice oryzenin than in the glutinous.

XII. The Silver Salt of Oryzenin.

Hitherto it has been repeatedly said that a protein or its decomposition product combines with heavy metals to make its salt, and Hirschstein[31] [a b], reported that, especially concerning casein, an insoluble precipitate obtained by adding silver nitrate to an ammonium solution of casein always contains a constant quantity of silver oxide that is chemically equivalent. The authors undertook the following examinations to compare the difference of combination with silver between the two kinds of oryzenin.

Eight-tenths g. of oryzenin was dissolved in 50 ccm of $^1/_{25}$ normal NaOH solution, the solution was neutralized with nitric acid using phenolphthalein as an indicator. 8 ccm of 20 % silver nitrate solution were added to the mixture to form a precipitate. The precipitate was filtered, washed, dried and weighed in the usual way. The total nitrogen was estimated according to Kjeldahl's method using 0.2 g. of the dried dark brownish precipitate. 0.4 g. of the substance was fused with a fusing mixture, dissolved in nitric acid and the silver of the solution was determined as silver nitrate by the gravimetric method. The results are tabulated in Table XXIX.

Table XXIX.

Combination of silver with the two kinds of oryzenin.

	Common rice oryzenin		Glutinous rice oryzenin	
	N in 0.2 g. (mg.)	Total N (%)	N in 0.2 g. (mg.)	Total N (%)
Sample I	30.389	Average 15.2435	28.6818	Average 14.6315
II	30.085		29.3647	

	$AgCl_2$ in 0.4 g. (mg.) Amount of dried matter	$AgCl_2$ in 0.4 g. (mg.) Amount of dried matter
Sample I II	13.40 12.80 Average 3.27 % Silver 2.47 %	27.20 27.90 Average 6.88 % Silver 5.18 %
Ratio between N and Ag	$\frac{Ag}{N} \times 100$ 16.07	35.48

In respect to the quantity of nitrogen the silver salts of common rice oryzenin predominate over those of glutinous, but in the quantity of silver the latter predominates over the former. This indicates that the carb xyl group which combines with silver is more abundant in the glutinous than in the common. The ratio between silver and nitrogen of glutinous is greater than that of the common for the reason that the former contains more silver and less nitrogen.

XIII. The Composition of Acetyl Oryzenin.

For the purpose of studying the constitutional differences of the two kinds of oryzenins, the authors acetylated the oryzenin and obtained pyrrols and their derivatives from the acetyl oryzenin.

(1) According to Troensegaard's method[32], oryzenin was acetylated as follows:— 4 g. of oryzenin were kept over night with 20 ccm of glacial acetic acid. The solution was heated in a boiling water bath with a reflux condenser. 3 ccm of acetyl chloride were added through the condenser and the bath was kept boiling. After 3 hours, 3 ccm of the reagent were added as formerly, 3 ccm more of the reagent were added twice at intervals of 3 hours and 1 hour respectively. Thus after the addition of 12 ccm of acetyl chloride, the boiling was ceased when a solution of acetyl protein was obtained. 20 ccm of acetic anhydride were added to the solution and the mixed solution was evaporated to about 20 ccm under a diminished pressure. Again, 10 ccm of acetic anhydride were added. To the mixture were added 4 g. of newly fused and pulverized natrium acetate accompanied by a good shaking. The mixture was heated at 132-135° C. for 5 minutes in an oil bath. After its cooling, 30 ccm of dry chloroform were added and the whole was stood over night.

The chloroform solution freed from insoluble matter by filtration was then poured into 150 ccm of dry ether. By this treatment, the acetyl oryzenin produced was precipitated; the precipitate was washed

twice with ether and dried over sulphuric acid under diminished pressure. A very hygroscopic brownish powder was prepared by successive crushings.

(2) The nitrogen content of the compound was estimated by Kjeldahl's method and the acetyl group by Wenzel's method[33].

Table XXX.

Content of nitrogen in acetylized oryzenins, weight of the acetyl group and ratio of acetyl to nitrogen.

Kind of rice		Amount of N (g.)	% of N	Acetyl group (g.)	% of acetyl group	Acetyl / N
Saitama	Common	0.0189368	9.468	0.032997	32.997	3.48
	Glutinous	0.0109634	5.482	0.038074	38.074	6.94
Ibaragi	Common	0.0179402	8.970	0.039087	39.087	4.35
	Glutinous	0.0173357	8.668	0.041881	41.881	4.83
Niigata	Common	0.0199335	9.967	0.038074	38.074	3.82
	Glutinous	0.0136046	9.302	0.041881	41.881	4.50
Mie	Common	0.0173357	9.668	0.036605	36.605	4.22
	Glutinous	0.0166113	8.305	0.042842	42.842	5.15

As the nitrogen content of the common rice acetyl oryzenin surpasses that of the glutinous and the acetyl group is the reverse, a very significant difference is observed in the proportions of acetylgroup to nitrogen.

(3) According to Troensegaard's method, the acetyl oryzenin was hydrolyzed and the separation of decomposition products was achieved. The sample was hydrolyzed by amylalcohol purified with diazobenzosulfonic acid and metallic natrium. A Jena glass flask of 250 ccm capacity was stoppered with a cork that had a reflux condenser, a dropping funnel, hydrogen gas inlet and thermometer. 40 ccm amylalcohol was measured into the flask. The top of the reflux condenser was connected to an absorption flask of standard H_2SO_4 and the absorption flask was sucked very slowly by a pump. Passing hydrogen gas through the inlet, the amylalcohol was heated to 80°C., then 2 g. of acetyl oryzenin were added with constant shaking. When the mixture was heated to 105°C., the heating was stopped and 1 g. of metallic natrium was added. Within an interval of 15 minutes, 2 g. more of metallic natrium suspended in xylol were dropped slowly from the dropping

funnel. After 10 minutes' vigorous reaction, the mixture was heated gently and then boiled for 20 minutes. The following 15 minutes were given for the cooling with constant shaking. 20 ccm of ether and then 12 ccm of ice water were added and the decomposition was completed.

The ammonia evolved by the hydrolysis was absorbed in the standard H_2SO_4 of the absorption flask connected to the reflux condenser. This ammonia nitrogen is called A.

The ether amylalcohol layer of the reaction product was separated and the residue was washed twice each time with 20 ccm of a mixture of ether and amylalcohol (3 : 1). The whole ether amylalcohol solution was collected in a separation funnel and was shaken three times each time with 10 ccm 33 % KOH solution. The KOH solution was added to the water layer separated from the ether amylalcohol layer. The water layer was neutralized carefully with conc. HCl, during which treatment, the whole was cooled in a freezing mixture. Then the solution was extracted 8–10 times using 50 ccm of ether each time. The whole ether solution was neutralized with potassium carbonate solution, the ether was evaporated and the nitrogen (D1) of the residue was estimated according to Kjeldahl's method.

The water solution from which the nitrogen (D1) had been separated was saturated with potassium carbonate, extracted with 94 % alcohol 8–10 times. The whole alcohol solution was neutralized with HCl. After one night's still standing, the precipitate was filtered, the filtrate evaporated under diminished pressure, and the nitrogen (D2) of the residue was estimated as before. The nitrogen (D3) of the alcohol insoluble residue was estimated as before.

The separated ether amylalcohol solution was extracted 10 times each with 10 ccm of 30 % sodium biphosphate solution.

The solution of phosphate was saturated with potassium carbonate, extracted 8–10 times with ether. Before the last extraction with ether, 10 ccm of 50 % KOH solution were added. The nitrogen content of the whole ether solution was estimated (C1) after the removal of ether as before. To the mother liquor nitrogen (C1) was added 3.1 ccm of 85 % phosphoric acid solution. Where the neutralization of KOH by phosphoric acid solution occured, the mother liquor was extracted 8 times with 94 % alcohol. The alcohol of the whole solution was evaporated under diminished pressure and the nitrogen (C2) of the residue was determined as before. The difference of total nitrogen and the sum (A), (B), (C1), (C2) and (D) was called the nitrogen (C3).

The ether amylalcohol solution extracted with phosphate solution was treated under diminished pressure to drive off the amylalcohol, the residue was dissolved in the smallest possible quantity of alcohol. A precipitation was formed when a large amount of ether was added to the alcoholic solution. The mixture was allowed to stand over night and filtered. The nitrogen (B1) of the solution and the nitrogen (B2) of the precipitate were determined respectively according to Kjeldahl's method. The nitrogen thus separated is arranged as follows in the table.

Table XXXI. (a)

Percentage of nitrogen in different kinds of nitrogen derived from acetyl oryzenin, taking acetyl oryzenin as 100

Nitrogen	Ibaragi		Niigata		Mie	
	Common	Glutinous	Common	Glutinous	Common	Glutinous
A	0.930	0.870	1.113	1.063	1.063	0.946
B1	0.216	0.133	0.173	0.133	0.169	0.102
B2	0.066	0.066	0.083	0.035	0.093	0.035
C1	0.050	0.059	0.083	0.166	0.083	0.183
C2	0.216	0.282	0.299	0.133	0.232	0.232
C3	0.351	0.494	0.375	0.197	0.482	0.731
D1	0.314	0.263	0.100	0.066	0.199	0.066
D2	3.937	4.053	5.648	5.615	3.884	3.851
D3	2.890	2.458	2.093	1.894	2.458	2.159

(b)

Percentage of nitrogen calculated on the basis of total nitrogen as 100.

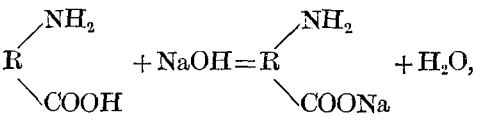
A	10.368	10.037	11.167	11.428	12.263	11.391
B1	2.408	1.534	1.736	1.430	1.950	1.228
B2	0.736	0.761	0.833	0.376	1.073	0.421
C1	0.558	0.681	0.832	1.784	0.957	2.203
C2	2.408	3.253	3.000	1.430	2.676	2.793
C3	3.913	5.609	3.762	2.118	5.561	8.802
D1	3.500	3.064	1.003	0.709	2.296	0.795
D2	43.891	46.758	56.667	60.363	44.808	46.370
D3	32.218	28.357	21.000	20.361	28.357	25.996

There is a marked difference between the common and the glutinous rice acetyl oryzenin in the quantities of base produced by hydrolysis, pyrrole, pyrrolidin and glyoxalin, pyrrolic acid, proteol and H_2O-K_2CO_3 soluble substance. The former is predominant in the quantities of base produced by hydrolysis, pyrrole, pyrrolic acid and H_2O-K_2CO_3 soluble substance compared with the latter, while the latter exceeds in pyrrolidin and glyoxalin, and proteol. The authors have already shown that the glutinous rice oryzenin predominates in quantity of histidin over the common. Comparing the constitution of histidin and glyoxalin, the identity of the two facts or the predominance of histidin and glyoxalin in the glutinous is easily acceptable.

SUMMARY.

(1) There was a difference between the quantities of HCl needed to precipitate the two proteins when dissolved in an alkali solution. Therefore, a difference should exist between the quantities of constitutional groups of the two proteins when combined with acid or with alkali. The theories of Robertson, Zsigmondy, Hardy and Pauli postulate that the following types of reaction would occur;

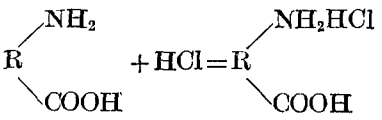
when with alkali,



$$R\begin{cases}NH_2\\COOH\end{cases} + NaOH = R\begin{cases}NH_2\\COONa\end{cases} + H_2O,$$

or $$-COH:N- + NaOH = -CONa^{++} + {}''\overset{H}{\underset{OH}{N}}-$$

and when with acid,



$$R\begin{cases}NH_2\\COOH\end{cases} + HCl = R\begin{cases}NH_2HCl\\COOH\end{cases}$$

or $$-CO-NH- + HCl = -COH^{++} + {}''\overset{H}{\underset{Cl}{N}}-.$$

Accepting their theories it is to be considered that amino groups are small in the protein having weak power of combination with acid and that carboxyl groups are small in the protein having weak power of combination with alkali. In that case, the common rice protein that was precipitated by a small quantity of acid from its alkali solution should contain a small quantity of carboxyl groups and the glutinous rice protein requiring large quantities of acid should contain large quantities of carboxyl groups.

(2) The fact that the iso-electric point of common rice oryzenin is nearer to alkali than that of glutinous rice oryzenin indicates that the amino groups of the former are more numerours than those of the latter. On the contrary, the iso-electric point of glutinous rice oryzenin being nearer to acid than that of common, indicates that the carboxyl groups of the former are superior to those of the latter.

(3) If the authors follow Robertson's opinion and accept that the difficulty of solubility of protein in an alkali solution is due to the constitutional difference of protein such as R. $CH\begin{smallmatrix}NH_2\\COOH\end{smallmatrix}$ and R. $CH\begin{smallmatrix}NH\\CH\end{smallmatrix}CO$, the more insoluble common rice protein must exist in a large quantity in the R. $CH\begin{smallmatrix}NH\\CH\end{smallmatrix}CO$ group and in less quantity in the R. $CH\begin{smallmatrix}NH_2\\COOH\end{smallmatrix}$ group. The reverse would hold true with respect to the glutinous rice protein.

(4) The refractive index of alkali oryzenin solution was observed. The index of the common is higher than that of the glutinous. This fact indicates that the former is composed more densely than the latter in optical structure.

(5) Rotatory power of organic compounds is increased by assymmetric carbon atoms, complexity of molecular structures, saturation compounds and especially by the presence of $-CO.R$ groups. In al-

kali protein solution, this group is decreased by neutralization of carboxyl groups and by the transition of keto-form, i. e. $-\underset{\underset{-CO}{|}}{CH_2}$ to enol-form, i. e. $-\underset{\underset{C(OH)}{\|}}{CH}$ in the molecular structure. The higher rotatory power of common rice oryzenin solution would be caused by the assymmetric carbon atoms and $-CO.R$ groups, while the lower rotatory power of glutinous rice oryzenin solution might be caused by the richness of carboxyl groups which would be neutralized by alkali and by the transition of keto-form to enol-form in the molecular structure.

(6) According to Cohnheim's method, the quantity of HCl combined with oryzenin of the common rice was greater than that of the glutinous. This is due to the abundance of amino group which combines with HCl more easily in the former than in the latter.

(7) In the elemental composition, the greater quantity of nitrogen of the common rice oryzenin is the reason of its basic nature. The lower ratio of carbon to oxygen of the glutinous rice oryzenin as compared with that of the common rice oryzenin shows the predominance of acidity and of the carboxyl groups of the former.

(8) The ash content of the common rice oryzenin is higher than that of the glutinous. There are strange tendencies that the higher sulphur content and lower phosphorus content are found in the common rice oryzenin and vice versa in the glutinous rice oryzenin.

(9) On the determination of amino acids, it is observed that in common rice oryzenin there is a predominance of ammonia, arginin and lysin nitrogen, while in the glutinous there is an excess of monoamino, histidin and cystin nitrogen. The quantities of ammonia nitrogen in the glutinous rice oryzenin are about 87 % of those of the common, arginin nitrogen 73 %, lysin nitrogen about 60 %, while monoamino nitrogen of the common are 96 % of glutinous, histidin and cystin nitrogen ca. 60 %.

(10) The contents of tyrosin and tryptophan were estimated after Fürth and his co-workers without any distinct difference between the two kinds of oryzenins being found.

(11) Jodoprotein was prepared after Blum and Straus. It was seen that the glutinous combines with more iodine than the common.

This is due to histidin which possesses the imidazol ring as the two oryzenins do not differ in quantity of tyrosin.

(12) The free amino nitrogen was determined after Sörensen's method. The quantity in the common predominates over that of glutinous. In oryzenin alkali solution, free amino nitorogen increased by exposure to ultra-violet ray. The velocity of increase of the glutinous was greater than that of the common.

(13) The decomposition of oryzenin by pancreatin was observed. The glutinous rice oryzenin decomposed more rapidly than the other. This is because the polymerization degree of the common is more complicated than that of the glutinous.

(14) The silver salt of oryzenin was prepared. The quantity of nitrogen of the common predominated while the case of silver was the contrary. This is due to the fact that the glutinous contains more carboxyl groups than the common.

(15) An acetylation of oryzenin was carried out. In respect to nitrogen content the common rice acetyl oryzenin predominates over the glutinous. In regard to the acetyl groups the reverse holds true. This seems to be due to the abundance of hydroxyl group which is replaced by acetyl group in the glutinous.

(16) There was a marked difference between the common and the glutinous rice acetyl oryzenin in the quantities of base produced by hydrolysis, pyrrol, pyrrolidin and glyoxalin, pyrrolic acid, proteol and $H_2O-K_2CO_3$ soluble substance. The former was predominant in the quantities of base produced by hydrolysis, pyrrol, pyrrolic acid and $H_2O-K_2CO_3$ soluble substance, and the latter showed larger quantities of pyrrolidin and glyoxalin, and proteol than the former. The glutinous rice oryzenin predominated over the common in the quantity of histidin. Comparing the constitution of histidin and glyoxalin, the identity of the two predominancies of histidin and glyoxalin contents in the glutinous was easily accepted.

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Contents.