Chemical Studies on the Brewing Barley

By

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Introduction

The use of barley in Japan is very wide, not only as food for man and animals, but also for the brewing of beer. The consumption for the brewer's purpose, increases year after year with the improvement of the fermentation industry. Consequently, barley is now ranking among the most important one in our agricultural products. The fact is seen from the following statistical tables.

TABLE I.

The production of barley and naked barley[1].

	Barley		Naked barley	
	Koku	Yen	Koku	Yen
1882	5,896,642		4,563,102	
1915	10,253,615		8,299,731	
1916	9,532,162		7,919,519	
1917	9,168,844	56,864,300	8,197,099	80,934,748
1918	8,368,370	106,683,652	7,777,430	132,769,186
1919	9,835,075	159,958,314	7,620,695	175,630,526
1920	8,289,859	125,701,293	8,297,090	191,045,594
1921	9,028,075	82,766,511	7,053,681	91,232,114
1922	8,771,948	80,164,620	7,153,753	91,974,279
1923	7,595,296	68,569,897	5,856,154	75,867,548
1924	8,075,776	83,133,523	5,738,982	93,624,350
1925	8,826,712	108,352,563	7,778,636	133,700,724

It can be seen that the production of barley in this country is about 8.8 million Koku values at 10 million Yen per annum. The import and export of it is seen from the Table II.

[Jour. Facul. Agric., Hokkaido Imp. Univ. Sapporo, Vol. XXIII, Pt. 2. 1928]

TABLE II.

The import and export of barley[2].

	Import				Export	
	Barley		Malt		Barley	
	Tan	Yen	Tan	Yen	Pound	Yen
1918			84,438	1,422,002	91,816	7,854
1919	205,297	1,546,045	82,531	1,508,039	90,497	9,244
1920	102,822	957,506	104,724	1,927,990	52,895	8,622
1921	5,604	29,189	19,519	351,204		
1922	4,996	36,577	67,990	880,918		
1923	28,656	164,492	45,898	698,499		
1924	16,972	85,069	108,655	1,757,036		

The consumption of barley between July, 1922 and June, 1923 was as follows.

TABLE III.

The consumption of barley between July, 1922 and June, 1923[3].

	Barley		Naked barley	
	Koku	%	Koku	%
For food-stuff	5,439,727	64.827	5,763,211	74.009
feeding-stuff	1,851,644	23.067	748,083	11.623
seed	241,404	2.877	233,780	3.632
milling	23,738	0.283	20,224	0.314
soja	97,450	1.161	222,411	3.456
brewing beer	315,576	3.761	—	—
cake and *ame* (rice-gelatine)	81,015	0.965	10,437	0.162
miso	279,639	3.332	360,318	5.599
other purpose	60,846	0.725	77,454	1.203
Total	8,391,039	100.000	6,435,918	100.000

The quantity of barley exported now is very small, while larger quantity of malt than of barley is imported. Among the quantities consumed, that for a food for man is the largest followed by that for the purpose of feeding domestic animals. The quantity for brewing beer is the third and those for soja and miso are not small. How-

ever, from the standpoint of the technical industry, the amount of the consumption of barley for brewing is the largest. The import of malt is disproportionately larger than the import of barley, indicating that there must be some differences in the barley and the malt produced at home and abroad. The very large consumption of barley for brewing purposes tells us how a study of the barley might be useful for the brewing. Needless to say studies on brewing and also on barley are very important and desirable in the present. This is the reason of the present investigation. It is also intended to find out physico-chemical differences of barleys according to their species.

Investigations upon barley which have hitherto been carried out and are already numerous, can be classified into 9 sorts; (1) upon the method of the estimation, (2) upon the nutrition and the nutritive value, (3) upon the relations among the soil, the manure and the growth of the plants, (4) upon the relations between the growing period and the constituents of the plants, (5) upon the fermentation, (6) upon the starches, (7) upon the fats, (8) upon the proteins and (9) upon the enzymes.

In the whole course of the investigations, Prof. Tetsutaro Tadokoro, Nogakuhakushi has given the author constant leadings and kind advices for which he is deeply grateful. He wishes to express his hearty thanks to him. The author is obliged to Mr. Murai Nogakushi and Mr. Hanada Nogakushi for the troubles they have taken on the barleys used in the investigation.

Materials of the investigation.

The barley generally used in this country is Golden Melon, but in Hokkaido in addition to this, Chevalier and Hokudai No. 1 are widely grown. The author has selected in his study these three species as they are important in brewing. The Golden Melon was cultured in Ibaragi Province, the Chevalier and Hokudai No. 1 were both cultured pure in the Sapporo Experimental Field of Dai Nippon Brewery Co., Ltd. All were produced in 1923, 1924 and 1925.

Their 1000 kernel weights and specific gravities were measured as follows.

TABLE IV.

The real and specific weight of barley grains.

	1000 kernel weight (g)			Specific gravity		
	Chevalier	Golden Melon	Hokudai No. 1	Chevalier	Golden Melon	Hokudai No. 1
1923	—	—	—	2.2864	2.4471	2.2503
1924	36.1724	45.1963	41.6608	2.7563	2.3276	2.3094
1925	46.8614	50.5071	53.7134	2.5909	2.7525	2.6338

There is no remarkable differences or relationship between the 1000 kernel weight and the specific gravity according to the species of barley.

The barley was powdered by means of a stone mill, and was sifted through a 80 mesh sieve. The following table shows the general analysis of the flour here obtained.

TABLE V.

The general analysis of the flour.

1925	Water	Ash (water free %)	Total nitrogen (water free %)	Protein nitrogen (water free %)
Chevalier	12.4633	3.6176	2.4327	2.2639
Golden Melon	4.6380	2.5586	1.7750	1.7628
Hokudai No. 1	12.2174	1.5721	2.3195	2.0854

The total and the protein nitrogen was the most copious in Chevalier, medium Hokudai No. 1 and small in Golden Melon.

The fats of the flour were extracted with ether for 3 days by means of a Soxhlet's apparatus. The defatted flour was dried in the open air. The general analysis of the defatted flour was carried out.

TABLE VI.

The general analysis of defatted flour.

	1923			1924			1925		
	Chevalier	Golden Melon	Hokudai No. 1	Chevalier	Golden Melon	Hokudai No. 1	Chevalier	Golden Melon	Hokudai No. 1
Water	—	—	—	9.435	8.590	10.085	12.404	12.847	12.032

Ash (water free)	—	—	—	2.491	2.087	2.743	3.425	2.547	2.501
Total nitrogen (water free)	1.901	1.765	2.020	1.955	1.567	2.220	2.498	2.084	2.251
Protein nitrogen) (water free)	—	—	—	1.884	1.532	2.148	2.453	2.037	2.206

The same tendency was observed in the case of defatted flour as with the original flour. Both the total and the protein nitrogen were the most copious in Chevalier, medium in Hokudai No. 1 and small in Golden Melon.

1. THE FATS.

On fats of barley, very few reports have been published. König[4], Lermer[4] and R. Meyer[4] stated that the fats of barley were yellow or yellowish brown in colour, Stellwaag[4], Wallerstein[4] and Sedlmeyer[5] reported that the cholesterin content of the fat was 4.70–6.08 %, and that of lecithin 3.06–4.24 %. According to R. Meyer and J. Sedlmeyer the physico-chemical constants of the fats were as follows.

TABLE VII.

The physico-chemical constants of fats of barley.

Specific gravity	Acid value	Saponification value	Iodine number	Hehner value	Author
0.94174 (0.9145)	25	280	90	86	R. Meyer
—	17	191	—	—	J. Sedlmeyer

R. Meyer also gave the following figures on the fatty acids of barley.

TABLE VIII.

The physico-chemical constants of fatty acids of barley fats.

Saponification value	Iodine number	Refractive index (30°C.)
280	63.5	1.4745

J. Sedlmeyer studied the change of fats of barley when the grain was malted, and stated that by the process the fat content decreased, the acid value increased from 17 to 32, and the saponification value did not change from 191.

The material of the investigation was the fats extracted by ether

from the flour. The ether of the fats was first driven off completely.

The fats thus obtained were at room temperature a yellowish liquid in which yellowish white solid of fats were mingled. All smelled very characteristic.

A. Method of experiments.

The author used the official and tentative method of the Association of Official Agricultural Chemists[6] in his experiment.

a) The specific gravity was determined at 15° C. using a pycnometer.

b) The refractive index was determined at 40° C. using a Zeiss' Butyro-Refractometer.

c) The saponification value was determined using alcoholic KOH solution and 1/2 normal HCl with phenolphthalein as an indicator.

d) The iodine number was determined by Hanus' method.

e) The Hehner value, Reichert-Meissl value and soluble acid were determined by the usual method.

f) The acid value was determined by treating the fats with alcohol.

g) The Polenske number was determined by Leffmann and Beam's method.

B. The experimental results.

TABLE IX.

The physico-chemical constants of fats of barley.

	1923		
	Chevalier	Golden Melon	Hokudai No. 1
Specific gravity (15°C.).....	0.89128	0.95031	0.99717
Refractive index (40°C.)	1.4695	1.4694	1.4698
Saponification value	178.635	111.059	188.456
Iodine value (Hanus)	104.564	79.890	85.770
Hehner value.....	—	—	—
Reichert-Meissl value	7.585	1.319	3.245
Acid value	23.774	30.335	22.663
Polenske number	—	—	—
Soluble acid (as Butyric acid).	—	—	—

	1924		
Specific gravity (15°C.).....	0.91212	0.91136	0.84800
Refractive index (40°C.)	1.4693	1.4724	1.4693
Saponification value.....	136.540	123.052	143.270
Iodine value (Hanus)	99.898	93.656	104.246
Hehner value.....	92.407	83.107	91.963
Reichert-Meissl value	3.366	3.341	4.939
Acid value	26.426	26.505	26.174
Polenske number	0.775	2.077	1.453
Soluble acid (as Butyric acid).	—	—	—
	1925		
Specific gravity (15°C.).....	0.89993	0.88444	0.85960
Refractive index (40°C.)	1.4717	1.4723	1.4704
Saponification value.....	172.855	114.703	175.546
Iodine value (Hanus)	108.320	83.314	106.635
Hehner value.....	95.085	94.263	93.420
Reichert-Meissl value	3.120	1.975	3.163
Acid value	29.558	17.250	37.032
Polenske number	3.152	2.693	3.954
Soluble acid (as Butyric acid).	0.452	0.147	0.447

From the table the following facts are seen.

(1) The results of specific gravity agree well with those of R. Meyer's, but the author can not find constant difference.

(2) The refractive index of Golden Melon is the greatest, the smallest Hokudai No. 1, Chevalier lying between the two.

(3) The asponification value is quite contrary to the refractive index.

(4) The iodine value increases in the order Golden Melon, Hokudai No. 1 and Chevalier.

(5) The Hehner value is the greatest in Chevalier, but the other two are indifferent.

(6) The Reichert-Meissl value is quite the same as the saponification value.

(7) The acid value is very unstable, but is between 17 to 37, which coincides well with Meyer's and Sedlmeyer's results.

(8) The Polenske number shows no definite tendency.

(9) The soluble acid seems to be the same as that of the iodine value, but as the result was obtained with only one year's sample, no decided statement can be made.

C. Discussion.

Considering the above results, the author is able to find constant differences in the fats according to species of the barley as follows.

TABLE X.

The constant differences according to species.

	Chevalier	Golden Melon	Hokudai No. 1
Refractive index	Medium	Great	Small
Saponification value .	Medium	Small	Great
Iodine value	Great	Small	Medium
Reichert-Meissl value	Medium	Small	Great
Soluble acid	Great	Small	Medium

The fats of Golden Melon always gives the smallest numbers with the one exception of refractive index which is the largest.

2. THE PROTEINS.

R. Wahl[7] studied the brewing quality, the agricultural position and the nitrogen content of barley and classified the products of U. S. A. into 3 classes, namely (a) the six-rowed barley with relatively high albumin content (10.5–14.00), medium size and weight of barley (25–32 g for 1000 head), medium thickness of husks, (b) the six-rowed barley of relatively less albumin content (below 10.5), of large size and weight of head (1000 kernel weight 35–45 g), with thick husks, and (c) the two-rowed barley with relatively low albumin content (below 11.5), of large size and weight of head (1000 kernel weight 35–45 g), with thin husks. C. Wenglein[8] stated that although individual exceptions might occur, both starch content and extract in barley, as a general rule decreased when the protein content increased. A. Cluss[9] stated that barleys low in albumin by no means always excelled as to quality and quantity of the beer produced, even highly albuminous barleys may be used to advantage. A. Le Clerc and R. Wahl[10] stated that the two-row barleys were richer in starch, extract, bran and endosperm, and had a higher

bushel and 1000 grain weight and a higher coefficient of mealiness and degree of dissolution than six-row varieties, but contained less protein, fibre, pentosans, hulls, sulphur, embryo and steely grains; six-row barley malts were highest in protein, lecithin, soluble protein and embryo, but lowest in starch, extract, bran, weight per bushel, and weight per 1000 grains. Two-row barley malts were highest in weight per bushel, extract and coefficient of mealiness, lowest in fibre, pentosans, hulls and embryo. O. Wenglein[11] studied the relations of starch of barley to protein and size of berry. Th. B. Osborne[12] reported the existence of water soluble globulin and proteose, NaCl solution soluble leucosine, 75% alcohol solution soluble hordein and dilute alkali solution soluble protein in barley flour, and Th. B. Osborne and S. H. Clapp[13] hydrolyzed the hordein, and estimated the content of alanin, valin, leucin, prolin, phenylalanin, glutamic acid, tyrosin, arginin, histidin, tryptophan and ammonia, and compared the results with those of gliadin. A. Kleinschmidt[14] obtained similar results by hydrolyzing the hordein and stated that hordein, gliadin and zein were proteins of same class, but were different chemically and physically. W. Rommel[15] carried out malting experiments on barley with varying protein content and E. Grabner[16] reported the correlations between the valuable characteristics of brewing barley. W. Kraft[17] compared hordein and bynin and found the two to be almost identical, but only the rotatory power was different. L. Lindet and L. Amann[18] reported that $[\alpha]_D = -137.5°$. C. Wenglein[19] described the method to apply the analytical results for practical brewing, L. Adler[20] estimated the amino acid and polypeptide nitrogen of barley, malt and beer by formol titration, H. Leberle and H. Lüers[21] found that the nitrogen content of barley were different according to the protein content, the place of production, the method of storing and the method of malting. F. Koritschoner[22] determined the ammonia nitrogen of barley, H. Schjerning[23] studied the change of protein substance by brewing, H. Schjerning and J. Hempel[24] reported that the existence of protein substance gave no serious effect upon the malting power of barley. H. C. Eckstein and H. S. Grindley[25] hydrolyzed the feeding stuff, determined the humin nitrogen and for barley determined it as 3.9. Johns and Finks[26] reported the existence of lysin in hydrolytic products of hordein, Th. B. Osborne and L. B. Mendel[27] determined the nutritive value of protein of barley, H. F. E. Hulton[28] discussed

the relations between the protein content and the brewing value of barley. Th. B. Osborne and L. B. Mendel[29] reared white rats with proteins of barley, rye, oats and wheat and found that the nutrition and growth were superior in the case of barley. H. Lüers and M. Landaner[30] obtained similar results with Van Slyke by hydrolysis of leucosine prepared by Osborne's method. W. Windisch, W. Dietrich and A. Mehlitz[31] precipitated the proteins from the water extract of barley by the addition of $SnCl_2$, $HgCl_2$, ferric acetate, uranyl acetate and $MgSO_4$, thus separated albumin I, albumin II, denuclein, propepton and pepton, carried out the ultrafiltration using Bechhold's apparatus of water extract and reported the quantitative relations between dispersity and the content of each protein. C. Consolani[32] stated that the good brewing barley should not contain more than 12 % of protein. W. F. Hoffman and R. A. Gortner[33] studied the combining power of prolamins with acid and alkali, and reported the combination following Freundlich's adsorption formula below pH 2.5 and over pH 10.5. They also stated that the intersections of acid and alkali combination curves showed the iso-electric point of the protein. Concerning the analysis of hordein they compared their results with Osborne's as follows.

TABLE XI.

The analysis of hordein.

	C	H	N	S	O
Osborne (1895)	54.29	6.80	17.21	0.83	20.87
Hoffman and Gortner..			16.70	0.76	

The Hausmann number (Osborne and Harris, 1903)

Ammonia nitrogen	Basic nitrogen	Non-basic nitrogen	Humin nitrogen
23.31	4.47	69.96	1.34

The Van Slyke number (Hoffman and Gortner, 1925)

Ammonia N	Humin N	Arginin N	Histidin N	Cystin N	Lysin N	Non-basic N
23.38	1.44	6.22	10.36	1.38	3.02	54.03

E. Takahashi and K. Shirahama[34] separated the hordein from

naked barley, then carried out elementary analysis, hydrolysis and studies on the change of surface tension of hordein solution by titration with HCl.

A. Estimation of water-, NaCl-, alcohol-, and alkali-solution-soluble nitrogens.

Five g of defatted and dried barley flour were mixed with 50 cc. of water, the mixture was shaken for 30 minutes by a shaking machine and then it was placed overnight in an ice-box. The mixture was then centrifuged for 10 minutes by a centrifugal machine. The nitrogen in a given quantity of the supernatant solution was determined by Kjeldahl's method. The residue was treated with 10 % NaCl solution instead of water and the 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.

TABLE XII.

The distribution of nitrogen.

		1923	1924	1925
Total nitrogen	Chevalier	1.9011%	1.9554%	2.4980%
	Golden Melon	1.7653	1.5674	2.0844
	Hokudai No. 1	2.0199	2.2202	2.2509
Water soluble nitrogen	Chevalier	0.3055	0.2991	0.3417
	Golden Melon	0.3055	0.3065	0.3316
	Hokudai No. 1	0.3123	0.3947	0.3982
10 % NaCl solution soluble nitrogen	Chevalier	0.2007	0.2269	0.2828
	Golden Melon	0.2122	0.2524	0.2605
	Hokudai No. 1	0.2376	0.2908	0.2699
70 % alcohol solution soluble nitrogen	Chevalier	0.4418	0.4538	0.6306
	Golden Melon	0.4583	0.2963	0.5921
	Hokudai No. 1	0.5092	0.4882	0.6571
0.2 % NaOH solution soluble nitrogen	Chevalier	0.5601	0.7013	0.8248
	Golden Melon	0.5142	0.5926	0.7343
	Hokudai No. 1	0.6111	0.8517	0.8910

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

TABLE XIII.

The distribution of nitrogen calculated on a basis of total nitrogen as 100.

		1923	1924	1925
Water soluble nitrogen	Chevalier	16.071	15.294	13.679
	Golden Melon	17.308	19.555	15.909
	Hokudai No. 1	15.462	17.778	17.691
10 % NaCl solution soluble nitrogen	Chevalier	11.607	11.603	11.321
	Golden Melon	12.019	16.102	12.499
	Hokudai No. 1	11.765	13.100	11.989
70 % alcohol solution soluble nitrogen	Chevalier	23.661	23.205	25.243
	Golden Melon	25.962	18.904	28.409
	Hokudai No. 1	25.210	21.989	29.192
0.2 % NaOH solution soluble nitrogen	Chevalier	29.464	35.863	33.019
	Golden Melon	29.129	37.807	35.227
	Hokudai No. 1	30.252	38.364	39.585

The tables show that the amount of nitrogen soluble in 10 % NaCl solution is the smallest and the amount increases according to the order of the water soluble, the 70 % alcohol solution soluble and the 0.2 % NaOH solution soluble nitrogen.

The quantities of 10 % NaCl solution soluble nitrogen decreases in the order Golden Melon, Hokudai No. 1 and Chevalier. The 0.2 % NaOH solution soluble nitrogen decreases in the order Hokudai No. 1, Golden Melon and Chevalier.

B. Preparation and purification of proteins.

To 600 g of the defatted and dried flour was added 3000 cc. of water and the mixture was shaken well. Then it was placed in an ice-chamber. The upper liquid was decanted, and after the same treatment was repeated 3 times, the residue was filtered through a filter paper.

To the residue was added a NaCl solution so that the whole was made up to 3000 cc. and 10 % NaCl. The mixture was shaken well and placed overnight in an ice-chamber. The upper liquid was obtained by a decantation and the residue filtered. The same treatment was repeated 3 times and the whole solution thus obtained was

filtered several times until no iodine reaction to starch was observed by a Buchner's funnel containing purified filter pulp of 1–2 cm. thickness. The clear filtrate thus obtained was then acidified with dilute HCl, whereupon the protein precipitated. The protein was washed well with water until no chlorine reaction was observed in the washing. It was washed with absolute alcohol and ether in a centrifugal machine and dried in a sulphuric acid desiccator of diminished pressure. The dried protein was powdered with an agate mortar and was again kept in a sulphuric acid desiccator.

The residue that was extracted with 10 % NaCl solution was washed with water thoroughly until the washing became chlorine free. To it was added alcohol until the whole was made up to 70 % alcohol. The following treatment was the same as with the 10 % NaCl solution. The clear filtrate was evaporated under diminished pressure at below 60° C. till hordein had precipitated as a gummy substance. The hordein was dissolved in 70 % alcohol and precipitated by pouring the solution into 8 times its volume of cold water. The same treatment was repeated 3 times. The hordein was dissolved in 70 % alcohol and precipitated by pouring the solution into 8 times its volume of absolute alcohol. The hordein was washed quickly with absolute alcohol and ether, dried in a sulphuric acid desiccator of diminished pressure. The hordein thus obtained was a yellowish white powder.

The residue from the hordein was dried in the open air. From the residue was then extracted the 0.2 % NaOH solution soluble protein with 0.2 % NaOH solution using the method described above. To the clear filtrate was added diluted acetic acid. The protein precipitated as a white amorphous mass. Then the protein was washed with water, alcohol and ether as stated above, dried in a sulphuric acid desiccator of diminished pressure and then was powdered.

The residue was used for the preparation of starch.

The ash content of the proteins was determined as the following table indicates. These are calculated on the water free substance.

TABLE XIV.

The ash content of proteins.

		1923	1925
10 % NaCl solution soluble protein	Chevalier	0.55	0.859
	Golden Melon	0.40	—
	Hokudai No. 1	0.90	—
Hordein	Chevalier	0.30	0.369
	Golden Melon	0.20	0.262
	Hokudai No. 1	0.40	0.554
0.2 % NaOH solution soluble protein	Chevalier	0.65	0.778
	Golden Melon	0.60	0.527
	Hokudai No. 1	1.35	0.810

The ash content of the proteins increases in the order hordein, 10 % NaCl solution soluble protein and 0.2 % NaOH solution soluble protein. As for the species of the barley, Golden Melon has the minimum ash content, Chevalier medium, and Hokudai No. 1 the maximum. This appears to be a constant relation found among the species of the barley and among the kind of proteins.

C. The elementary analysis of proteins.

From the results of elementary analysis of proteins, one can deduce nothing about the amino acids constituting the protein molecule, but from the quantities of each element of proteins some differences may be expected in the protein themselves.

The author estimated the carbon and hydrogen by the ordinary method using an electric furnace. Nitrogen is estimated by Kjeldahl's method and sulphur by Denis-Benedict's method.

TABLE XV.

The elementary composition of proteins.

Carbon and hydrogen.

1923

	Sample (g)	CO_2 (g)	H_2O (g)	Carbon %	Hydrogen %
Chevalier 10 % NaCl solution soluble protein	0.0958	0.1775	0.0706	50.531	8.247
	0.0711	0.1322	0.0564	50.709	8.876
	0.0952	0.1754	0.0729	50.248	8.250
			mean	50.498	8.548

Golden Melon 10 % NaCl solution soluble protein	0.0762	0.1404	0.0580	50.250	8.510
	0.0780	0.1438	0.0592	50.279	8.433
	0.0746	0.1375	0.0546	50.268	8.132
	0.0884	0.1628	0.0670	50.226	8.421
			mean	50.256	8.374
Hokudai No. 1 10 % NaCl solution soluble protein	0.0718	0.1284	0.0525	48.772	8.124
	0.0768	0.1375	0.0560	48.828	8.391
	0.0746	0.1333	0.0519	48.733	7.730
			mean	48.778	8.181
Chevalier hordein	0.1041	0.2116	0.0762	55.436	8.133
	0.0995	0.2038	0.0741	55.861	8.275
			mean	55.644	8.204
Golden Melon hordein	0.0830	0.1808	0.0650	59.189	8.701
	0.1022	0.2313	0.0747	61.724	8.121
	0.0993	0.2202	0.0752	60.469	8.414
			mean	60.461	8.409
Hokudai No. 1 hordein	0.0885	0.1952	0.0692	60.154	8.688
	0.0801	0.1763	0.0581	60.027	8.059
			mean	60.046	8.373
Chevalier 0.2 % NaOH solution soluble protein	0.1010	0.1979	0.0709	53.438	7.800
	0.0764	0.1498	0.0524	53.474	7.621
	0.0888	0.1757	0.0578	53.960	7.233
			mean	53.624	7.551
Golden Melon 0.2% NaOH solution soluble protein	0.0733	0.1371	0.0485	51.101	7.352
	0.0759	0.1418	0.0498	50.952	7.290
	0.0719	0.1450	0.0464	53.736	7.170
			mean	51.969	7.271
Hokudai No. 1 0.2 % NaOH solution soluble protein	0.0834	0.1624	0.0573	53.107	7.501
	0.0981	0.1923	0.0664	53.461	7.521
	0.1047	0.2043	0.0741	53.218	7.864
			mean	53.262	7.628

Nitrogen.

	1923			1924			1925		
	Chevalier	Golden Melon	Hokudai No. 1	Chevalier	Golden Melen	Hokudai No. 1	Chevalier	Golden Melon	Hokudai No. 1
10% NaCl solution soluble protein	15.955	15.556	16.100	15.565	15.262	16.378	15.818	15.583	16.263
Hordein	14.304	14.569	13.991	14.386	14.864	13.799	14.458	14.799	13.833
0.2% NaOH solution soluble protein	15.499	14.608	16.293	15.797	14.016	16.273	15.279	14.369	16.023

Sulphur

10% NaCl solution soluble protein	1.149	1.197	1.128	—	—	—	0.793	0.751	0.853
Hordein	0.787	0.926	0.801	—	—	—	0.950	0.863	0.812
0.2% NaOH solution soluble protein	0.717	0.701	0.657	0.688	0.603	0.698	0.847	0.991	0.928

The results from 1923 barley proteins are tabulated as follows.

TABLE XVI.

The results of analysis of 1923 barley proteins.

		C	H	N	O & S
10% NaCl solution soluble protein	Chevalier	50.496	8.548	15.955	25.001
	Golden Melon	50.256	8.374	15.556	25.814
	Hokudai No. 1	48.778	8.181	16.100	26.941
Hordein	Chevalier	55.644	8.204	14.304	21.848
	Golden Melon	60.461	8.409	14.569	16.826
	Hokudai No. 1	60.046	8.373	13.991	17.590
0.2% NaOH solution soluble protein	Chevalier	53.624	7.551	15.499	23.326
	Golden Melon	51.969	7.271	14.608	26.152
	Hokudai No. 1	53.262	7.628	16.293	23.177

Among the species of the barley, there is a tendency that the content of carbon and hydrogen of the protein always follow and that of oxygen is always contrary. The content of the nitrogen of the protein is markedly different among the species of the barley. The nitrogen of the 10% NaCl solution soluble protein and the 0.2% NaOH solution soluble protein is the maximum in Hokudai No. 1, medium in Chevalier and the minimum in Golden Melon while that of the

hordein is greatest in Golden Melon, medium in Chevalier and smallest in Hokudai No. 1. The content of nitrogen of the hordein is the smallest, the 0.2 % NaOH solution soluble protein medium and the 10 % NaCl solution soluble protein the greatest.

The sulphur content of the proteins is not regular, but it can be seen that of the hordein is the highest and that of the 0.2 % NaOH solution soluble protein the lowest.

D. The free amino nitrogen.

The free amino nitrogen of a protein is thought by Loeb[35], Pauli[36], Robertson[37] and the author[38] to give a protein its basic nature as the form of its existence in protein molecule will require. Therefore, when the colloidal nature of a protein is studied, the content of free amino nitrogen must be handled very carefully as paralleled by the carboxyl group of a protein which gives to it its acidic nature.

Thus the exact determination of free amino nitrogen of a protein must not be omitted when its physico-chemical properties are to be discussed. The author conducted the estimation according to the Sörensen's formol titration method as follows.

TABLE XVII.

The free amino nitrogen content of proteins.

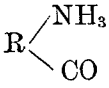
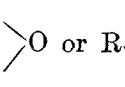
	1923			1924			1925		
	Chevalier	Golden Melon	Hokudai No. 1	Chevalier	Golden Melon	Hokudai No. 1	Chevalier	Golden Melon	Hokudai No. 1
10% NaCl solution soluble protein	3.325	3.850	3.500	2.611	3.358	2.850	3.149	3.955	3.575
Hordein	3.500	3.150	3.075	2.250	—	2.113	2.343	2.987	2.275
0.2% NaOH solution soluble protein	3.780	3.325	3.200	3.418	3.288	3.250	3.421	3.086	3.031

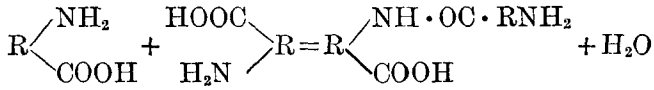
The free amino nitrogen calculated on a basis of total nitrogen as 100.

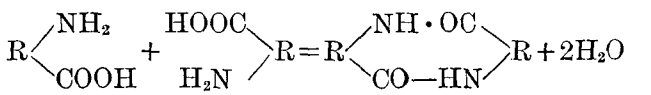
10% NaCl solution soluble protein	20.840	24.749	21.739	17.096	22.002	17.401	19.908	25.380	21.982
Hordein	24.470	21.621	21.978	15.640	—	15.313	16.205	20.184	16.446
0.2% NaOH solution soluble protein	24.389	22.761	19.640	21.637	23.459	19.972	22.390	21.477	18.916

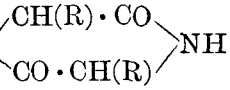
The table shows that the amount of free amino nitrogen of the 10 % NaCl solution soluble protein is the greatest in Golden Melon, medium in Hokudai No. 1 and the smallest in Chevalier, and that of the 0.2 % NaOH solution soluble protein, the greatest in Chevalier, medium in Golden Melon and the smallest in Hokudai No. 1. The amount of free amino nitrogen of the hordein is always greater in Chevalier than in Hokudai No. 1, and in Golden Melon it is irregular. The hordein contains less free amino nitrogen than the 10 % NaCl solution soluble protein and the 0.2 % NaOH solution soluble protein, while the 10 % NaCl solution soluble protein seems to contain more than the 0.2 % NaOH solution soluble protein.

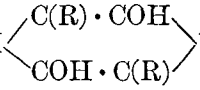
E. The determination of amino acids produced by the hydrolysis of proteins.

Concerning the structure of a protein, besides E. Fischer's polypeptide theory, starting from Ssadikow and Zelensky's study[39] on the feather of goose, K. Shibata[40], O. Herzog[41], E. Abderhalden[42] and Karrer concluded that a pyrazine ring and other ring structures must exist in protein. Therefore, there must be such anhydrides as  or  , called inner salts by Winkelflech or polyamino acid or inner salts (anhydrides) produced by the following reactions.





Also there must be diketopiperazins such as 

(keto form) and  (enol form). Notwithstanding these older and newer theories, the study on the sorts and the quantities of amino acids constructing a protein is an important item for the investigation of protein. The quantities of basic amino acids such as histidin, lysin, arginin and cystin may have some effect

upon the basic nature of protein. These are unnegligible facts which give some idea concerning the structure of protein molecule.

The author undertook the following experiments according to Van Slyke's method[43]. To 1 g of protein was added 10 cc. of 20 % HCl; the mixture was boiled and hydrolyzed for 8 hours on a sand bath under a reflux condensor. 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 a diminished pressure at below 40° C. To the concentrated filtrate were added 18 cc. of concentrated 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[44]. The water solution thus obtained was concentrated to 100 cc. under diminished pressure at below 40° C. With 25 cc. of the solution arginin nitrogen was determined, and with 10 cc. each the total nitrogen, cystin sulphur, and using Van Slyke's micro apparatus, the free amino nitrogens were determined.

TABLE XVIII.

The kinds of nitrogen found in protein.

	Barleys of 1923.								
	Total N	Ammonia N	Melanin N	Non-basic N	Basic N	Arginin N	Histidin N	Lysin N	Cystin N
10 % NaCl solution soluble protein									
Chevalier	15.955	0.986	0.438	9.393	5.138	2.741	0.530	1.549	0.318
Golden Melon	15.556	1.099	0.495	8.348	5.658	2.561	1.328	1.446	0.320
Hokudai No. 1	16.100	1.215	0.451	8.980	5.554	2.766	0.642	1.899	0.642
Hordein									
Chevalier	14.304	2.915	0.349	8.156	2.884	0.723	1.406	0	0.755
Golden Melon	14.569	2.154	0.304	9.673	2.438	0.650	1.089	0	0.699
Hokudai No. 1	13.991	2.646	0.427	8.822	2.036	0.815	0.502	0	0.179

0.2 % NaOH solution soluble protein									
Chevalier	15.499	2.190	0.410	10.159	2.740	1.095	0.013	1.292	0.341
Golden Melon	14.608	1.658	0.546	9.978	2.426	1.294	0.431	0.281	0.320
Hokudai No. 1	16.293	1.941	0.406	9.657	4.287	1.084	1.929	0.558	0.716
Barleys of 1924.									
0.2 % NaOH solution soluble protein									
Chevalier	15.797	1.923	0.406	10.476	2.992	1.311	0.075	1.146	0.460
Golden Melon	14.061	1.554	0.516	8.587	3.359	1.344	0.561	1.097	0.357
Hokudai No. 1	16.273	1.864	0.394	9.872	4.143	1.492	1.359	0.926	0.366
Barleys of 1925.									
Hordein									
Chevalier	14.458	2.339	0.234	8.908	2.977	0.748	1.994	0	0.235
Golden Melon	14.799	2.488	0.276	9.696	2.339	0.680	0.718	0	0.941
Hokudai No 1.	13.833	2.961	0.216	8.711	1.945	0.869	0.499	0	0.577
0.2 % NaOH solution soluble protein									
Chevalier	15.279	1.141	0.527	11.122	2.489	0.660	0.125	1.282	0.321
Golden Melon	14.369	1.811	0.553	9.723	2.282	0.766	0.748	0.601	0.167
Hokudai No. 1	16.023	1.453	0.516	10.458	3.596	0.969	1.429	0.931	0.267

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

TABLE XIX.

Barleys of 1923.									
10 % NaCl solution soluble protein									
Chevalier	100.00	6.180	2.745	58.872	32.203	17.179	3.322	9.769	1.933
Golden Melon	100.00	6.550	3.182	53.896	36.372	16.482	8.472	8.847	2.571
Hokudai No. 1	100.00	7.561	2.801	55.776	34.497	17.180	3.891	11.796	1.534
Hordein									
Chevalier	100.00	20.379	2.440	57.019	20.162	5.054	9.830	0	5.278
Golden Melon	100.00	14.785	2.087	66.394	16.734	4.461	7.475	0	4.798
Hokudai No. 1	100.00	18.921	3.052	63.055	14.552	5.825	5.825	0	5.139
0.2 % NaOH solution soluble protein									
Chevalier	100.00	14.130	2.645	65.546	17.678	7.065	0.084	8.329	2.200
Golden Melon	100.00	11.350	3.738	68.305	16.607	8.858	2.950	2.608	2.190
Hokudai No. 1	100.00	11.913	2.492	59.271	26.312	6.653	11.839	3.426	4.394
Barleys of 1924.									
0.2 % NaOH solution soluble protein									
Chevalier	100.00	12.173	2.570	66.316	18.940	8.299	0.475	7.254	2.912
Golden Melon	100.00	11.087	3.681	61.265	23.965	9.589	4.002	7.827	2.547
Hokudai No. 1	100.00	11.455	2.421	60.665	25.459	9.168	8.351	5.690	2.249

Barleys of 1925.

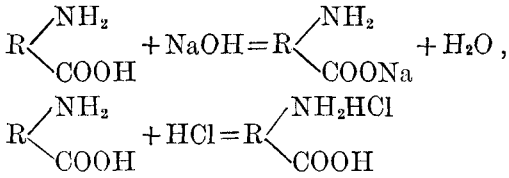
Hordein									
Chevalier	100.00	16.178	1.618	61.613	20.591	5.174	13.792	0	1.625
Golden Melon	100.00	16.812	1.865	65.518	15.805	4.595	4.852	0	6.358
Hokudai No. 1	100.00	21.405	1.561	62.973	14.060	6.282	3.607	0	4.171
0.2 % NaOH solution soluble protein									
Chevalier	100.00	7.468	3.449	72.973	16.290	4.320	0.818	9.052	2.101
Golden Melon	100.00	12.603	3.884	67.659	15.881	5.331	5.206	4.183	1.162
Hokudai No. 1	100.00	9.068	3.220	65.269	22.443	6.047	8.918	5.810	1.666

From the above tables, differences are observed according to the barley species among the content of the hydrolytic products of the 10 % NaCl solution soluble protein, of the hordein and of the 0.2 % NaOH solution soluble protein.

In the case of the 10 % NaCl solution soluble protein, the ammonia nitrogen decreases in the order of Hokudai No. 1, Golden Melon and Chevalier; melanin nitrogen in the order Golden Melon, Hokudai No. 1 and Chevalier; non-basic nitrogen in the order Chevalier, Hokudai No. 1 and Golden Melon; basic nitrogen in the order Chevalier, Hokudai No. 1 and Golden Melon; arginin nitrogen in the order Hokudai No. 1, Chevalier and Golden Melon; lysin nitrogen in the order Hokudai No. 1, Golden Melon and Chevalier; histidin nitrogen in the order Golden Melon, Hokudai No. 1 and Chevalier; cystin nitrogen in the order Golden Melon, Chevalier and Hokudai No. 1, but an absolute statement can not be made as the results are obtained with only one year's product. In the case of the hordein, ammonia nitrogen is at the maximum in Golden Melon; non-basic nitrogen decreases in the order of Golden Melon, Hokudai No. 1 and Chevalier; basic nitrogen decreases Chevalier, Golden Melon and Hokudai No. 1; arginin nitrogen decreases Hokudai No. 1, Chevalier and Golden Melon; histidin nitrogen decreases Chevalier, Golden Melon and Hokudai No. 1. In the case of the 0.2 % NaOH solution soluble protein, melanin nitrogen decreases in the order of Golden Melon, Chevalier and Hokudai No. 1; non-basic nitrogen is superior in Chevalier to Hokudai No. 1; basic nitrogen maximum in Hokudai No. 1; arginin nitrogen greater in Golden Melon than Chevalier; histidin nitrogen decreases Hokudai No. 1, Golden Melon and Chevalier; lysin nitrogeu is richer in Chevalier than Hokudai No. 1; cystin nitrogen is superior in Chevalier to Golden Melon.

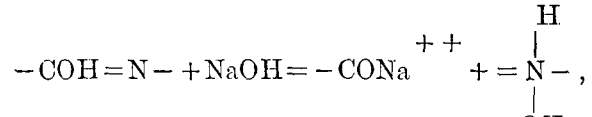
E. The change of surface tension and turbidity of protein alkali solution by titration with HCl.

On the principles of reactions between protein and acid or alkali, there are many theories, such as Pauli's, Robertson's and Loeb's. These are in short that it follows these reactions:

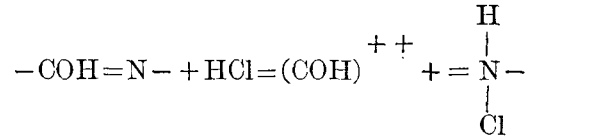


$$R\begin{cases}NH_2\\COOH\end{cases} + NaOH = R\begin{cases}NH_2\\COONa\end{cases} + H_2O,$$

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

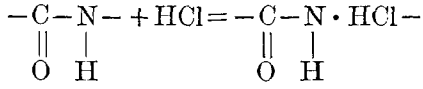


$$-COH=N- + NaOH = -CONa^{++} + = \underset{\displaystyle OH}{\overset{\displaystyle H}{\overset{|}{\underset{|}{N}}}} -,$$



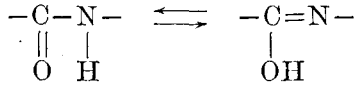
$$-COH=N- + HCl = (COH)^{++} + = \underset{\displaystyle Cl}{\overset{\displaystyle H}{\overset{|}{\underset{|}{N}}}} -$$

By the combination of acid to desaminoprotein, the NH-group of peptid linking acts as if it were NH_2-group as follows.



$$-\underset{\displaystyle O}{\overset{\displaystyle \|}{C}}-\underset{\displaystyle H}{\overset{|}{N}}- + HCl = -\underset{\displaystyle O}{\overset{\displaystyle \|}{C}}-\underset{\displaystyle H}{\overset{|}{N}}\cdot HCl-$$

And in the case of the combination of alkali, the laktam form changes to laktim form.



$$-\underset{\displaystyle O}{\overset{\displaystyle \|}{C}}-\underset{\displaystyle H}{\overset{|}{N}}- \rightleftarrows -\underset{\displaystyle OH}{\overset{|}{C}}=N-$$

Therefore, it seems to be sure that the physico-chemical changes of a protein alkali solution when titrated with HCl will give some idea on the iso-electric point of a protein, because according to Robertson, Zsigmondy, Hardy, Pauli and Loeb, a protein solution must show at the iso-electric point its maximum turbidity and surface tension, at the same time its minimum osmotic pressure and viscosity.

The author for the purpose of searching the iso-electric point of protein estimated the changes of surface tension and turbidity of protein alkali solution by titration with HCl. One tenth g of protein was dissolved in 10 cc. of 1/50 normal NaOH solution. To 1 cc. of the solution were added 9 cc. of redistilled water, to the mixture was

titrated 1/100 normal HCl solution. The surface tension of the titrated solution was determined by De Nouy's torsionmeter, and the turbidity by Dubosque's nephelometer. The surface tension is dyne per sq. cm. at 15° C. and the turbidity is expressed in specific value calculated taking the turbidity resulting from the minimum HCl titration as 1.

TABLE XX.

The surface tension and the turbudity of protein alkali solution titrated with HCl.

Barleys of 1923.											
10 % NaCl solution soluble protein											
1/100 N HCl cc.					1.8	1.9	2.0	2.1	2.2	2.3	2.4
Surface tension											
Chevalier					66.38	66.71	**67.40**	66.03	65.79	65.45	65.45
Golden Melon					66.71	**67.05**	66.71	65.45	65.45	65.45	65.45
Hokudai No. 1					66.38	66.71	67.05	**67.74**	66.03	66.03	66.03
Turbidity											
Chevalier					1	3.62	**3.94**	3.08	2.33	2.12	—
Golden Melon					1	**3.00**	1.96	1.31	0.93	—	—
Hokudai No. 1					—	1	1.03	**1.29**	1.24	1.22	—
Hordein											
1/100 N HCl cc.	1.65	1.70	1.75	1.80	1.85	1.90	1.95	2.00	2.05	2.10	2.15
Surface tension											
Chevalier	—	51.13	50.11	50.45	50.45	51.47	54.04	**55.56**	53.28	52.50	52.16
Golden Melon	—	48.41	48.41	49.09	49.09	49.43	51.81	52.50	**53.28**	52.16	—
Hokudai No. 1	—	47.38	47.72	48.41	50.45	53.62	53.96	**55.22**	53.28	52.50	52.50
Turbidity											
Chevalier	—	1	1.66	2.70	4.45	7.56	14.22	**19.33**	7.60	3.65	1.64
Golden Melon	1	1.20	2.10	3.06	4.50	10.60	17.42	30.32	**37.94**	17.33	—
Hokudai No. 1	—	1	1.16	1.87	3.65	5.00	12.14	**24.61**	15.40	9.01	4.31
Barleys of 1924.											
10 % NaCl solution soluble protein											
1/100 N HCl cc.			2.00	2.05	2.10	2.15	2.20	2.25	2.30	2.35	2.40
Surface tension											
Chevalier			66.82	67.50	67.84	68.18	67.84	**69.20**	68.18	65.45	65.45
Golden Melon			66.82	66.82	66.82	67.50	**68.18**	66.82	66.82	66.13	66.13
Hokudai No. 1			68.18	68.18	68.52	68.18	68.86	68.86	**69.54**	68.52	68.18

Turbidity											
Chevalier			1	1.263	1.734	1.934	2.901	**3.919**	3.592	3.412	3.241
Golden Melon			1	1.282	2.024	2.857	**3.296**	2.966	2.966	2.867	2.069
Hokudai No. 1			1	1.418	2.002	2.502	4.289	5.475	**5.763**	5.283	2.465

Hordein

1/100 N HCl cc.	1.60	1.65	1.70	1.75	1.80	1.85	1.90	1.95	2.00	2.05	2.10
Surface tension											
Chevalier	—	—	47.73	45.68	49.09	51.13	**52.50**	52.50	49.09	49.09	47.73
Hokudai No. 1	51.47	49.77	51.82	51.82	51.13	50.79	50.79	**53.18**	50.79	—	—
Turbidity											
Chevalier	—	—	1	1.053	1.019	1.132	**1.283**	1.249	1.138	1.100	0.916
Hokudai No. 1	1	2.182	5.237	4.015	5.538	6.156	5.704	**12.223**	1.408	—	—

0.2 % NaOH solution soluble protein

1/100 N HCl cc.			2.00	2.10	2.20	2.25	2.30	2.35	2.40	2.45	2.50
Surface tension											
Chevalier			66.82	66.82	66.13	66.13	66.47	**67.16**	64.77	64.77	65.45
Golden Melon			67.50	66.82	66.82	66.82	66.13	**67.16**	65.79	65.11	64.77
Hokudai No. 1			64.77	66.47	66.47	66.13	66.13	66.47	**67.16**	66.13	66.13
Turbidity											
Chevalier			1	1.818	4.958	6.000	6.923	**8.307**	6.784	6.671	5.337
Golden Melon			1	1.935	3.518	5.783	5.205	**6.645**	6.645	6.313	5.997
Hokudai No. 1			1	2.500	5.555	7.407	9.456	11.820	**11.820**	98.50	9.029

Barleys of 1925.

10 % NaCl solution soluble protein

1/100 N HCl cc.			1.70	1.75	1.80	1.85	1.90	1.95	2.00	2.05	2.10
Surface tension											
Chevalier			60.34	61.36	61.36	61.36	62.04	**62.73**	61.70	61.70	60.34
Golden Melon			62.04	62.04	62.73	64.77	**65.45**	63.41	62.04	62.73	62.04
Turbidity											
Chevalier			1	1.290	1.382	2.154	4.308	**4.535**	3.522	3.374	3.374
Golden Melon			1	1.28	2.07	2.53	**3.30**	2.75	2.70	2.46	1.72

Hordein

1/100 N HCl cc.	1.70	1.75	1.80	1.85	1.90	1.95	2.00	2.05	2.10	2.15	2.20
Surface tension											
Chevalier	49.77	48.41	48.41	49.09	52.50	**55.22**	53.18	53.18	51.13	—	—
Golden Melon	—	—	43.29	41.59	41.93	42.61	**46.06**	45.00	44.32	42.27	42.27
Hokudai No. 1	44.66	42.61	44.32	45.68	46.70	**47.73**	44.32	45.00	44.32	—	—

Turbidity											
Chevalier	1	1.07	2.14	3.13	5.78	7.54	3.52	3.11	1.76	—	—
Golden Melon	—	—	1	1.33	1.33	2.22	3.60	3.24	2.32	1.82	1.15
Hokudai No. 1	1	1.28	2.33	4.37	4.95	4.95	4.66	3.34	2.12	—	—
0.2 % NaOH solution soluble protein											
1/100 N HCl cc.	1.80	1.85	1.90	1.95	2.00	2.05	2.10	2.15	2.20	2.25	2.30
Surface tension											
Chevalier	56.59	58.63	58.98	59.98	60.00	61.36	60.68	60.00	60.00	—	—
Golden Melon	—	—	62.04	62.04	61.36	60.68	62.04	63.07	62.04	62.04	62.04
Hokudai No. 1	—	—	62.04	62.04	61.36	62.04	62.73	62.04	63.41	62.04	—
Turbidity											
Chevalier	1	1.93	3.73	5.01	9.70	13.86	10.86	8.78	4.24	—	—
Golden Melon	—	—	1	3.75	2.62	4.63	11.11	13.07	9.58	7.98	6.68
Hokudai No. 1	—	—	1	1.93	2.76	4.04	6.06	8.08	8.08	7.54	—

In the case of the 10 % NaCl solution soluble protein, the quantitias of HCl required to attain the greatest surface tension and turbidity decrease in the order Hokudai No. 1, Chevalier and Golden Melon. In the case of the hordein, that of Golden Melon requires the maximum amount of HCl, next Hokudai No. 1, while Chevalier requires the minimum amount. Using the 0.2 % NaOH solution soluble protein, the quantities of HCl required decreases in the order Hokudai No. 1, Golden Melon and Chevalier. These are the marked differences of species, and the iso-electric points of the proteins would differ from each other for the above stated reasons.

G. The specific rotatory power of protein alkali solution.

The rotatory power of an organic substance is caused by its asymmetric carbon and the optical activity of a compound is the algebraic sum of each independent asymmetric carbon if there are many such carbons in the molecule. Not only carbon, but other atoms which are asymmetric show optical activities. According to Kaufmann[45], all atoms can rotate the plane of polarized light, and when there is no asymmetric carbon, the centroasymmetric molecule has an optical activity. The homologous substances have a limit of rotatory power and in unsaturated compounds, nearer the double binding to asymmetric carbon, the rotatory power is greater. Thus with the complexity of molecular structure and increase of saturation

degree, the rotatory power become greater. Therefore, it can be deduced that the more complex the structure of a protein, the greater may be its specific rotatory power.

One tenth g of protein was dissolved in 15 cc. of 1/50 normal NaOH solution. After 24 hours, using a 100 mm. observation tube the rotatory power of the solution was measured by mean of Haensch-Schmidt half shadow polariscope at 18° C.

TABLE XXI.

The rotatory and specific rotatory power of protein alkali solution at 18° C.

	1923		1924		1925	
	Ventzke degree	Sp. rot. power	Ventzke degree	Sp. rot. power	Ventzke degree	Sp. rot. power
10 % NaCl solution soluble protein						
Chevalier	−2.5	−129.75	−2.7	−140.13	—	—
Golden Melon	−3.0	−155.70	−3.1	−160.89	—	—
Hokudai No. 1	−2.4	−124.56	−2.1	−108.99	—	—
Hordein						
Chevalier	−4.0	−207.60	−4.1	−222.79	−4.0	−207.60
Golden Melon	−3.5	−181.65	—	—	−3.1	−160.89
Hokudai No. 1	−3.7	−192.03	−3.7	−192.03	−3.7	−192.03

According to the table, the specific rotatory power of the 10 % NaCl solution soluble protein alkali solution decreases in the order of Golden Melon, Chevalier and Hokudai No. 1, and that of the hordein in an alkaline solution decreases in the order Chevalier, Hokudai No. 1 and Golden Melon.

H. The refractive index of protein alkali solution.

The refractive index of an organic substance may also have a relation to the structure of the substance.

One tenth g of protein was dissolved in 10 cc. of 1 normal NaOH solution, and after 24 hours the refractive index of the solution was determined at 20° C. by a Goeltz's refractometer as follows.

TABLE XXII.

The refractive index of protein alkali solution.

		1923	1924	1925
Hordein	Chevalier	1.3458	1.3456	1.3458
	Golden Melon	1.3458	—	1.3458
	Hokudai No. 1	1.3460	1.3459	1.3460
0.2% NaOH solution soluble protein	Chevalier	1.3455	1.3453	1.3460
	Golden Melon	1.3458	1.3455	1.3462
	Hokudai No. 1	1.3460	1.3456	1.3462

It seems to be, from the table, that in cases of both hordein and 0.2% NaOH solution soluble protein the refractive index increases in the order Chevalier, Golden Melon and Hokudai No. 1. However, the differences are so small that one cannot state the fact exactly.

I. The determination of the quantities of NaOH combined with protein.

For the same reason stated in Part F, the quantities of NaOH combined with protein are the indirect proof of the difference of content of carboxyl groups of the protein from the other.

The author carried out the following experiments according to Hoffman and Gortner[33]. The electromotive force of NaOH solutions of various concentrations, such as 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40 and 0.50 normal were measured with a calomel saturated KCl solution cell at 20° C. The corresponding pH values were calculated by the formula

$$\text{pH}=\frac{\text{e.m.f.}(v.)-0.249(v.)}{0.0581} \text{ (at } 20^\circ \text{ C)}.$$

The mathematical relation between the normality of alkali (x) and the pH value (y) was obtained by the method of interpolation and the values of y corresponding to $x=0.05$, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40 and 0.50 were calculated.

TABLE XXIII.

Electromotive force (e.m.f.) and the pH values observed and calculated of various NaOH solutions.

	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.50
e.m.f. (mv.)	981.06	996.82	1006.7	1012.6	1018.4	1020.5	1022.4	1026.4	1032.3
pH values observed	12.592	12.863	13.033	13.135	13.235	13.271	13.304	13.372	13.474
$y=12.422+4.155\ x-4.284\ x^2$.									
calculated	12.619	12.795	12.949	13.082	13.193	13.283	13.351	13.399	13.428

In a given quantity of NaOH solution of the above mentioned concentrations protein was dissolved so that the solution became 1 % solution of protein. After an equilibrium between protein and alkali was obtained, the electromotive force of the solution was measured as stated above. The pH value of the measured electromotive force was calculated and the corresponding normality of NaOH was calculated by the above equation. The difference between the original normality and the obtained normality indicated the normality of the NaOH which combined with the added protein.

TABLE XXIV.

The normality of the NaOH combining with the protein added.

Original normality	0.20	0.25	0.30	0.35	0.40	0.50
1923, Chevalier hordein	0.0459	0.0680	0.1071	0.1122	0.1462	0.2312
1923, Golden Melon hordein	0.0170	0.0255	0.0442	0.0595	0.0850	0.1275
1923, Hokudai No. 1 hordein	0.0323	0.0425	0.0476	0.0595	0.0680	0.1071
1925, Chevalier hordein	0.0220	0.0270	0.0780	—	—	0.1649
1925, Golden Melon hordein	0.0051	0.0255	0.0476	0.0595	0.0680	0.1275
1925, Hokudai No. 1 hordein	—	0.0085	0.0272	0.0612	0.0646	0.0816
1923, Chevalier 0.2 % NaOH solution soluble protein	0.0187	0.0255	0.0444	0.0782	0.0867	0.1309
1923, Golden Melon 0.2% NaOH solution soluble protein	0.0187	0.0395	0.0612	0.0782	0.1071	0.1190
1923, Hokudai No. 1 0.2% NaOH solution soluble protein	—	0.0119	—	0.0578	0.0867	0.1649
1925, Chevalier 0.2% NaOH solution soluble protein	0.0476	0.0748	—	0.0786	0.0935	0.1411
1925, Golden Melon 0.2% NaOH solution soluble protein	0.018 7	0.0289	0.0459	0.0578	0.0850	0.1666
1925, Hokudai No. 1 0.2% NaOH solution soluble protein	—	0.0235	—	0.0578	0.0850	0.1326

The table shows that the normality of alkali combined with hordein is always the greatest in Chevalier, intermediate in Golden Melon and the smallest is by Hokudai No. 1. However, the same normality with 0.2 % NaOH solution soluble protein shows no constant difference.

J. The conductometric titration of protein alkali solution with HCl.

One tenth g of protein was dissolved in 10 cc. of 1 normal NaOH solution. After 24 hours, 1 cc. of the solution was diluted to 11 cc. with distilled water. The electric conductivity of the solution was measured by means of a cell whose cell constant was 0.24 at 20° C. after each addition of 1/5 normal HCl solution. The conductivity cell was kept in a thermostat, and the resistance used was 12 ohms. From the readings (a) of Wheatstone bridge, the values of $(100-a)/a$ were obtained by means of a table.

TABLE XXV.

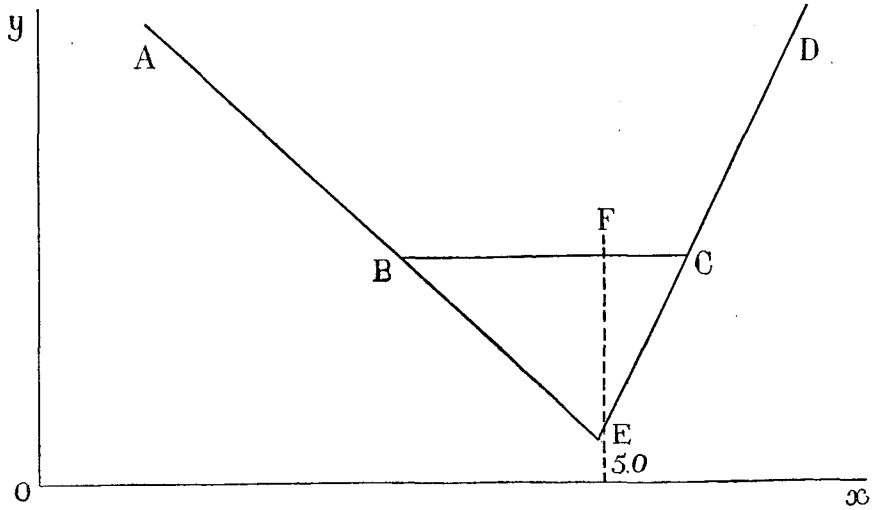
The value of $(100\text{-}a)/a$.

Hordein	1923			1924			1925		
HCl added cc.	Chevalier	Golden Melon	Hokudai No. 1	Chevalier	Golden Melon	Hokudai No. 1	Chevalier	Golden Melon	Hokudai No. 1
4.0	0.451	0.487	0.633	0.473	—	0.482	0.490	0.485	0.484
1	439	471	619	459	—	468	476	468	466
2	430	460	604	446	—	457	463	459	458
3	417	446	594	433	—	444	451	446	447
4	405	435	587	421	—	434	439	435	435
5	397	422	576	412	—	421	422	423	422
6	393	414	568	403	—	411	413	413	412
7	395	409	566	402	—	409	411	408	406
8	396	409	575	405	—	409	412	408	408
9	396	411	577	408	—	411	416	412	410
5.0	396	414	577	408	—	413	416	412	414
1	396	414	574	408	—	413	416	413	414
2	408	421	588	420	—	421	417	416	420
3	428	436	615	442	—	446	434	436	439
4	446	467	653	472	—	466	454	464	462
5	482	487	684	491	—	487	478	485	484

0.2% NaOH solution soluble protein									
4.0	0.465	0.549	0.445	0.455	0.467	0.462	0.462	0.471	0.475
1	452	535	438	439	455	448	451	458	461
2	444	520	430	427	443	435	438	443	449
3	432	506	416	416	429	423	427	432	436
4	423	494	410	406	518	512	417	422	425
5	410	485	398	398	407	402	409	409	415
6	406	475	392	295	398	396	401	400	405
7	402	472	388	394	399	394	398	396	401
8	401	472	390	396	399	398	396	395	400
9	401	475	390	397	398	398	397	396	400
5.0	401	478	391	396	398	398	397	396	398
1	405	487	395	402	405	402	399	395	399
2	429	508	407	420	427	420	418	409	417
3	443	534	426	443	437	441	440	433	441
4	467	575	450	468	472	465	463	453	462
5	493	606	473	490	495	490	489	476	486

The results of the tables have been drawn in figure, taking the quantities of HCl on the x-axis and the values of $(100\text{-}a)/a$ on the y-axis.

Fig. 1.



The general form of the curve is shown in fig. 1. The straight

line AB is the conductivity curve of neutralization of free NaOH by HCl according to Kolthoff[46], and the straight line CD is the conductivity curve of free HCl. The two straight lines AB and CD intersect at E whose value on the x-axis is about 5.0. The quantity of HCl equivalent to the used NaOH is 5.0 cc. The two facts coincide very well, and the author's consideration about the two straight lines AB and CD is verified. The straight line BF which is parallel to the x-axis shows the reaction between the NaOH combining with protein and the added HCl. The straight line FC which is also parallel to the x-axis shows the combination of the added HCl with protein and the length of BF indicates the quantity of NaOH combining with protein and the length of FC indicates the quantity of HCl combining. It seems to the author that a more precise investigation of the straight line BFC may be very interesting and important for the study of the structure of protein.

K. The kinetics of the enzymatic decomposition of protein.

The fact that an enzymatic decomposition of a protein has a relation to its constitution is accepted by many investigators. In recent days, Waldschmidt-Leitz[47] has studied the structure of proteins and has stated that the relation with enzymes, especially between erepsin and trypsin and proteins is most important in the study of the structure of proteins. Sörensen[48] in his recent lecture set forth the relations between the structure of proteins and pepsin and trypsin. He stated also that there may be heterocyclic rings such as diketopiperazin and pyrrole and so on in a protein molecule, but the most important method of combination of amino acids may be the method of peptide linking in a protein molecule. Ssadikow[49] classified the methods of investigations into the structure of proteins in the two sorts, the one being the study of decomposition products by the weak hydrolysis of protein caused by a weak agent such as enzymes, and the second being the synthesis of protein-like substance with heterocyclic complexes. He named the mother substances of the protein composition the "proteone" and divided the structural types into 4, namely pepsinlabil-trysinlabil, pepsinlabil-trypsinfest, pepsinfest-trypsinlabil and pepsinfest-trypsinfest.

Concerning the kinetics of the action of enzyme, there are studies by J. H. Northrup[50] and by P. Rona and H. Kleinmann[51]. According to A. C. Anderson[52] though it may be very difficult, the

action of trypsin and pepsin is a complete reaction. The reaction equation of n-th order is

$$\left(\frac{1}{n-1}\right)\left(\frac{1}{(a-x)^{n-1}}-\frac{1}{a^{n-1}}\right)=kt.$$

The decomposition of protein by enzyme is an equibimolecular reaction according to P. Rona and H. Kleinmann, n of the above equation becomes 2. Thus the equation is

$$k=\frac{1}{t}\cdot\frac{x}{a(a-x)}\ .$$

However, the Schütz law is

$$x=k\sqrt{p\cdot t}$$

where p is the quantity of enzyme, t is the time of reaction.

Therefore, the author intending to make the equation express the reaction as exactly as possible, made the following combination of the above two equations.

$$\frac{1}{t^{k'}}\cdot\frac{x}{a(a-x)}=k\ .$$

(1) The decomposition by trypsin.

To 0.625 g of protein were added 200 cc. of water, the mixture was shaken well, and to it were added 5 cc. of 1/2 normal NaOH solution. The mixture was shaken well and kept still for 24 hours. Then the solution was made up to 250 cc. and the protein solution used in the experiments was prepared. To the mixture of 6.0 cc. of the solution, 19.5 cc. of water and 7.5 cc. of Sörensen's 1/15 M phosphate mixture[53] (pH=7.731) were added with 2 cc. of 1/7000 Grübler's trypsin solution. The solutions were at once placed in a thermostat of $40°\pm0.5°$ C. and the decomposition continued for 15, 30, 45, 60, 90, 120, 150, 180 and 210 minutes respectively. After the given time, the action of trypsin was stopped adding 1.25 cc. of 1/25 normal HCl solution. The HCl was neutralized with 1.25 cc. of 1/25 normal NaOH solution and the undigested protein was precipitated by an addition of 12.5 cc. of 10 % trichloracetic acid solution. The use of trichloracetic acid for the purpose of precipitating the undigested protein was originated by A. Hiller and D. D. Van Slyke[54]. The control was made with the same treatment on a solution of 0 minute's action of trypsin. Five minutes after the addition of trichloracetic acid, the turbidities of the solutions were compared by means of a

Dubosque's nephelometer at room temperature. The following figures were lengths of the nephelometric tube in mm. and the means of 2-4 experiments. The undigested protein was calculated from the means and the digested protein was obtained by subtraction.

TABLE XXVI.

The decomposition of protein by trypsin.

Time (minutes)	0	15	30	45	60	90	120	150	180	210
1923, Chevalier 10% NaCl solution soluble protein										
Means	30	32.6	33.2	33.8	34.4	35.3	36.5	37.05	37.7	38.0
Undigeted	100	92.08	90.36	88.76	87.21	84.98	82.19	80.97	79.57	78.95
Digested	0	7.98	9.64	11.24	12.79	15.02	17.81	19.03	20.43	21.05
1923, Golden Melon 10% NaCl solution soluble protein										
Means	30	33.8	34.6	35.4	35.6	36.4	37.0	38.0	38.7	39.0
Undigested	100	88.76	86.68	84.80	84.27	82.42	81.08	78.95	77.52	76.92
Digested	0	11.24	13.32	15.20	15.73	17.58	18.92	21.05	22.48	23.08
1925, Chevalier 10% NaCl solution soluble protein										
Means	30	32.1	33.25	33.8	34.45	35.2	36.5	37.2	37.55	38.05
Undigested	100	93.45	90.22	88.76	87.05	85.23	82.19	80.65	79.89	78.84
Digested	0	6.55	9.18	11.24	12.95	14.77	17.81	19.35	20.11	21.16
1925, Golden Melon 10% NaCl solution soluble protein										
Means	30	33.58	34.65	35.12	35.58	36.42	37.35	37.9	38.52	39.0
Undigested	100	89.04	86.58	85.42	84.31	82.37	80.32	79.16	77.88	76.97
Digested	0	10.96	13.42	14.58	15.69	17.63	19.68	20.84	22.12	23.03
1923, Chevalier hordein										
Means	30	31.4	32.1	32.6	33.05	34.0	35.4	35.9	36.7	37.2
Undigested	100	95.56	93.46	92.02	90.77	88.23	84.74	83.57	81.74	80.64
Digested	0	4.46	6.54	7.98	9.23	11.77	15.26	16.43	16.26	19.36
1923, Golden Melon hordein										
Means	30	30.8	31.3	32.1	32.8	33.5	34.1	34.6	35.2	36.0
Undigested	100	97.40	95.85	93.46	91.46	89.34	87.98	86.71	85.23	83.33
Digested	0	2.60	4.15	6.54	8.54	10.66	12.02	13.29	14.77	16.67
1924, Chevalier hordein										
Means	30	31.4	32.1	32.5	33.0	34.0	35.6	36.0	36.75	37.3
Undigested	100	95.54	93.46	92.21	90.91	88.23	84.27	83.33	81.63	80.43
Digested	0	4.46	6.54	7.79	9.09	11.77	15.73	16.67	18.37	19.57

1924, Hokudai No. 1 hordein

Means	30	30.9	31.5	32.0	32.65	33.2	34.2	34.7	35.9	36.5
Undigested	100	97.09	95.24	93.75	91.88	90.36	87.72	86.45	83.56	82.19
Digested	0	2.91	4.76	6.25	8.12	9.64	12.28	13.55	16.44	17.81

1925, Chevalier hordein

Means	30	31.45	31.65	32.3	32.88	33.65	34.56	35.27	36.11	36.67
Undigested	100	95.39	94.78	92.83	91.24	89.15	86.80	85.06	83.03	81.81
Digested	0	4.61	5.22	7.12	8.76	10.85	13.20	14.94	16.92	19.19

1925, Golden Melon hordein

Means	30	30.9	31.3	31.9	32.7	33.5	34.2	34.75	35.2	35.95
Undigested	100	97.09	95.84	94.04	91.74	89.55	87.72	86.33	85.23	83.45
Digested	0	2.91	4.16	5.96	8.26	10.45	12.28	13.67	14.77	16.55

1925, Hokudai No. 1 hordein

Means	30	30.9	31.45	32.0	32.7	33.25	34.35	35.0	36.0	36.4
Undigested	100	97.08	95.39	93.75	91.74	90.22	87.34	85.71	83.33	82.42
Digested	0	2.92	4.61	6.25	8.26	9.78	12.66	14.29	16.67	17.58

1923, Chevalier 0.2% NaOH solution soluble protein

Means	30	30.8	31.4	31.7	32.1	32.7	33.1	33.4	33.7	34.0
Undigested	100	97.40	95.54	94.49	93.45	91.74	90.63	89.52	89.02	88.24
Digested	0	2.60	4.46	5.51	6.55	8.26	9.37	10.48	10.98	11.76

1923, Golden Melon 0.2% NaOH solution soluble protein

Means	30	30.7	31.2	31.6	31.95	32.45	33.0	33.25	33.6	34.0
Undigested	100	97.72	96.15	94.99	93.89	92.45	90.91	90.22	89.52	88.24
Digested	0	2.28	3.85	5.01	6.11	7.55	9.09	9.78	10.48	11.76

1923, Hokudai No. 1 0.2% NaOH solution soluble protein

Means	30	30.75	31.1	31.55	32.0	32.45	32.95	33.2	33.65	34.05
Undigested	100	97.45	96.46	95.09	93.75	92.45	91.05	90.36	89.15	88.10
Digested	0	2.44	3.54	4.91	6.25	7.55	8.95	9.64	10.85	11.90

1924, Chevalier 0.2% NaOH solution soluble protein

Means	30	30.8	31.4	31.7	32.2	32.7	33.2	33.6	33.85	34.2
Undigested	100	97.40	95.54	94.64	93.17	91.74	90.36	89.28	88.62	87.72
Digested	0	2.60	4.46	5.36	6.83	8.26	9.64	10.72	11.38	12.28

1924, Golden Melon 0.2% NaOH solution soluble protein

Means	30	30.7	31.3	31.6	32.0	32.5	32.9	33.35	33.7	34.0
Undigested	100	97.72	95.84	94.99	93.75	92.21	91.19	89.95	89.02	88.23
Digested	0	2.28	4.16	5.01	6.25	7.79	8.81	10.05	10.98	11.77

1924, Hokudai No. 1 0.2% NaOH solution soluble protein										
Means	30	30.75	31.3	31.6	32.1	32.55	32.9	33.35	33.7	34.1
Undigested	100	97.56	95.84	94.99	93.46	92.16	91.19	89.95	89.02	87.98
Digested	0	2.44	4.16	5.01	6.54	7.84	8.81	10.05	10.98	12.02
1925, Chevalier 0.2% NaOH solution soluble protein										
Means	30	30.8	31.15	31.7	32.15	32.7	33.0	33.2	33.8	34.2
Undigested	100	97.40	96.38	94.64	93.31	91.74	90.91	90.36	88.76	87.72
Digested	0	2.60	3.62	5.36	6.69	8.26	9.09	9.64	11.24	12.28
1925, Golden Melon 0.2% NaOH solution soluble protein										
Means	30	30.75	31.25	31.65	32.05	32.6	33.1	33.4	33.7	34.05
Undigested	100	97.56	96.00	94.78	93.60	92.02	90.63	89.82	89.02	88.10
Digested	0	2.44	4.00	5.22	6.40	7.98	9.37	10.18	10.98	11.90
1925, Hokudai No. 1 0.2% NaOH solution soluble protein										
Means	30	30.7	31.4	31.65	32.3	32.65	33.25	33.7	33.9	34.15
Undigested	100	97.72	95.54	94.78	92.88	91.88	90.22	89.02	88.50	87.85
Digested	0	2.28	4.46	5.22	7.12	8.12	9.78	10.98	11.50	12.15

(2) The decomposition by pepsin.

The decomposition of protein when the reaction of the medium was alkaline was described in section (1). For the purpose of confirming whether the decomposition is the same or not when the reaction of the medium was acid the experiments with pepsin were carried out as follows.

The same protein solution was used in this case as that of the section (1). The quantities of protein solution and water used were also the same as in section (1), but 7.5 cc. of 1/5 M HCl and 1/5 M KCl mixture[53] of pH value 1.2 were used instead of phosphate mixture. The mixture of 6.0 cc. of a NaOH solution made by a dilution of 5 cc. of 1/2 normal NaOH solution to 250 cc., 19.5 cc. of water and 7.5 cc. of the above buffer solution has a pH value of 2.0. Instead of trypsin solution, 2 cc. of 1/7000 Merck's pepsin solution were used. To stop the enzyme action 2.5 cc. of 1/10 normal NaOH solution were added. The other treatments were all the same as those in the experiments of section (1).

TABLE XXVII.

The decomposition of protein by pepsin.

Time (minutes)	0	15	30	45	60	90	120	150	180	210
1923, Chevalier 10% NaCl solution soluble protein										
Means	30	33.35	34.04	34.9	35.5	36.3	36.9	37.3	37.7	38.05
Undigested	100	89.95	88.13	85.96	84.51	82.64	81.30	80.43	79.57	78.84
Digested	0	10.05	11.87	14.04	15.49	17.36	18.70	19.57	20.43	21.16
1924, Hokudai No. 1 10% NaCl solution soluble protein										
Means	30	32.5	33.4	34.05	34.45	35.25	35.7	36.15	36.9	37.3
Undigested	100	92.31	89.82	88.10	87.08	85.11	84.03	82.99	81.30	80.43
Digested	0	7.69	10.18	11.90	12.92	14.89	15.97	17.01	18.70	19.57
1925, Chevalier hordein										
Means	30	32.0	32.7	33.5	34.0	34.7	35.2	35.7	36.4	37.05
Undigested	100	93.75	91.74	89.52	88.23	86.45	85.23	84.03	82.42	80.97
Digested	0	6.25	8.26	10.48	11.77	13.55	14.77	15.97	17.58	19.03
1925, Golden Melon hordein										
Means	30	31.5	32.05	32.5	33.1	33.95	34.65	35.3	35.6	36.25
Undigested	100	95.24	93.60	92.31	90.43	88.36	86.58	84.99	84.27	82.76
Digested	0	4.76	6.40	7.69	9.57	11.64	13.42	15.01	15.73	17.24
1925, Hokudai No. 1 hordein										
Means	30	31.65	32.3	33.0	33.7	34.4	35.0	35.6	36.05	36.5
Undigested	100	94.78	92.88	90.91	89.02	87.21	85.71	84.27	83.22	82.19
Digested	0	5.22	7.12	9.09	10.98	12.79	14.29	15.73	16.78	17.81
1923, Chevalier 0.2% NaOH solution soluble protein										
Means	30	31.2	31.9	32.4	33.1	33.8	34.5	35.05	35.45	36.05
Undigested	100	96.15	94.04	92.59	90.63	88.76	86.96	85.59	84.63	83.22
Digested	0	3.85	5.96	7.41	9.37	11.24	13.04	14.41	15.37	16.78
1923, Golden Melon 0.2% NaOH solution soluble protein										
Means	30	31.05	31.6	32.2	32.7	33.2	33.9	34.55	35.05	35.55
Undigested	100	96.62	94.94	93.17	91.74	90.36	88.50	86.83	85.59	84.39
Digested	0	3.38	5.06	6.83	8.26	9.64	11.50	13.17	14.41	15.61

(3) The calculation of the experimental results.

The two constants k and k' of the author's equation were cal-

culated by the method of least squares as follows.

$$\frac{1}{t^{k'}} \cdot \frac{x}{a(a-x)} = k$$

$$\therefore \quad -k' \log t + \log x - \log \{a(a-x\} = \log k$$

or $$\log k + k' \log t - \log x + \log \{a(a-x)\} = 0$$

For example, the calculations of the 10 % NaCl solution soluble protein of Chevalier produced in 1923 in the case of trypsin are shown as follows.

TABLE XXVIII.

The values of k and k', the observed and calculated values of x, and their differences.

Observation equations:—

$$\log k + k' \ 1.17609 - 0.90200 + 3.96388 = 0$$
$$\log k + k' \ 1.47712 - 0.98408 + 3.95598 = 0$$
$$\log k + k' \ 1.65321 - 1.05077 + 3.94822 = 0$$
$$\log k + k' \ 1.77815 - 1.10687 + 3.94057 = 0$$
$$\log k + k' \ 1.95424 - 1.17667 + 3.92932 = 0$$
$$\log k + k' \ 2.07918 - 1.25066 + 3.91482 = 0$$
$$\log k + k' \ 2.17609 - 1.27944 + 3.90832 = 0$$
$$\log k + k' \ 2.25527 - 1.31027 + 3.90075 = 0$$
$$\log k + k' \ 2.32222 - 1.32325 + 3.89735 = 0$$

Normal equations:—

$$9 \log k + 16.87157 \ k' + 24.97823 = 0$$
$$16.87157 \log k + 32.8143 \ k' + 46.2829 = 0$$

Solving these equations, the author get

$$k' = 0.45522 \ ,$$
$$k = 0.00023522 \ .$$

Therefore, the required equation is

$$\frac{x}{100\,(100-x)} t^{-0.45522} = 0.00023522.$$

From the equation, the author calculated the values of x, and tabulated the results as follows comparing with those obtained by experiments.

Time (minutes)	0	15	30	45	60	90	120	150	180	210
Observed	0	7.98	9.64	11.24	12.79	15.02	17.81	19.03	20.43	21.05
Calculated	0	7.42	9.90	11.74	13.16	15.41	17.21	18.70	20.00	21.14
Difference	0	+0.56	−0.34	−0.50	−0.37	−0.39	+0.60	+0.33	+0.43	−0.09

1923, Golden Melon 10% NaCl solution soluble protein

$$\frac{x}{100(100-x)}\, t^{-0.33783}=0.00048559$$

Observed	0	11.24	13.32	15.20	15.73	17.58	18.92	21.05	22.48	23.08
Calculated	0	10.81	13.28	14.95	16.22	18.17	19.66	20.88	21.91	22.80
Difference	0	+0.43	+0.04	+0.25	−0.49	−0.59	−0.74	+0.17	+0.57	+0.28

1925, Chevalier 10% NaCl solution soluble protein

$$\frac{x}{100(100-x)}\, t^{-0.49973}=0.0001876$$

Observed	0	6.55	9.18	11.24	12.95	14.77	17.81	19.35	20.11	21.16
Calculated	0	6.77	9.31	11.16	12.67	15.09	17.03	18.66	20.09	21.35
Difference	0	−0.22	−0.13	+0.08	+0.28	−0.32	+0.78	+0.69	+0.02	−0.19

1925, Golden Melon 10% NaCl solution soluble protein

$$\frac{x}{100(100-x)}\, t^{-0.34030}=0.00047748$$

Observed	0	10.96	13.42	14.58	15.69	17.63	19.68	20.84	22.12	23.03
Calculated	0	10.71	13.19	14.85	16.13	18.08	19.58	20.80	21.85	22.76
Difference	0	+0.25	+0.23	−0.27	−0.44	−0.45	+0.10	+0.04	+0.27	+0.27

1923, Chevalier hordein

$$\frac{x}{100(100-x)}\, t^{-0.62264}=0.000087057$$

Observed	0	4.46	6.54	7.98	9.23	11.77	15.26	16.43	18.26	19.36
Calculated	0	4.49	6.75	8.52	10.02	12.54	14.65	16.46	18.09	19.54
Difference	0	−0.03	−0.21	−0.54	−0.79	−0.77	+0.61	−0.03	+0.17	−0.18

1923, Golden Melon hordein

$$\frac{x}{100(100-x)}\, t^{-0.75592}=0.000036514$$

Observed	0	2.60	4.15	6.54	8.54	10.66	12.02	13.29	14.77	16.67
Calculated	0	2.75	4.56	6.09	7.42	9.88	12.23	13.88	15.61	17.21
Difference	0	−0.15	−0.41	+0.45	+1.12	+0.78	−0.21	−0.59	−0.84	−0.54

1924, Chevalier hordein

$$\frac{x}{100(100-x)}\, t^{-0.64795}=0.000076142$$

Observed	0	4.46	6.54	7.79	9.09	11.77	15.73	16.67	18.37	19.57
Calculated	0	4.24	6.48	8.28	9.81	12.41	14.59	16.49	18.19	19.73
Difference	0	+0.22	+0.06	−0.49	−0.72	−0.64	+1.14	+0.18	+0.18	−0.16

1924, Hokudai No. 1 hordein

$$\frac{x}{100(100-x)}\,t^{-0.69237}=0.000052142$$

Observed	0	2.91	4.76	6.25	8.12	9.64	12.28	13.55	16.44	17.81
Calculated	0	3.29	4.99	6.79	8.17	10.54	12.57	14.37	16.00	17.48
Difference	0	−0.38	−0.23	−0.54	−0.05	−0.90	−0.29	−0.82	+0.44	+0.33

1925, Chevalier hordein

$$\frac{x}{100(100-x)}\,t^{-0.65368}=0.000067343$$

Observed	0	4.61	5.22	7.12	8.76	10.85	13.20	14.94	16.92	19.19
Calculated	0	3.80	5.85	7.50	8.91	11.31	13.34	15.11	16.71	18.16
Difference	0	+0.81	−0.63	−0.38	−0.15	−0.46	−0.14	−0.17	+0.21	+1.03

1925, Golden Melon hordein

$$\frac{x}{100(100-x)}\,t^{-0.74044}=0.000039076$$

Observed	0	2.91	4.16	5.96	8.26	10.45	12.28	13.67	14.77	16.55
Calculated	0	2.82	4.62	6.14	7.49	9.86	11.92	13.77	15.45	17.00
Difference	0	+0.09	−0.46	−0.18	+0.77	+0.59	+0.36	−0.10	−0.68	−0.45

1925, Hokudai No. 1 hordein

$$\frac{x}{100(100-x)}\,t^{-0.75793}=0.000037842$$

Observed	0	2.92	4.61	6.25	8.26	9.78	12.66	14.29	16.67	17.58
Calculated	0	2.86	4.74	6.34	7.76	10.28	12.47	14.44	16.23	17.95
Difference	0	+0.06	−0.13	−0.09	+0.50	−0.50	+0.19	−0.15	+0.44	−0.37

1923, Chevalier 0.2% NaOH solution soluble protein

$$\frac{x}{100(100-x)}\,t^{-0.56596}=0.00006669$$

Observed	0	2.60	4.41	5.51	6.55	8.26	9.47	10.48	10.98	11.76
Calculated	0	2.99	4.38	5.43	6.34	7.85	9.09	10.20	11.19	11.37
Difference	0	−0.39	+0.08	+0.08	+0.21	+0.41	+0.38	+0.28	−0.21	+0.29

1923, Golden Melon 0.2% NaOH solution solubie protein

$$\frac{x}{100(100-x)}\,t^{-0.63841}=0.000045016$$

Observed	0	2.28	3.85	5.01	6.11	7.55	9.09	9.78	10.48	11.76
Calculated	0	2.47	3.79	4.76	5.79	7.37	8.73	10.14	11.02	11.79
Difference	0	−0.19	+0.06	+0.25	+0.32	+0.18	+0.36	−0.36	−0.54	−0.03

1923, Hokudai No. 1 0.2% NaOH solution soluble protein

$$\frac{x}{100(100-x)}\,t^{-0.64561}=0.000043604$$

Observed	0	2.44	3.54	4.91	6.25	7.55	8.95	9.64	10.85	11.90
Calculated	0	2.44	3.77	5.40	5.78	7.38	8.75	9.97	11.08	12.10
Difference	0	0	−0.23	−0.49	+0.49	+0.17	+0.20	−0.33	−0.23	−0.20

1924, Chevalier 0.2% NaOH solution soluble protein

$$\frac{x}{100(100-x)}\, t^{-0.61845} = 0.000054065$$

Observed	0	2.60	4.46	5.36	6.83	8.26	9.64	10.72	11.38	12.28
Calculated	0	2.89	4.24	5.38	6.37	8.04	9.45	10.70	11.83	12.86
Difference	0	−0.20	+0.22	−0.02	+0.46	+0.22	+0.19	+0.02	−0.45	−0.58

1924, Golden Melon 0.2% NaOH solution soluble protein

$$\frac{x}{100(100-x)}\, t^{-0.63937} = 0.00004572$$

Observed	0	2.28	4.16	5.01	6.25	7.79	8.81	10.05	10.98	11.77
Calculated	0	2.52	3.86	4.95	5.90	7.51	8.89	10.12	11.23	12.25
Difference	0	−0.24	+0.30	+0.06	+0.35	+0.38	−0.08	−0.07	−0.25	−0.48

1924, Hokudai No. 1 0.2% NaOH solution soluble protein

$$\frac{x}{100(100-x)}\, t^{-0.61490} = 0.00005165$$

Observed	0	2.44	4.16	5.01	6.54	7.84	8.81	10.05	10.98	12.02
Calculated	0	2.66	4.02	5.09	6.02	7.59	8.98	10.11	11.26	12.15
Difference	0	−0.22	+0.14	−0.08	+0.52	+0.25	−0.17	−0.06	−0.28	−0.13

1925, Chevalier 0.2% NaOH solution soluble protein

$$\frac{x}{100(100-x)}\, t^{-0.61089} = 0.00005136$$

Observed	0	2.60	3.62	5.36	6.69	8.26	9.09	9.64	11.24	12.28
Calculated	0	2.66	4.02	5.10	6.03	7.62	8.96	10.15	11.24	12.20
Difference	0	−0.06	−0.40	+0.26	+0.66	+0.64	+0.13	−0.51	0	+0.08

1925, Golden Melon 0.2% NaOH solution soluble protein

$$\frac{x}{100(100-x)}\, t^{-0.61842} = 0.000047699$$

Observed	0	2.44	4.00	5.22	6.40	7.98	9.37	10.18	10.98	11.90
Calculated	0	2.63	3.97	5.08	6.03	7.67	9.07	10.31	11.42	12.46
Difference	0	−0.19	+0.03	+0.14	+0.37	+0.31	+0.30	−0.13	−0.44	−0.56

1925, Hokudai No. 1 0.2% NaOH solution soluble protein

$$\frac{x}{100(100-x)}\, t^{-0.63685} = 0.000045331$$

Observed	0	2.28	4.46	5.22	7.12	8.12	9.78	10.98	11.50	12.15
Calculated	0	3.26	4.06	5.23	6.26	8.01	9.52	10.86	12.08	13.23
Difference	0	−0.98	+0.40	−0.01	+0.86	+0.09	+0.26	+0.12	−0.58	−1.08

The above are the results of trypsin, and the results of pepsin are calculated by the same method as follows.

1923, Chevalier 10% NaCl solution soluble protein

$$\frac{x}{100(100-x)}\,t^{-0.34201}=0.0004384$$

Observed	0	10.05	11.87	14.04	15.49	17.36	18.70	19.57	20.43	21.16
Calculated	0	9.99	12.33	13.92	15.14	17.02	18.46	19.64	20.64	21.52
Difference	0	+0.06	−0.46	+0.12	+0.35	+0.34	+0.24	−0.07	−0.21	−0.36

1924, Hokudai No. 1 10% NaCl solution soluble protein

$$\frac{x}{100(100-x)}\,t^{-0.39943}=0.00028615$$

Observed	0	7.69	10.18	11.90	12.92	14.89	15.97	17.01	18.70	19.57
Calculated	0	7.78	10.02	11.57	12.80	14.72	16.22	17.47	18.55	19.50
Difference	0	−0.09	+0.16	+0.33	+0.12	+0.17	−0.25	−0.46	+0.15	+0.07

1925, Chevalier hordein

$$\frac{x}{100(100-x)}\,t^{-0.46737}=0.00018956$$

Observed	0	6.25	8.26	10.48	11.77	13.55	14.77	15.97	17.58	19.03
Calculated	0	6.29	8.59	10.10	11.38	13.44	15.08	16.46	17.67	18.75
Difference	0	−0.04	−0.24	+0.38	+0.39	+0.11	−0.31	−0.49	−0.09	+0.28

1925, Golden Melon hordein

$$\frac{x}{100(100-x)}\,t^{-0.63991}=0.000074122$$

Observed	0	4.76	6.40	7.69	9.57	11.64	13.42	15.01	15.73	17.24
Calculated	0	4.02	6.13	7.84	9.24	11.65	13.69	15.46	17.05	18.42
Difference	0	+0.74	+0.27	−0.15	+0.33	−0.01	−0.27	−0.45	−1.32	−1.17

1925, Hokudai No. 1 hordein

$$\frac{x}{100(100-x)}\,t^{-0.52728}=0.00013319$$

Observed	0	5.22	7.12	9.09	10.98	12.79	14.29	15.73	16.78	17.81
Calculated	0	5.26	7.41	9.02	10.34	12.50	14.26	15.75	17.07	18.25
Difference	0	−0.04	−0.29	+0.07	+0.64	+0.29	+0.03	−0.02	−0.29	−0.44

1923, Chevalier 0.2% NaOH solution soluble protein

$$\frac{x}{100(100-x)}\,t^{-0.61003}=0.000079402$$

Observed	0	3.85	5.96	7.41	9.37	11.24	13.04	14.41	15.37	16.78
Calculated	0	3.98	5.95	7.49	8.80	11.00	12.84	14.44	15.87	17.16
Difference	0	−0.13	+0.01	−0.08	+0.57	+0.24	+0.20	−0.03	−0.50	−0.38

1923, Golden Melon 0.2% NaOH solution soluble protein

$$\frac{x}{100(100-x)}\,t^{-0.63073}=0.00006353$$

Observed	0	3.38	5.06	6.83	8.26	9.64	11.50	13.17	14.41	15.61
Calculated	0	3.38	5.15	6.55	7.75	9.79	11.51	13.03	14.47	15.62
Difference	0	0	−0.09	+0.28	+0.51	−0.15	−0.01	+0.14	−0.06	−0.01

From the results of the experiments and the calculations, the author is able to find that the decomposition of proteins of brewing barley by trypsin and pepsin is expressed by the equation of the general form

$$\frac{x}{100\,(100-x)}\,t^{-k'}=k\,,$$

where k and k' are the constants characteristic to the protein used.

L. Discussions.

From the experiments on the proteins of brewing barley, the author is able to tabulate the marked differences as follows.

TABLE XXIX.

The marked differences of the proteins of barley.

The 10% NaCl solution soluble protein	
The free amino nitrogen content	Golden Melon>Hokudai No. 1>Chevalier
The non-basic nitrogen content	Chevalier>Hokudai No. 1>Golden Melon
The quantities of HCl required for the maximum surface tension and turbidity	Hokudai No. 1>Chevalier>Golden Melon
The specific rotatory power	Golden Melon>Chevalier>Hokudai No. 1
The hordein	
The free amino nitrogen content	Chevalier>Hokudai No. 1
The non-basic nitrogen content	Golden Melon>Hokudai No. 1>Chevalier
The basic nitrogen content	Chevalier>Golden Melon>Hokudai No. 1
The quantities of HCl required for maximum surface tension and turbidity	Golden Melon>Hokudai No. 1>Chevalier
The specific rotatory power	Chevalier>Hokudai No. 1>Golden Melon
The 0.2% NaOH solution soluble protein	
The free amino nitrogen content	Chevalier>Golden Melon>Hokudai No. 1
The quantities of HCl required for maximum surface tension and turbidity	Hokudai No. 1>Golden Melon>Chevalier

The quantities of HCl required for the maximum surface tension and turbidity shows the quantities of alkali combined with the carboxyl group which are substituted by the titrated HCl. Therefore, the smaller quantities of HCl shows the smaller quantities of carboxyl groups of the protein molecule. From the facts of the above table, i.e. the quantities of HCl, the non-basic nitrogen content, the free amino nitrogen content and the basic nitrogen content, it can be deduced that the pH value of the iso-electric point of the 10% NaCl solution soluble protein decreases in the order Golden

Melon, Chevalier and Hokudai No. 1; that the pH value of the hordein decreases Chevalier, Hokudai No. 1 and Golden Melon, while that of the 0.2% NaOH solution soluble protein decreases in the order Chevalier, Golden Melon and Hokudai No. 1.

It is also seen that the more basic the protein is, the greater the specific rotatory power of the alkali protein solution, that is, this indicates that the basic protein has a more complex structure than the acidic protein. Thus in the case of the 10% NaCl solution soluble protein, the structure becomes more complex in the order Golden Melon, Chevalier and Hokudai No. 1, in the case of hordein, it is more complicated in the order Chevalier, Hokudai No. 1 and Golden Melon, and in the case of the 0.2% NaOH solution soluble protein, the order is Chevalier, Golden Melon and Hokudai No. 1.

The experimental values and the calculated values of the decomposition of protein by enzymes agreed very well and the same decomposition were observed when the medium of reaction was acid or alkaline respectively by the use of trypsin and pepsin. Therefore, the equation which denotes the decomposition process of protein by an enzyme must be as follows.

$$\frac{x}{100\,(100-x)}\,t^{-k'}=k\,,$$

where k is the reaction constant. The value of k of the author's experiments increases in the following order.

The 10% NaCl solution soluble protein	Chevalier, Golden Melon
The hordein	Golden Melon, Hokudai No. 1, Chevalier
The 0.2% NaOH solution soluble protein	Hokudai No. 1, Golden Melon, Chevalier

The values of k' are opposed to the values of k and they decrease in the above order. The pH values of the iso-electric points of the proteins coincide very well with the values of k. Thus as stated above the pH values of the iso-electric points increases in the above order. Therefore, it can be said that the more complex the structure of a protein is, the more easily decomposed it is by the action of an enzyme.

The fact that k and k' are of opposite values indicates that there is some relation between the two. If the author draws figures taking

log k on y-axis and k' on x-axis, he obatins 3 straight lines representing the 10 % NaCl solution soluble protein, the hordein and the 0.2 % NaOH solution soluble protein. Therefore, the equations of the 3 straight lines must be

$$y=mx+b\,.$$

Taking log k as y and k' as x, for the purpose of determining the m and b of the equation, the method of least squares results as follows.

10 % NaCl solution soluble protein (trypsin)

Observation equations:—

$$3.32105=0.34030\ m+b$$
$$3.72676=0.49973\ m+b$$
$$3.31373=0.33783\ m+b$$
$$3.62872=0.45522\ m+b$$

Normal equations:—

$$4\ b+1.63308\ m+13.99026=0$$
$$1.63308\ b+0.68689\ m+\ 5.76387=0$$
$$m=-2.5822$$
$$b=-2.44333$$

Therefore, the required equation is

$$\log k=-2.5822\ k'-2.44333.$$

Hordein (trypsin)

By the same method as above, solving 7 observation equations, the values of m and b are

$$m=-2.7705$$
$$b=-2.34335$$
$$\log k=-2.7705\ k'-2.34335.$$

0.2 % NaOH solution soluble protein (trypsin)

From 9 observation equations, the values of m and b are calculated.

$$m=-2.0709$$
$$b=-3.00766$$
$$\log k=-2.0709\ k'-3.00766.$$

These 3 straight lines intersect each other, and the intersection points of the 10 % NaCl solution soluble protein and the hordein, and of the hordein and the 0.2 % NaOH solution soluble protein are calculated as follows.

The 10 % NaCl solution soluble protein and the hordein	The hordein and the 0.2% NaOH solution soluble protein
$k'=0.5309$	$k'=0.9495$
$\log k=-3.81421$	$\log k=-4.97407$
$k=0.00015338$	$k=0.000010615$

The equations of decomposition denoted by these two intersection points are

$$\frac{x}{100\,(100-x)}\,t^{-0.5309}=0.00015338 \qquad (1)$$

$$\frac{x}{100\,(100-x)}\,t^{-0.9495}=0.000010615 \qquad (2)$$

Substituting for t 15, 30, 45, 60, 90, 120, 150, 180 and 210, the values of x are calculated from the two equations.

TABLE XXX.

The calculated values of x.

t	15	30	45	60	90	120	150	180	210
(1)	6.07	8.53	10.37	11.88	14.50	16.30	17.99	19.46	20.78
(2)	1.38	2.61	3.79	4.92	7.07	9.09	11.00	12.81	14.54

The 3 straight lines are figured in Fig. 2 and the 2 decomposition curves are figured in Fig. 3. That the intersections were obtained in Fig. 2 shows that the 3 proteins, at least those in the barley, have some continuous constitution. It also seems that the 3 proteins gradually merge with each other either qualitatively or quantitatively taking as the limit the proteins whose decompositions are expressed by the curves made by the intersection points.

According to the solubility of the proteins in the 10 % NaCl solution, in the 70 % alcohol or in the 0.2 % NaOH solution, no proteins exceed the author's so-called limiting proteins. These facts seem to prove the author's opinion concerning the constitution of the proteins deserve careful attention.

The values of k' at the intersections of the 3 straight lines were 0.5309 and 0.9495 which approximate 0.5 and 1.0. If it is 0.5, the equation take on the form of Schütz law, and if 1.0, the equation becomes like the form of the bimolecular reaction. Many investigators have already proved the correctness of Schütz law and of the bimolecular equation. Thus it seems that the investigators limited their results to a specific protein or the author's limiting proteins.

Fig. 2.

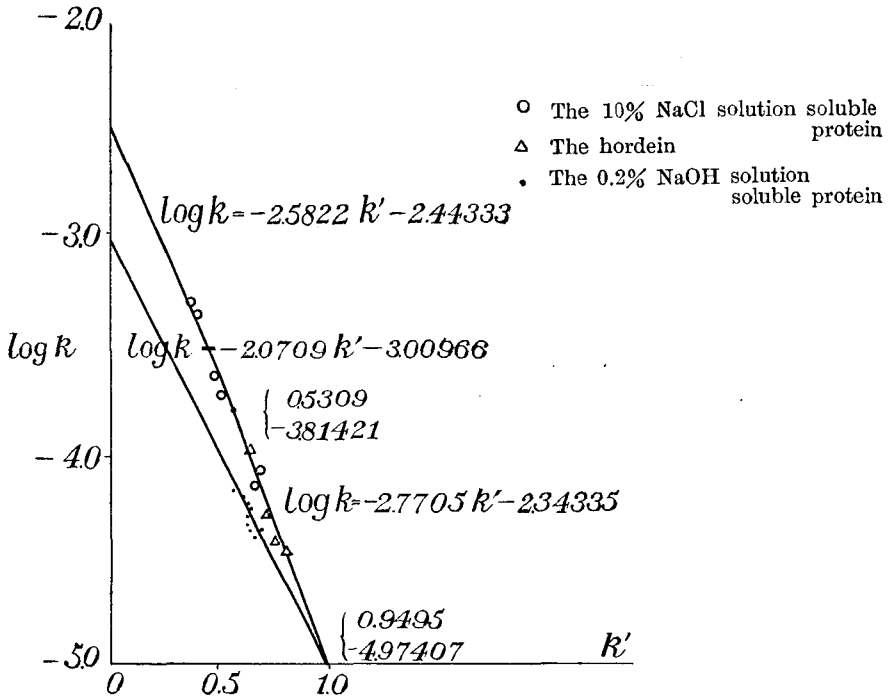
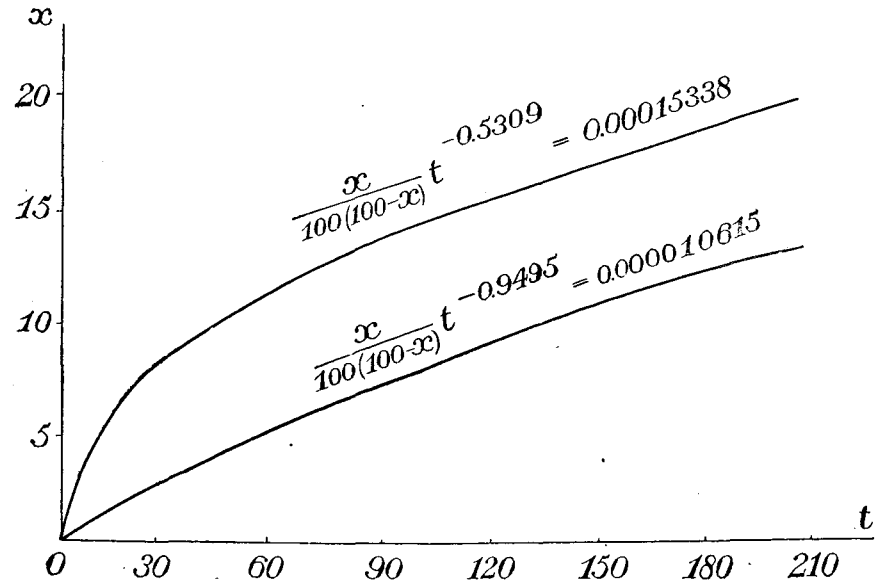

−2.0
−3.0
−4.0
−5.0
log k
0
0.5
1.0
k′
o The 10% NaCl solution soluble protein
Δ The hordein
• The 0.2% NaOH solution soluble protein
log k=−2.5822 k′−2.44333
log k=−2.0709 k′−3.00966
0.5309
−3.81421
log k=−2.7705 k′−2.34335
0.9495
−4.97407

Fig. 3.


x
20
15
10
5
0
30
60
90
120
150
180
210
t
x/100(100−x) t^−0.5309 = 0.00015338
x/100(100−x) t^−0.9495 = 0.000010615

Therefore, the author's limiting proteins had to resemble, at least on the relation to the action of enzyme, the typical and the representative proteins investigated up to recent days. To expect a general constitution of the two kinds of proteins seems to be most proper.

4. THE STARCHES.

Many investigations concerning the starches of brewing barley carried out by such authors as Lintner, Wenglein, Ewers, Schubert, Schwarcz, Kastscha, Ling, Ling and Nanji and Harper have been mainly connected with methods to determine quickly the starch content or the barley granules. Reichard[55] studied the relations between the starch content and the extract. Vine[56] stated that the hardness of barley grain was proportional to the starch content of it. Daish[57] studied the starch of barley grain changed to glucose through soluble starch, dextrin and maltose by the process of malting. However, no studies have been carried out on barley starches according to the species. T. Tadokoro *et. al.*[58] have made precise investigations on the differences of the starches of glutinous and common rices.

The starches for the investigations were separated from the residue of protein preparation.

The residue was treated with 0.2 % NaOH solution, the supernatant solution being changed every day. Thus the Millon reaction to protein was completely removed and the residue was washed till the washing water gave no reaction with phenolphthalein. The pure white starch was filtered through a silk mesh. The starch thus separated was washed several times with distilled water. Finally the starch was treated with alcohol and ether. The air-dried starch was powdered by means of an agate mortar. The water and ash content of the starch was estimated with the following results.

TABLE XXXI.

The water and ash content of starch.

	1923		1924		1925	
	Water	Ash (water free)	Water	Ash (water free)	Water	A h (water free)
Chevalier	14.317	0.033	14.147	0.198	13.831	0.186
Golden Melon	12.679	0.077	14.707	0.138	13.415	0.165
Hokudai No. 1	12.813	0.076	16.838	0.235	13.588	0.114

A. The viscosity of starch solution.

Twenty-five-hundredths g, one half g and one g (calculated as water free) respectively of starch were suspended in 40 cc. of water. The mixture were heated on a boiling water bath for exactly 10 minutes with occasional stirrings. After the heating, the solution was poured into a measuring flask of 50 cc. and distilled water was added to the mark. The solution was cooled for 1 hour in tap water. The viscosity of the solution was measured by means of Ostwald's viscosimeter at 15° C. The following figures are the specific viscosities calculated taking the viscosity of water as 1 as the basis.

TABLE XXXII.

The viscosity of starch solution at 15° C.

	1923			1924			1925		
	0.5%	1.0%	2.0%	0.5%	1.0%	2.0%	0.5%	1.0%	2.0%
Chevalier	1.155	1.395	3.123	1.147	1.490	3.105	1.147	1.356	2.035
Golden Melon	1.130	1.328	2.137	1.098	1.344	1.716	1.142	1.219	1.674
Hokudai No. 1	1.095	1.265	1.967	1.081	1.242	1.574	1.109	1.195	1.614

According to the table, it can be seen that the specific viscosisty of the Chevalier starch solution was the greatest, that of the Golden Melon intermediate, and that of the Hokudai No. 1 was the smallest.

To find the mathematical relation between the viscosities and the concentration of the starch solutions, the author applied interpolation. Thus he calculated the functions between the specific viscosities η and the concentrations c. Also the mean values of the functions when c was 0 to 2 were calculated, which seemed to represent more clearly the differences of viscosities of the 3 kinds of starches.

TABLE XXXIII.

The function between η and c, and the mean value of the function when c was 0 to 2.

		Function	Mean value of function (c=0-2)
1923	Chevalier	$\eta=1+0.3905\ c-0.3265\ c^2+0.331\ c^3$	1.617
	Golden Melon	$\eta=1+0.2268\ c+0.031\ c^2+0.0696\ c^3$	1.407
	Hokudai No. 1	$\eta=1+0.1378\ c+0.0815\ c^2+0.0456\ c^3$	1.338
1924	Chevalier	$\eta=1+0.3348\ c-0.2285\ c^2+0.2936\ c^3$	1.617
	Golden Melon	$\eta=1-0.046\ c+0.578\ c^2-0.188\ c^3$	1.349
	Hokudai No. 1	$\eta=1+0.0436\ c+0.275\ c^2-0.076\ c^3$	1.257
1925	Chevalier	$\eta=1+0.2445\ c+0.0865\ c^2+0.0250\ c^3$	1.410
	Golden Melon	$\eta=1+0.4316\ c-0.3780\ c^2+0.1653\ c^3$	1.260
	Hokudai No. 1	$\eta=1+0.2936\ c-0.2040\ c^2+0.1053\ c^3$	1.232

B. The saponification value.

Rosenthaler[59] estimated the saponification values of many starches and stated that this represented the saponification of amylopectin, the ester of phosphoric acid. Therefore, the high saponification value of a starch indicates that if the constitution of amylopectin was the same the amylopectin content is high or if the amylopectin content was the same the constitution of amylopectin is different. The saponification value seems to denote that there are some differences in the constitution of starch. One g of starch was heated for 1 hour with 20 g of ethyl alcohol and 5 cc. of 1/10 normal alcoholic KOH solution in connection with a reflux condensor in a water bath. Thereupon the unexpended KOH was titrated with 1/10 normal HCl solution to a permanently colorless solution. The figures of the table are the mg of expended KOH to 1 g of starch.

TABLE XXXIV.

The saponification value of starch.

	1923	1924	1925
Chevalier	2.945	3.085	3.085
Golden Melon	2.665	2.665	2.805
Hokudai No. 1	3.085	4.348	4.488

The saponification value of the Hokudai No. 1 starch was the greatest, that of the Chevalier intermediate, and that of the Golden Melon was the smallest.

C. The adsorption of iodine by starch.

To 0.5 g of starch were added 20 cc. of iodine potassium iodide solution of various concentrations, such as ca. 1/10, 1/25, 1/50, 1/75, 1/100 and 1/200 normal. The mixture was shaken for 30 minutes on a shaking machine. After 30 minutes' standing of the mixture, it was centrifuged for 5 minutes. A given quantity of the supernatant solution was taken and the solution was titrated with sodium thiosulphate solution. One cc. of the solution was equivalent to 0.001458 g of iodine. The following figures are the means of 3 determinations.

From the figures the two constants k and $1/n$ of Freundlich's adsorption isotherm $\frac{x}{m}=k\,(a-x)^{\frac{1}{n}}$ were calculated by means of the method of least squares.

TABLE XXXV.

The adsorption of iodine.

a=quantities of iodine contained in 20 cc. of iodine solution (g).
x=quantities of iodine adsorbed (g).
m=0.5 g.

a	$a-x$	x	x/m	log $a-x$	log x/m
1924, Chevalier starch					
0.01239	0.00029	0.01210	0.02420	$\bar{4}$.4623980	$\bar{2}$.3838154
0.02479	0.00286	0.02193	0.04386	$\bar{3}$.4563660	$\bar{2}$.6420686
0.03305	0.00598	0.02707	0.05414	$\bar{3}$.7767012	$\bar{2}$.7335183
0.04957	0.01572	0.03385	0.06770	$\bar{2}$.1964525	$\bar{2}$.8305887
0.09914	0.04024	0.05872	0.11744	$\bar{2}$.6046589	$\bar{1}$.0698160
0.24786	0.14434	0.10352	0.20704	$\bar{1}$.1593867	$\bar{1}$.3160543
1924, Golden Melon starch					
0.01239	0.00035	0.01204	0.02408	$\bar{4}$.5440680	$\bar{2}$.3816565
0.02479	0.00286	0.02193	0.04386	$\bar{3}$.4563660	$\bar{2}$.6420686
0.03305	0.00636	0.02669	0.05338	$\bar{3}$.8034571	$\bar{2}$.7273786
0.04957	0.01482	0.03475	0.06950	$\bar{2}$.1708482	$\bar{2}$.8419848
0.09914	0.03624	0.06290	0.12580	$\bar{2}$.5591882	$\bar{1}$.0996836
0.24786	0.11722	0.13064	0.26128	$\bar{1}$.0690017	$\bar{1}$.4171062

1924, Hokudai No. 1 starch					
0.01239	0.00035	0.01224	0.02408	$\bar{4}$.5440680	$\bar{2}$.3816565
0.02479	0.00268	0.02211	0.04422	$\bar{3}$.4281348	$\bar{2}$.6456187
0.03305	0.00636	0.02669	0.05338	$\bar{3}$.8034571	$\bar{2}$.7273786
0.04957	0.01539	0.03418	0.06836	$\bar{2}$.1872386	$\bar{2}$.8348021
0.09914	0.04053	0.05881	0.11762	$\bar{2}$.6056282	$\bar{1}$.0704812
0.24786	0.15090	0.14696	0.29392	$\bar{1}$.1786892	$\bar{1}$.4682291
1925, Chevalier starch					
0.01239	0.00029	0.01210	0.02420	$\bar{4}$.4623980	$\bar{2}$.3838154
0.02479	0.00256	0.02223	0.04446	$\bar{3}$.4082400	$\bar{2}$.6479695
0.03305	0.00604	0.02701	0.05402	$\bar{3}$.7810369	$\bar{2}$.7325546
0.04957	0.01662	0.03295	0.06590	$\bar{2}$.2206310	$\bar{2}$.8188854
0.09914	0.03820	0.06094	0.12188	$\bar{2}$.5820634	$\bar{1}$.0859324
0.24786	0.14497	0.10289	0.20578	$\bar{1}$.1612781	$\bar{1}$.3134032
1925, Golden Melon starch					
0.01239	0.00044	0.01205	0.02410	$\bar{4}$.6484527	$\bar{2}$.3280170
0.02479	0.00256	0.02223	0.04446	$\bar{3}$.4082400	$\bar{2}$.6479695
0.03305	0.00619	0.02686	0.05372	$\bar{3}$.7916906	$\bar{2}$.7301360
0.04957	0.01443	0.03516	0.07032	$\bar{2}$.1592663	$\bar{2}$.8470789
0.09914	0.03776	0.06138	0.12276	$\bar{2}$.5770320	$\bar{1}$.0890569
0.24786	0.14632	0.10154	0.20308	$\bar{1}$.1653037	$\bar{1}$.3076672
1925, Hokudai No. 1					
0.01239	0.00023	0.01216	0.02432	$\bar{4}$.3617278	$\bar{2}$.3859636
0.02479	0.00204	0.02275	0.04559	$\bar{3}$.3096302	$\bar{2}$.6580114
0.03305	0.00525	0.02780	0.05560	$\bar{3}$.7201593	$\bar{2}$.7450748
0.04957	0.01335	0.03622	0.07244	$\bar{3}$.1254813	$\bar{2}$.8599784
0.09914	0.03747	0.06167	0.12334	$\bar{2}$.5736837	$\bar{1}$.0911039
0.24786	0.14624	0.10172	0.20344	$\bar{1}$.1650662	$\bar{1}$.3084363

Values of k and $1/m$ of Freundlich's formula.

Concentration	1924				1925			
	of iodine high		low		high		low	
	$1/n$	k	$1/n$	k	$1/n$	k	$1/n$	k
Chevalier	0.25968	0.20124	0.50065	0.55754	0.25056	0.19064	0.51330	0.57984
Golden Melon	0.28006	0.23233	0.63945	1.03520	0.33984	0.30738	0.46805	0.52564
Hokudai No. 1	0.27459	0.21696	0.64218	0.97013	0.26686	0.22995	0.42807	0.47494

When the concentration of iodine was low, that is when the quantities of iodine in 20 cc. of the solution were 0.01239–0.04957 g, both $1/n$ and k were the greatest in the Golden Melon starch, intermediate in the Hokudai No. 1 starch, the smallest in the Chevalier starch. That is, the tendency and the capacity of the Golden Melon starch to adsorb iodine was the greatest, and those of the Chevalier starch was the smallest. But when the concentration was high, or the quantities of iodine in 20 cc. of the solution were 0.04957–0.24876 g, there was no difference according to the species.

D. The acetylation of starch.

The acetylation of an organic substance seems to give some idea concerning its constitution. The author intending to discover something of its constitution, carried out the acetylation of starch according to Tadokoro's method. Five g of starch were mixed with 40 g of acetic anhydride and 0.5 g of $ZnCl_2$ and heated gently for 2 hours on a sand bath with a reflux condensor. When all the combined starch became a transparent, dark brown solution, it was poured into water and acetyl starch was precipitated. The precipitate was washed many times with water untill the water had no reaction to acetic acid, the precipitate was washed with alcohol and ether, and dried for 8 hours at 100° C. The dried acetyl starch was then stored in a sulphuric acid desiccator.

The acetyl group of acetyl starch was determined by Wenzel's method[60], the specific rotatory power of acetyl starch (0.2 g of material dissolved in 15 cc. of pyridine) was measured by Goeltz's polariscope after 5 hours at 25° C. and the melting point of acetyl starch was determined by means of Thiele's apparatus[61]. The necessary corrections were made.

TABLE XXXVI.

The acetyl group, specific rotatory power and melting point of acetyl starch.

	Acetyl group		
	Chevalier	Golden Melon	Hokudai No. 1
1923	49.064	48.042	46.767
1924	49.727	48.406	47.738
1925	50.049	49.727	49.395

	Specific rotatory power (+)		
1923	131.62	133.64	138.81
1924	137.25	138.75	140.25
1925	136.50	137.25	138.00
	Melting point (corrected)		
1923	151	154	160
1924	156	162	168
1925	155	160	166

The content of acetyl group was the greatest in the Chevalier acetyl starch, intermediate in the Golden Melon acetyl starch and the smallest in the Hokudai No. 1 acetyl starch. The specific rotatory power and the melting point of acetyl starch were just the contrary.

In 1927, E. Peiser[62] by the study of chlorine substitution of acetyl starch reported that the aldehyde group of starch molecule which is situated in its middle was combined more weakly than the others and the alcohol group neighbouring that aldehyde group was in combination instead of free state. E. Peiser also reported that the aldehyde group and the neighbouring alcohol group parted easily from their acetyl group and easily took chlorine. The author carried out the chlorine substitution according to E. Peiser. Four g of acetyl starch were suspended in 60 cc. of dry toluol, to the mixture were added 6.5 g of phosphorouspentachloride. The mixture was heated in an oil bath for 1.5 hours at 105° C. The colorless solution was poured into water while still hot. Thereupon, the reaction substance was precipitated and the precipitate was washed several times with water, then washed with alcohol several times. It was dried at 100° C. for 3 hours and stored in a sulphuric acid desiccator. The chlorine content of the substance was measured by Koranyi and Ruszuyak's micro-method[63]. The results are the means of 3 determinations.

TABLE XXXVII.

The chlorine content of chlorinated acetyl starch.

	Chevalier	Golden Melon	Hokudai No. 1
1924	0.368%	0.269%	0.247%

By the table it is seen that the quantity of chlorine substituted in the chlorine substitution of acetyl starch was the greatest in the

Chevalier acetyl starch, intermediate in the Golden Melon acetyl starch and the smallest in the Hokudai No. 1 acetyl starch.

E. Discussion.

The viscosity of a colloid changes proportionately to the magnitude and the dispersion degree. The maximum viscosity was observed in the Chevalier starch solution, intermediate in the Golden Melon starch solution, and the minimum viscosity in the Hokudai No. 1 starch solution. The content of acetyl group of acetyl starch also had the same tendency with the viscosity. From the two facts here mentioned, it can be deduced that the content of OH group in the Chevalier starch is the greatest, intermediate in the Golden Melon starch and the smallest in the Hokudai No. 1 starch. As all starches are considered to be built up from glucose, the difference of OH group content seems to express the difference of the magnitude of starch molecule.

In acetylation the content of acetyl group and the quantity of chlorine substituted seems to indicate the magnitude of the acetyl starch molecule. Therefore, it is possible to say that the molecule of the Chevalier acetyl starch is the smallest, the Golden Melon acetyl starch molecule intermediate, and the Hokudai No. 1 acetyl starch molecule the greatest. If the specific rotatory power and the melting point of a substance decrease with the decrease of magnitude of the molecule, the experimental results agree very well with this assumption.

Therefore, it seems (1) that the molecular weight of the Chevalier starch is the greatest and also that the molecule is the most loosely composed of the 3 kinds of starches, (2) that the smallest and the hardest is the Hokudai No. 1 starch, and (3) that the Golden Melon starch is situated between these two in respect to the molecular weight and the mode of composition.

4. CONCLUSIONS.

1. The general analysis of barley grain was carried out. The total nitrogen and the protein nitrogen were the most abundant in Chevalier, medium in Hokudai No. 1 and the smallest in Golden Melon.

2. The fats, the proteins and the starches were separated and purified.

3. The fats of Golden Melon always have the smallest saponi-

fication value, iodine value, Reichert-Meissl value and soluble acid. But the refractive index was the largest.

4. The distribution of the 4 kinds of nitrogen, that is the water-soluble, the 10 % NaCl-solution-soluble, the 70 % alcohol-solution-soluble and the 0.2 % NaOH-solution-soluble nitrogen, was determined.

5. The ash content of the proteins increases in the order, hordein, 10 % NaCl-solution-soluble-protein and the 0.2 % NaOH-solution-soluble-protein. As for the species of the barley, Golden Melon has the minimum ash content, Chevalier medium, and Hokudai No. 1 the maximum.

6. The author carried out (a) the elementary analysis of the proteins, (b) the determination of the amount of free amino nitrogen of the proteins, (c) the hydrolysis of the proteins according to Van Slyke, (d) the determination of the changes of the turbidity and the surface tension of the protein alkali solution when titrated with HCl, (e) the specific rotatory power of the proteins in an alkaline solution, (f) the determination of the refractive index of the protein alkali solutions, (g) the determination of the combining power of proteins with alkali and (h) the conductometric titration of protein alkali solution with HCl.

7. The decomposition of proteins by enzymes seems to follow the equation

$$k=\frac{1}{t^{k'}}\cdot\frac{x}{a\,(a-x)}\,.$$

8. The pH value of the iso-electric point of the 10 % NaCl-solution-soluble-protein decreases in the order Golden Melon, Chevalier and Hokudai No. 1, that of the hordein decreases Chevalier, Hokudai No. 1 and Golden Melon, while that of the 0.2 % NaOH-solution-soluble-protein decreases Chevalier, Golden Melon and Hokudai No. 1.

9. The pH values of the iso-electric points and the values of k of the above equation increases in the following order.

The 10 % NaCl solution soluble protein	Chevalier, Golden Melon
The hordein	Golden Melon, Hokudai No. 1, Chevalier
The 0.2 % NaOH solution soluble protein	Hokudai No. 1, Golden Melon, Chevalier

Therefore, it can be said that the more complex the structure of

the protein, the more easily decomposed it is by the action of an enzyme.

10. The viscosities of the starch solutions, the saponification values of the starches, the adsorptive power of the starches using iodine solutions, the acetylation of the starches and the chlorination of the acetyl starches were carried out.

11. It seems (a) that the molecular weight of the Chevalier starch is the greatest and also that its molecule is the most loosely composed of the 3 kinds of starches, (b) that the smallest and the hardest is the Hokudai No. 1 starch molecule, and (c) that the Golden Melon starch is situated between these two in respect to the molecular weight and the mode of composition.

Literatures.

(1) Beureau of Agric. and Forest. Japan:—The Statistics of the Beureau of Agric. and Forest., **1**, 10-11, March, 1926.
(2) Beureau of Agric. and Com., Japan:—The Statistics of the Beureau of Agric. and Com., **35**, March, 1920; **36**, March, 1921; **39**, March. 1924; **40**, March, 1925. Beureau of Com. and Ind., Japan:—The Statistics of the Beureau of Com. and Ind., **1**, 164, March, 1926.
(3) Dept. of Provisions, Beureau of Agric. and Com., Japan:—Materials for the Investigation of Provision, No. 4, Investigations on use and consumption of rice and barley, 12, 13, Sept., 1925.
(4) E. Abderhalden:—Biochemisches Handlexikon, Bd. III, 56, 1911.
(5) J. Sedlmeyer:—Z. ges. Brauw., 1912, 191-193; by Chem. Abstr., **16**, 2956-2957, 1922.
(6) Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, 2nd ed. 239-251, 1921.
(7) R. Wahl:—Amer. Brew. Rev., June, 1, 1907; by Chem. Abstr., **1**, 2810, 1907.
(8) C. Wenglein:—Z. Ges. Brauw., **30**, 157-160; by Chem. Abstr., **3**, 939, 1909.
(9) A. Cluss:—Wochschr. Brauw., **26**, 81-86, 97-104, 117-120; by Chem. Abstr., **3**. 1437. 1909.
(10) A. Le Clerc and R. Wahl:—U. S. Dept. Agr. Bur., Bull., **124**, 7-74; by Chem. Abstr., **3**, 1570, 1909.
(11) O. Wenglein:—Z. Ges. Brauw., **31**, 257-261; by Chem. Abstr., **3**, 2608, 1909.
(12) Th. B. Osborne:—J. Amer. Chem. Soc.. **17**, 539-567, 1895.
(13) Th. B. Osborne and S. H. Clapp:—Amer. J. Physiol., **19**, 117-124, 1907; by Chem. Abstr., **1**, 1869, 1907.
(14) A. Kleinschmidt:—Z. Physiol. Chem., **54**, 110-118, 1907.
(15) W. Rommel:—Wochschr. Brauw., **26**, 357-358; by Chem. Abstr., **4**, 638, 1910.
(16) E. Grabner:—J. Landwirt., **57**, 321-350; by Chem. Abstr., **4**, 1218, 1910.
(17) W. Kraft:—Z. Ges. Brauw., **33**, 193-195, 205-207; by Chem. Abstr., **5**, 756, 1911.
(18) L. Lindet and L. Amann:—Compt. rend., **145**, 253-255, 1907; J. Chem. Soc., **92**, 1, 1095, 1907.
(19) C. Wenglein:—Z. Ges. Brauw., **35**, 65-67, 80-83; by Chem. Abstr., **7**, 2087, 1913.

(20) L. Adler:—Z. Ges. Brauw., **37**, 105-108, 117-121, 129-133; by Chem. Abstr., **8**, 2025, 1914.
(21) H. Leberle and H. Lüers:—Z. Ges. Brauw., **37**, 321-324; by Chem, Abstr., **8**, 2919, 1914.
(22) F. Koritschoner:—Brau.-Malzind., **15**, 282-284; by Chem. Abstr., **8**, 3483, 1914.
(23) H. Schjerning:—Compt. rend. trav. lab. Carlsberg, **11**, 45-105, 1914; by Chem. Abstr., **9**, 689, 1915.
(24) H. Schjerning and J. Hempel:—Ibid., **11**, 338-378, 1917; by Chem. Abstr., **11**, 3373, 1917.
(25) H. C. Eckstein and H. S. Grindley:—J. Biol. Chem., **37**, 373-376, 1919.
(26) C. O. Johns and A. J. Finks:—J. Biol. Chem., **38**, 63-66, 1919.
(27) Th. B. Osborne and L. B. Mendel:—J. Biol. Chem., **41**, 275-295, 1920.
(28) H. F. E. Hulton:—J. Inst. Brewing, 1922, 33-142; by Chem. Abstr., **16**, 1482, 1922.
(29) Th. B. Osborne and L. B. Mendel:—Carnegie Inst. Washington Year Book, **19** 389-401, 1920; by Chem. Abstr., **16**, 4255, 1922.
(30) H. Lüers and M. Landaner:—Biochem. Z., **133**, 598-602, 1922.
(31) W. Windisch, W. Dietrich and A. Mehlitz:—Wochschr. Brauw., **40**, 1-3, 7-8, 13-15, 19-21, 25-27, 31-33, 1923.
(32) G. Consolani:—Coltivatore **70**, 430-432, 464-465, 1924; by Chem. Abstr., **18**, 2578, 1925.
(33) W. F. Hoffman and R. A. Gortner:—Colloid Symposium Monograph, Vol. II, 209-368, 1925.
(34) E. Takahashi and K. Shirahama:—J. Agric. Chem. Soc. Japan, **2**, 470-485, 1926.
(35) J. Loeb:—Proteins and the Theory of Colloidal Behaviour, 1922.
(36) Wo. Pauli:—Kolloidchemie der Fiweißkörper. 1. Hälfte, 1920.
(37) T. B. Robertson:—Die physikalische Chemie der Proteine, 1912.
(38) T. Tadokoro, Y. Nakamura and S. Watanabe:—J. Coll. Agric., Hokkaido Imp. Univ., **14**, 129-169, 1925, Ibid., **16**, 73-89, 1926.
(39) Ssadikow and Zelensky:—Biochem. Z., **136**, 241, 1923, **147**, 30, 1924.
(40) K. Shibata:—Acta Phytochimica **2**, No. 2.
(41) O. Herzog:—Z. physiol. Chem., **134**, 299, 1924.
(42) E. Abderhalden:—Ibid., **139**, 196, 1924.
(43) Van Slyke:—J. Biol. Chem., **10**, 15-85, 1911-12.
(44) Van Slyke:—Ibid., **22**, 281, 1915.
(45) Kaufmann:—Beziehungen zwischen physikalischen Eigenschaften und chemischer Konstitution, 238, 1920.
(46) I. M. Kolthoff:—Konduktometrische Titrationen, 1923,
(47) Waldschmidt-Leitz:—Koll. Z., **40**, 295-300, 1926.
(48) S. P. L. Sörensen:—J. Chem. Soc., 1926, 2995-3009.
(49) Ssadikow:—Biochem. Z., **179**, 326-331, 1926.
(50) J. H. Northrup:—J. Gen. Physiol., **4**, 487-509, 1922; by Chem. Abstr., **16**, 2520-2521, 1922.
Naturwissenschaften, **11**, 713-719, 1923; by Chem. Abstr., **17**, 3678, 1923.
J. Gen. Pnysiol., **6**, 239-243, 1924; by Chem. Abstr., **18**, 844, 1924.
Ibid., **6**, 417-428, 439-452, 1924; by Chem. Abstr., **18**, 2532, 1924.
(51) P. Rona and H. Kleinmann:—Biochem. Z., **140**, 461-478; **150**, 444; **155**, 34-53, 1925; **159**, 146-174, 1925; **169**, 320-343, 1926; **177**, 89-106, 1926.

(52) A. C. Anderson:—Biochem. Z., **70**, 344-367, 1915; by Chem. Abstr., **9**, 2908, 1915.
(53) Clark:—The Determination of Hydrogen Ions, 1922.
(54) A. Hiller and D. D. Van Slyke:—J. Biol. Chem., **53**, 253, 1922.
(55) Reichard:—Z. ges. Brauw., **31**, 345-348, 357-360; by Chem. Abstr., **3**, 2603, 1909.
(56) H. C. A. Vine:—J. Inst. Brewing, **19**, 413-448; by Chem. Abstr., **8**, 983, 1914.
(57) A. J. Daish:—Biochem. J., **10**, 56-75, 1916; by Chem. Abstr., **10**, 1878, 1916.
(58) T. Tadokoro and S. Sato:—J. Coll. Agric., Hokkaido Imp. Univ., **13**, 1-65, 1923. T. Tadokoro:—Ibid., **16**, 91-123, 1926.
(59) Rosenthaler:—Pharm. Zentrlhalle, **66**, 631, 1925; by Chem. Abstr., **20**, 307, 1926.
(60) Meyer:—Lehrbuch d. organisch-chemische Methodik, **1**, 3658, 1922.
(61) Lasser-Cohn:—Arbeitsmethoden f. organisch-chemische Laboratorien, Allgem. Teil, 255, 1923.
(62) E. Peiser:—Z. physiol. Chem., **167**, 88-90, 1927.
(63) T. Takahata:—Biochemische Analyse, 388-390, 1927.