STUDIES ON THE PROTEINS AND OIL OF SOY BEAN

By

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Introduction

The soy bean is one of the most important food stuffs. It is considered as a very important source of protein for Japanese who live mainly on vegetables. Three articles of food, *tōfu* (soy bean curd), *miso* (soy bean paste) and soy sauce, have been indispensable in the Japanese diet for centuries. Though the processes of manufacturing these articles are different, the object of their use, from the viewpoint of nutrition, is chiefly to utilize the protein which is the first principal constituent of the soy bean containing nearly 40 percent. The oil, the second principal component of the soy bean containing about 20 percent, is much less important than protein in these foodstuffs. The utilization of the soy bean oil was not considered so important as that of the soy bean protein until some ten years ago. For last ten or more years, however, the demand of the oil has increased year after year and the oil became important not only for industrial purposes, but also as a food stuff. The residue of the soy bean freed from oil by pressure is called soy bean cake. It still contains about 5 to 7 percent oil unseparated. But all kinds of these residues contain nearly as much as 50 percent of protein, which are mainly utilized in their original form. It is to be much regreted that

the value of utility of the soy bean cake or meal is comparatively little. Let one see the statistics of soy beans and their oil. China is said to be the principal country supplying soy beans, though no reliable statistics can be obtained. The yield of soy beans in North and South Manchuria in 1928 is estimated at 5,420,000 short tons which corresponds to about a half of the entire yield of the world.[88]

The statistics of soy beans for the last few years in Japan[17][30] show their important economical value as follows:

TABLE I

Some statistics of soy beans in Japan

		1929	1928	1927	1926
Main land	Amount (*koku*)	2,655,139	2,976,924	3,263,178	2,998,606
	Value (yen)	42,751,677	49,276,460	49,973,843	50,842,818
Korea	Amount (*koku*)	3,990,965	3,810,641	4,747,062	4,351,537

The quantity and value of soy beans exported and imported are as follows:

TABLE II

Quantities and values of soy beans and oill cake exported and imported

Soy bean

	Exported		Imported	
	Quantities (100 *kin*)	Value (1000 yen)	Quantities (100 *kin*)	Value (1000 yen)
1930	37,033	355	7,153,294	36,664
1929	43,032	468	9,462,678	60,091
1928	42,857	473	7,800,957	49,688
1927	48,928	579	6,635,326	41,198
1926	37,164	432	7,021,021	49,028

Oil cake*

	Exported		Imported	
	Quantities (100 *kin*)	Value (1000 yen)	Quantities (100 *kin*)	Value (1000 yen)
1930	175,962	1,079	13,863,458	64,800
1929	591,582	4,001	14,829,044	57,731
1928	438,180	2,800	16,359,564	73,362
1927	220,772	1,371	19,764,658	88,471
1926	224,206	1,513	21,110,690	109,777

* This is considered as soy bean cake.

Next, let one see the economy of vegetable oils in the statistics of their export and import. Among imported oils, coconut oil is the most important (374,000 yen in 1928), and the other kinds are almost negligible. The four principal vegetable oils exported are as follows:

TABLE III**

The four principal vegetable oils exported from Japan

	1930	1929	1928	1927	1926	1925
Linseed oil	142	192	95	81	91	80
Coconut oil	35	38	7	11	10	42
Soy bean oil	4,359	2,236	1,625	1,704	3,045	2,878
Rape-seed oil	4,672	4,316	2,105	5,863	6,038	1,546

** Unit 1,000 yen

The production of each vegetable oil and its percentage in Japan are shown in units of their selling values in TABLE IV.

TABLE IV*

The production of vegetable oils and their percentages

	1929	1928	1927	1926	1925
Entire vegetable oil	44,347,827	41,092,703	39,926,965	44,233,310	45,412,897
Soy bean oil	13,963,580	13,710,316	11,151,277	13,386,947	13,923,496
%	31,49	33,36	27,93	30,26	30,66
Rape-seed oil	12,439,114	12,214,398	15,228,035	13,474,263	13,511,189
%	27,85	29,72	38,14	30,46	29,75
Linseed oil	2,945,224	2,733,100	1,584,332	1,520,004	1,904,994
%	6,64	6,65	3,97	3,44	4,19
Coconut oil	2,800,842	2,346,020	2,090,566	2,823,263	2,799,482
%	6,32	5,71	5,24	6,38	6,16
Sesame oil	2,398,899	2,645,601	2,443,698	2,182,166	3,760,426
%	5,41	6,44	6,12	4,93	6,08

* Unit yen

As can be seen clearly in this table, among vegetable oils the soy bean oil is a most important article ranking with the rape seed oil. Recently the percentage of the latter is decreasing gradually while the former is increasing. It is supposed that the soy bean oil will become the most important vegetable oil near future.

From the foregoing tables, one can see that the soy bean, the soy bean cake and meal, and the soy bean oil are important products in Japan. In addition to the three principal uses of soy bean protein in Japan as previously stated, it is very important to promote the utilization of every component of soy beans or of soy bean meal. Scientific investigations of soy beans have an intimate relation to the soy bean industry. In this thesis, the investigations of physico-chemical differences between glycinin and the denatured glycinin which is derived from glycinin and the principal component of soy bean meal, will be mainly described. Fundamental knowledge of the differences between these two proteins is very necessary for their intelligent utilization. As an accessory investigation, the soy bean oil was studied to learn some new fundamental facts in its decomposition by lipase and the changes of viscosity of the oil at different dilutions with various solvents.

Part I. Studies on Soy Bean Proteins

The reports of the main chemical investigations of soy bean proteins hitherto made by many authors may be abstracted as follows:

Osborne[60] extracted oil with petroleum ether from soy beans, and from the residue he extracted proteins with 10% saline solution. By the alternate treatment of dialysis and dissolution for several times, he prepared glycinin and gave the following elemental composition: C=52.12, H=6.93, N=17.15, S=0.79. He recognized that glycinin could be dissolved in a NaCl or $MgSO_4$ solution of more than 2% strength. It showed all colour reactions of protein. Beside glycinin, he separated some other proteins, i. e. phaseoline-like globulin which contained C=51.94, H=6.88, N=16.51, S=0.60, O=24.07, legumelin which belonged to albumins containing C=53.06, H=6.94, N=16.14, S=1.17, O=22.69, and proteose which contained C=48.76, H=6.28, N=16.14, S+O=22.82.

Muramatsu[61] deduced the following conclusion from his chemical investigation of soy bean proteins.

(1) The percentage of each soluble nitrogen to total nitrogen, when soy bean powder was extracted successively with H_2O, 10% NaCl and 0.2% NaOH was as follows: 86.02, 3.75, 0.23 respectively. The residual nitrogen was 7.93%.

(2) Each quantity of the extractable nitrogen in H_2O, NaCl and NaOH solutions was variable according to the kind of soy beans.

(3) Unripe soy beans contained far less water soluble protein than ripe ones. On the contrary, the amounts of NaOH soluble nitrogen and of nitrogen left in the residue were richer in unripe seeds than ripe ones.

(4) The protein which is soluble in a dilute solution of alkali, but neither soluble in water nor in saline solution belongs to glutelins. The percentage of the protein in soy beans amounted to 1.1 in dry condition. Muramatsu refined this substance and determined nitrogen to be only 11.72%. This small nitrogen percentage made the author think that this substance was contaminated with impurities, and that although it had the same soluble property as glutelin in an alkaline solution, it was not a true protein.

(5) Of the total water soluble nitrogen, 84.25% was globulin,

5.36% albumin, 4.36% proteose and the remaining 6.03% was non-protein.

(6) The larger part of the globulin of soy beans was extractable with water. This was caused by the fact that glycinin combined with mineral substances of soy beans and changed into soluble form in water.

(7) From 6 kilograms of soy bean flour dried in air, refined proteins were prepared in the following amounts in grams.

TABLE V

Muramatsu's result obtained in the preparation of soy bean proteins

Globulin	from water extract	200 g.
	from saline water extract	188 g.
		388 g.
Albumin	from water extract	66 g.
	from saline water extract	11 g.
		77 g.
Proteose from water extract		20.27 g

(8) 78.5% of the globulin of soy beans was glycinin and 21.5% was phaseolin.

(9) 78.79% of the albumin was legumelin and 21.21% was a new kind of albumin which was named by the author soy-legumelin. The result of its elemental analysis was as follows: C=52.98%, H=6.79%, N=15.14%, S=0.29%, O=24.80%.

Satō[72] made studies on soy beans to get some galalith-like substance for industrial use from soy bean protein. He made many experiments about glycinin which was prepared by the method outlined by Osborne.

In regard to the decomposition of soy bean proteins, there are following investigations. Osborne and Harris[63] decomposed the proteins of soy beans after the method of Hausmann. The results were as follows:

TABLE VI

Osborne and Harris's result obtained in the decomposition of soy bean proteins

		Total N	Ammonia N	Basic N	Non-basic N	N in MgO, pp.
		%	%	%	%	%
Glycinin	Protein %	17.75	2.11	3.95	11.27	0.12
	Total N %	100.00	12.1	22.6	64.7	—
Legumelin	Protein %	16.09	1.18	3.08	11.44	0.39
	Total N %	100 00	7.3	19.1	71.1	—
Phaseolin	Protein %	—	1.88	3.93	10.11	—

By the hydrolysis of glycinin, Osborne and Clapp obtained the following result:

TABLE VII

Osborne and Clapp's result obtained in the decomposition of glycinin

Glycocoll	0.97	Serine	—
Alanine	—	Tyrosine	1.86
Valine	0.68	Arginine	5.12
Leucine	8.45	Histidine	1.39
Proline	3.78	Lysine	2.71
Phenylalanine	3.83	Ammonia	2.56
Aspartic acid	3.86	Tryptophane	+
Glutamic acid	19.46		54.73
			(54.67?)

Nolau[56] hydrolyzed many kinds of seeds after the method of Van Slyke from the viewpoint of nutrition and reported the distribution of various forms of nitrogen. Grindley[32] made an analogous investigation. From their results, data on soy beans may be abstracted as follows:

TABLE VIII

Nollau and Grindley's data obtained with soy beans

	Nolau	Grindley
Ammonia N	12.97%	10.12
Melanine N	3.69	6.63
Cystine N	1.52	0.67
Arginine N	15.52	12.67
Histidine N	2.60	5.77
Lysine N	7.02	6.14
Monoamino-acid N	48.76	—
Amino N in the filtrate from bases	—	49.79
Non-amino N	7.12	8.56
Total	99.20	100.35

Kimura[41] determined the quantity mainly of diamino acids contained in *tōfu* and *kōri-tōfu* (frozen *tōfu*) as follows:

TABLE IX

Kimura's data obtained with *tōfu* and *kōri-tōfu*

Nitrogen in the form of	*Tofu*	*Kori-tofu*
Arginine	13.09	15.85
Histidine	6.87	3.66
Lysine	9.03	8.38
Humine	1.13	1.00
Ammonia	11.35	11.96

Hamilton and his coworkers[36] obtained the following results:

TABLE X

Hamilton's data obtained with soy bean protein

Total basic N	28.94	Ammonia N	9.38
Humine N	2.87	Arginine N	15.70
Cystine N	1.46	Histidine N	5.60
Lysine N	6.18	Monamino N	48.28

Onuki[59] prepared 5.47 g of crude β-hydroxyglutamic acid from 100 g of soy bean protein by the method of Dakin. Matsuyama and Mori[48] detected 0.83% tryptophane in soy bean protein. May and Rose[50] extracted proteins from oil-free soy bean flour with 0.2% NaOH solution. By the addition of acetic acid, they obtained proteins whose nitrogenous forms were determined to be as follows:

TABLE XI

May and Rose's result obtained with soy bean protein

Amide N	11.31	Humine N	1.84
Cystine N	1.04	Arginine N	14.57
Histidine N	5.92	Lysine N	8.26
Amino N	54.32		

Mashino[47] decomposed oil-free soy bean flour, soy bean meal (manufactured by the extraction method) and soy bean cake with 38.5% and 19.98% HCl, 19.61% H_2SO_4 and 19.65% NaOH respectively. At the end of a definite period of time, the quantity of separated nitrogen of either ammonia or of amino form was about equal in the different kinds of samples. In the hydrolysis with NaOH solution the ammonia produced was more than in the case with acids; the mean difference was 7.72% of the total nitrogen.

Shimo and Yanagawa[73] decomposed soy bean cake with hydrochloric acid to determine the distribution of different nitrogenous compounds and obtained the following result:

TABLE XII

Shimo and Yanagawa's result obtained with soy bean cake

Amide N	Humine N	Diamino N	Monoamino N
% 10.co	% 4.83	% 26.43	% 58.74

They detected five kinds of monoamino acid, i. e. glutamic and aspartic acids, leucine, proline and phenylalanine.

As above cited, there are many reports on soy bean proteins but not so many about the proteins of soy bean cake or meal. There can be found very few studies on the denaturation of soy bean proteins or on the comparison between a protein and its denatured protein. There is still required a fundamental knowledge of them, which is indispensable for their utilization.

I. Distribution of nitrogen for different solvents

The following experiments were undertaken to determine the nitrogen distribution in soy beans, soy bean cake and soy bean meal by the successive extraction of them with water, 10% saline solution and 0.2% sodium hydroxide solution.

Sample (1) Manchurian yellow soy beans, a mixture of various varieties.

(2) Soy bean meal which was manufactured by the extraction method at the Shimizu Mill of Hōnen Oil Co., Ltd.

(3) Soy bean cakes (round) manufactured by the pressing at three different mills in Dairen, Fukushuko (a), Doshuko (b) and Seitai (c).

(4) Soy bean cake (flat) manufactured in Harbin.

In the following experiments the soy bean was dried at below 40° for some hours to facilitate pulverization. Each sample was milled and sieved through 0.2 mm mesh. The following data resulted.

TABLE XIII

The data obtained with soy bean meal and cakes

	Water	Crude fat	Crude protein	Crude fiber	N free extr.	Ash.
Soy bean	8.71	20.20	42.96	6.00	25.80	5.04
,, ,, meal	7.96	1.40	52.36	7.04	33.49	5.71
,, ,, cake (a)	14.48	8.52	53.29	30.36		7.83
,, ,, ,, (b)	13.83	7.81	48.74	35.42		7.93
,, ,, ,, (c)	16.28	8.00	53.37	30.88		7.75
,, ,, ,, (flat)	12.22	6.45	52.55	34.54		6.49

After perfect extraction of oil, 15 g of each dry sample were taken to which 450 cc. of distilled water was added. Soy bean flour was triturated in a porcelain mortar with water added little by little to make the flour perfectly wet. Each sample was taken into a bottle, stoppered and shaken for 30 minutes with a shaking machine and then was kept in an ice-box. Next day, the extracted solution was separated by a centrifuge and the nitrogen was determined by the Kjeldahl method. To each residue, 400 cc. of distilled water was added and treated as in the previous case. This treatment was repeated while the separated solution showed Millon's reaction. Then the residue was extracted with 10% saline solution as in the case with distilled water. Thus the distribution of nitrogen in H_2O, 10% NaCl and 0.2% NaOH solutions was determined and the results are given in TABLE XIV which are recaptulated in TABLE XV. As shown in this table, almost four fifths of total nitrogen of soy bean was extracted with water, one tenth and one twentieth respectively were extracted with 10% NaCl and 0.2% NaOH. As much as 50% nitrogen of soy bean meal was extracted with 0.2% NaOH, and the quantities of nitrogen soluble in water and in saline solution and the quantity of residual nitrogen were 1/4, 1/10, 1/10 of the total nitrogen respectively. The nitrogen quantities of the former two types were less, while the NaOH soluble nitrogen and the residual nitrogen were comparatively richer in soy bean cake (round) than in meal.

TABLE XIV

The distribution of nitrogen in H_2O, 10% NaCl, 0.2% NaOH and residue determined with soy bean, soy bean meal and cakes

Soy bean

Solvent	Extraction time	1	2	3	Total
Water	% of dry matter	46.04	0.570	0.068	5.242
	% of total N	68.23	8.50	1.01	77.74
10% NaCl	% of dry matter	0.655	0.140	0.057	0.852
	% of total N	9.71	2.08	0.85	12.64
0.2% NaOH	% of dry matter	0.251	0.091	0.034	0.369
	% of total N	2.72	1.35	0.50	5.58
Residue	% of dry matter	—	—	—	0.273
	% of total N	—	—	—	4.05

Soy bean meal

Solvent	Extraction time	1	2	3	4	5	6	Total
Water	% of dry matter	1.477	0.391	0.223	0 089	—	—	2.180
	% of total N	17.85	4.72	2.69	1.08	—	—	26.34
10% NaCl	% of dry matter	0.787		0.196		—	—	5.983
	% of total N	9.51		2.37		—	—	11.88
0.2% NaOH	% of dry matter	3.023	—	8.17	0.282	0.092	0.049	42.63
	% of total N	36.53	—	9.87	3.41	1.11	0.59	51.51
Residue	% of dry matter	—	—	—	—	—	—	0.850
	% of total N	—	—	—	—	—	—	10.27

Soy bean cake (a)

Solvent	Extraction time	1	2	3	4	5	Total
Water	% of dry matter	0.517	0.205	0.168	0 157	0.070	1.117
	% of total N	6.90	2.73	2.25	2.09	0.94	14.91
10% NaCl	% of dry matter	0.409	0.154	0.096	—	—	0.659
	% of total N	5.46	2.06	1.28	—	—	8.80
0.2% NaOH	% of dry matter	2.598	0.867	0.276	0.119	0.098	3.958
	% of total N	34.68	11.57	3.68	1.59	1.31	52.83
Residue	% of dry matter	—	—	—	—	—	1.757
	% of total N	—	—	—	—	—	23.46

Soy bean cake (b)

Solvent	Extraction time	1	2	3	4	5	Total
Water	% of dry matter	0.656	0.287	0.131	0.099	—	1.173
	% of total N	7.80	3.41	1.56	1.18	—	13.57
10% NaCl	% of dry matter	0.845	0.133	0.108	—	—	1.086
	% of total N	10.05	1.58	1.28	—	—	12.91
0.2% NaOH	% of dry matter	2.619	1.027	0.860	0.151	0.095	4.752
	% of total N	31.14	12.21	10.23	1.80	1.13	56.51
Residue	% of dry matter	—	—	—	—	—	1.399
	% of total N	—	—	—	—	—	16.63

Soy bean cake (c)

Solvent	Extraction time	1	2	3	4	5	Total
Water	% of dry matter	0.688	0.209	0.160	0.098	—	1.155
	% of total N	8.06	2.45	1.87	1.15	—	13.53
10% NaCl	% of dry matter	0.356	0.209	0.086	—	—	0.651
	% of total N	4.17	2.45	1.00	—	—	7.62
0.2% NaOH	% of dry matter	2.679	0.971	0.602	0.393	0.108	4.753
	% of total N	31.37	11.37	7.05	4.60	1.72	55.66
Residue	% of dry matter	—	—	—	—	—	1.980
	% of total N	—	—	—	—	—	23.19

Soy bean cake (flat)

Solvent	Extraction time	1	2	3	4	Total
Water	% of dry matter	2.391	1.104	0.891	0.288	4.674
	% of total N	29.90	13.80	11.14	3.60	58.44
10% NaCl	% of dry matter	0.394	0.199	0.140	—	0.733
	% of total N	4.93	2.49	1.75	—	9.17
0.2% NaOH	% of dry matter	1.243	0.258	0.164	0.093	17.58
	% of total N	15.54	3.23	2.05	1.16	21.98
Residue	% of dry matter	—	—	—	—	8.33
	% of total N	—	—	—	—	10.41

TABLE XV

The comparative data of the distribution of nitrogen of different samples

		Total N	Water sol. N	10% NaCl sol. N	0.2% NaOH sol. N	Residue
Soy bean	% of dry matter	6.736	5.242	0.852	0.369	0.273
	% of total N	100.00	77.74	12.64	5.57	4.05
Soy bean meal...	% of dry matter	8.277	2.180	0.983	4.264	0.850
	% of total N	100.00	26.34	11.88	51.51	10.27
Soy bean cake (a)	% of dry matter	7.491	1.117	0.653	3.958	1.757
	% of total N	100.00	14.91	8.80	52.83	23.46
,, ,, ,, (b)	% of dry matter	8.410	1.173	1.086	4.752	1.399
	% of total N	100.00	13.95	12.91	56.51	16.63
,, ,, ,, (c)	% of dry matter	8.539	1.155	0.651	4.753	1.980
	% of total N	100.00	13.53	7.62	55.66	23.19
Mean of (a), (b) and (c)	% of dry matter	8.147	11.48	0.799	4.488	1.712
	% of total N	100.00	14.13	9.78	55.00	21.09
Soy bean cake (flat)	% of dry matter	7.998	4.674	0.733	1.758	0.833
	% of total N	100.00	58.44	9.17	21.98	10.41

The differences of the first and the last forms of nitrogen between these two samples were distinct. Such a remarkable change in nitrogen solubility could not be seen with soy bean cake (flat), i. g. the quantitative ratios of nitrogen soluble in H_2O, NaCl, NaOH and of the residual nitrogen were 6, 1, 2 and 1 respectively.

In short, through the industrial process of oil extraction, the saline water soluble nitrogen remains almost constant and nitrogen soluble in NaOH increases markedly both in soy bean meal and cake (round). As the principal part of the nitrogen of original soy beans belongs to globulins, it is supposed, from the above results, that glycinin is changed into glutelin form through the oil extraction process. This means a denaturation phenomenon of protein through an artificial process. Such a fact may bring an interesting problem in protein chemistry.

II. Differences of some chemical properties between the soy bean glutelin and the glutelin of soy bean meal, the denatured glycinin

It was made clear in the previous experiment that the soy bean meal was rich in NaOH soluble nitrogen (glutelin) and poor in water soluble. Some properties of glutelin of soy bean meal, the denatured glycinin, were investigated and compared with those of the natural glutelin of soy beans.

(i) Preparation of proteins

Soy beans and soy bean meal used were the same as in the previous experiment. The oily substance was perfectly extracted and the residue was treated as follows: Water soluble proteins were separated perfectly through repeated extraction with distilled water. Then, with 10% saline solution the separation of soluble proteins was perfectly done. Next, the washing with distilled water was repeated until chlorine could not be detected in the filtrate. To the residue, sodium hydroxide solution was added so as to bring 0.2% NaOH up to ten times the weight of the residue. After shaking for 30 minutes, the bottle was kept in an ice-box for one day. By centrifuging, the filtrate was first separated and then filtered through a thick pulp filter which had been obtained by repeated digestion and washing. The filtrate thus prepared from the extract of soy beans was golden yellowish in colour, while the filtrate from soy bean meal was somewhat deeper than the former. By the addition of acetic acid, the protein was precipitated, which was thoroughly washed and treated with alcohol and ether and dried in a desiccator in the usual way. The colour of the sodium hydroxide solution of the soy bean protein was lighter than that of protein of soy bean meal as above stated, while the former dried protein was always coloured light brown, and the latter protein was always snow-white. The dry proteins amounted to 1.0 % and 20.6 % of soy beans and soy bean meal respectively in an experimental result. For the sake of convenience, the glutelin of soy beans and of soy bean meal will hereafter be denoted by " N " for natural glutelin and " D " for denatured glycinin respectively.

(ii) Elemental analysis

Nitrogen was determined by the Kjeldahl method, sulphur by the Denis-Benedict method. Oxygen was determined by calculation. The result was as follows:

TABLE XVI

The result of elemental analysis obtained with "N" and "D"

	Dry matter taken in g.	CO_2	H_2O
"N"	0.13205	0.5642	0.1171
"D"	0.13824	0.2665	0.1027

From the data, the quantities of C and H were calculated and are tabulated with other components and also with Osborne's data obtained with glycinin in TABLE XVII.

TABLE XVII

Elemental compositions of natural glutelin, denatured and normal glycinins

	Nitrogen	Sulphur	Carbon	Hydrogen	Oxygen
"N"	15.54	0.49	52.96	8.87	22.14
"D"	16.61	0.46	52.50	7.42	23.01
Glycinin (Osborne)	17.53	0.97	52.12	6.93	22.63

Muramatsu extracted proteins with water and 10% NaCl, and without washing the residue, he added 0.2% NaOH to it and extracted soluble matter. By the addition of acetic acid he prepared a nitrogenous matter as given in the introduction. Muramatsu concluded that this substance was not protein. The present author determined nitrogen further with several preparations; the results of which were

almost same as given above. Therefore "N" and "D" were recognized as proteins. "D" may have originated in a part from the natural glutelin of soy beans, but the greater part of it must be considered to have originated from glycinin of soy beans. This supposition is due to the fact that, as shown in TABLE XV, in soy beans about as much as 90% of total nitrogen is of water and saline soluble form, and NaOH soluble nitrogen is only about 5%, while in soy bean meal water soluble nitrogen amounts only to 25% and NaOH soluble nitrogen amounts to as much as 50% of the total nitrogen. The facts that the sulphur content of "N" or "D" amounted to about one half that of glycinin and that the nitrogen content of glycinin was higher than that of either "N" or "D" are noticeable points. Each component of "D" differed in quantity from the same component of glycinin. The oxygen content of "D" was the highest of the three samples, while carbon and hydrogen contents of "D" were higher than that of glycinin. The author gave a consideration further for the differences of the components between both proteins with some theories hitherto reported. It[76] is reported that casein, when it is denatured to protonic acid, decreases its carbon and hydrogen contents and increases oxygen and that edestin, when it changes into bynedestin, increases its carbon content and decreases the nitrogen, while its hydrogen content remains almost constant. Sörensen and Jurgensen[75] and Quagliariello[66] ascertained quantitatively that, by heat coagulation, the acid combining capacity of protein was increased and the hydrogen ion concentration was decreased. According to Chick and Martin,[13] the heat coagulation of protein is caused by hydration and in acid solution the hydrogen ion accelerates the rate of denaturation. The heat coagulation of protein means not only the reaction of hydrolysis but also of dehydration. According to these thories, the denaturation of proteins does not mean only one of the reactions, hydration and dehydration. Moreover it is considered possible that, even if the denaturation is caused by hydration, it may not always occur in one kind of atomic group in protein molecule. The hydrolysis may happen in more than two different kinds of atomic group of protein molecule. The chemical changes caused by the oil extraction process, will be discussed later. Here, the author will infer the chemical changes from only the above data of elemental analysis. On the denaturation of glycinin, if only OH group be additionally combined with protein, the percentage of H will be decreased in the product, and it will be increased if OH group

be isolated from protein, for the percentages of H in OH group and in glycinin are 5.93 and 6.93 respectively. However, at the denaturation of proteins caused by heating, neither the combination of the sole group of OH (not OH + H) with protein nor its isolation from protein has been considered by any scholar. Therefore, for the sake of supposition, three chemisms, hydration, dehydration and deamination, are considered and examined in the denaturation of glycinin. Let glycinin be denatured only by hydration, the percentage of H will increase in the product over that in the original protein, for the percentage of H of H_2O is $2.016 \div 18.016 \times 100 = 11.19$ and that of glycinin is much less than 11.19%. The oxygen percentage of the product will increase, and that of carbon will decrease, for as much as 88.81% of H_2O is oxygen. Let the danaturation of glycinin be caused only by dehydration, the percentages of both hydrogen and oxygen atoms must change in a manner contrary to that in the case of hydration. If deamination occurs, the danatured protein should be higher than the original protein in the percentages of hydrogen and nitrogen, for they are $2.16 \div 16.024 \times 100 = 12.58$ and $14.008 \div 16.024 \times 100 = 87.42$ respectively. No valid conclusion can be deduced from the quantitative comparison of elemental composition of glycinin obtained by Osborne with that of the denatured glycinin obtained by the present author. However, as stated above, distinct differences were recognized in respect to the percentages of C, N and H between both the proteins. Therefore the denatured glycinin may possibly be supposed to have originated from glycinin through at least two predominant reactions, hydration and deamination.

(iii) Degree of turbidity of alkali solutions of protein, when titrated with HCl solution

(1) 0.6 gram of each of dry "N" and "D" was taken, to which 250 cc. of $1/75$ N NaOH was added and shaken well. Next day, the sample was filtered.* To each 10 cc. of the filtrate, a different amount of $1/25$ N HCl was added and the degree of turbidity of each sample was observed. Number of "+'s" in TABLE XVIII indicates the degree of turbidity. As shown in the table, the range of the turbidity of "D" was narrower than that of "N." To compare the ranges numerically, a Duboscq's nephelometer was used at 22°C. The result is shown in TABLE XIX.

*Where the solubility of "D" was always higher than that of "N".

TABLE XVIII

The degree of turbidity of alkali solutions of proteins for different quantities of HCl

1/25 N HCl cc.	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4
"N"	−	++	+ ++	++ ++	+ ++ ++	+ ++ ++	+ ++	++	+	+
"D"	−	−	++	+ ++	+ ++ ++	++ ++	++	−	−	

TABLE XIX

The degree of turbidity of alkali solutions of proteins for different quantities of HCl measured with a Duboscq's nephelometer

1/25 N HCl cc.	0	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3
"N"	32	11	15	60	60	60	18	8	8
"D"	60	60	60	44	44	46	60	60	60

TABLE XX

The degree of turbidity of alkali solutions of proteins for different quantities of HCl measured with a Duboscq's nephelometer

1/75 N HCl cc.	1.0	1.1	1.2	1.3	1.4
"N"	32.0	60.0	60.0	13.5	18.0
"D"	60.0	23.0	51.0	60.0	60.0

Furthermore, to each 1 cc. of the same filtrates, 9 cc. of redistilled water and a different amount of 1/75 N HCl were added and the degree of turbidity was determined at 22°C. as shown in TABLE XX. From

this result, it was recognized also that the range of turbidity of "N" was wider than that of "D." The maximum turbidity of each sample was determined as written under (2).

(2) One cc. of each of the same samples which was used in the preceding test was treated in the same manner as shown under (1) and the turbidity was determined at 22°C. The result is given in TABLE XXI.

TABLE XXI

The degree of turbidity of alkali solutions of proteins for different quantities of HCl measured with a Duboscq's nephelometer

	"N"			"D"		
1/75 N HCl cc.	1.15	1.20	1.30	1.10	1.15	1.20
	60.0 :	57.0	—	60.0 :	27.0	—
	—	47.0 :	60.0	—	42.5 :	60.0

The maximum turbidity of "N" appeared in the addition of 1.20 cc. of HCl while that of "D" appeared in the addition of 1.15 cc.

From the above results it was made clear that the range of turbidity of "N" was wider than that of "D", when their alkali solutions were titrated with HCl. That is, the isoelectric range of "N" was wider than that of "D." The quantity of HCl needed to bring the maximum turbidity of the solution was larger in "N" than "D." From these facts it may be considered that the deamination is greater, and the isolation of COOH group is less in the molecules of "N" than in those of "D", and that "N" is richer than "D" in keto group, while in enol group, the contrary holds true in comparing the two proteins.

(iv) Free amino nitrogen

For the determination of free amino nitrogen, Sörensen's formol titration method was used. 0.1 gram of dry sample was taken, to which 25 cc. of 1/5 N NaOH was added. To 20 cc. of the filtrate, 10 cc. of neutralized formaldehyde solution was added. A control solution was

made which was composed of 20 cc. of distilled water, 25 cc. of 1/5 N NaOH and 10 cc. of neutral formaldehyde solution. Each sample was titrated with 1/5 N HCl. The difference of the titration numbers corresponds to the amino nitrogen of the sample. Each cc. of 1/5 N NaOH is equivalent to 0.0028016 gram of nitrogen. The result is shown in the following table.

TABLE XXII

The result of determination of free amino nitrogen

	NaOH cc.	Free amino N	Total N %
"N"	0.60	0.00168	10.79
"D"	0.55	0.00154	9.27

As shown in this table "N" contained more amino nitrogen than "D", from which an assertion may be certainly made that the deamination occurs at the denaturation of glycinin which agrees with the supposition previously made. The author compared the specific rotations of alkali solutions of both proteins. "D" contained more nitrogen than "N," yet the rotation of "D" solution was always lower than that of "N".*

* 0.1 gram protein was dissolved in each 250 cc. of 1/25, 1/75, 1/200 N NaOH. Next day the rotations of the filirates were determined with a 100 mm. tube at 18°C. with following results:

		1/25 N	1/75 N	1/200 N.
"N"	Rot. power	−1.85	−1.8	−1.8
	$[\alpha]_D^{18}$	−158.5	−144.7	−144.7
"D"	Rot. power	−1.6	−1.6	−1.6
	$[\alpha]_D^{18}$	−138.4	−138.4	−138.4

Some chemical properties of the natural glutelin of soy beans were compared with those of the glutelin of soy bean meal which was the denaturation product of glycinin. The recaptulation is as follows:

The elemental compositions of both proteins were determined. To get some idea, the result was compared with Osborne's result obtained with glycinin. The sulphur content either of the natural glutelin or of the denatured glycinin was equivalent to about a half that of glycinin. The hydrogen content was highest in the natural glutelin, moderate in the denatured glycinin and lowest in glycinin. The oxygen content was highest in the denatured glycinin. The nitrogen content was highest in glycinin and lowest in the natural glutelin. From the above comparison and some theories hitherto proposed about the denaturation of proteins, the author considers that glycinin is denatured into glutelin at the oil extraction process through at least two principal chemisms, deamination and hydration.

The quantity of HCl which is necessary to bring the maximum turbidity of alkali solution of protein was less in the natural glutelin than in the denatured glycinin.

The content of free amino nitrogen was higher in the alkali solution of natural glutelin and the rotation was higher also in the solution than in that of the denatured glycinin. It is considered that, in connection with the result of elemental analysis, the lower content of amino nitrogen in the denatured glycinin may possibly indicate the deamination process and the lower rotation of alkali solution of the denatured glycinin seems to mean the hydration process at the denaturation of glycinin.

III. Influence of freezing upon the solution of denatured glycinin

Denatured proteins may be classified according to the conditions under which they were produced, as follows:

(a) Denaturation by certain chemical reagents.
(b) Heat denaturation.
(c) Denaturation caused by light or radiation.
(d) Denaturation caused by pressure.
(e) Denaturation caused by freezing.

Racemized protein, which is prepared by the treatment of protein with alkali solution, desamino protein which is prepared by the

treatment of protein with nitrous acid, or other various derivatives of protein belong to (a). Investigations of heat denaturation of protein were made by Söresen and Jurgensen,[75] and Quagliariello.[66] Heat coagulation of proteins and the relation between denaturation and coagulation were investigated by Chick and Martin, as already described in this paper. The congulation temperature of a protein is definite according to the kind of protein. The difference of temperature was utilized by Fredericqu and Haliberton to isolate various proteins. The denaturation by exposure to ultraviolet rays, or by radium radiation was studied by Fernan and Pauli[24] with the acid and alkali solutions of serum albumin. The coagulation by the latter treatment did not change the hydrogen ion concentration of the solution. Such a result can not be seen in heat coagulation. The denaturation by pressure was investigated by Bridgman.[12] Egg-white was coagulated by pressure. Kimura[41] determined the diamino acids of *tōfu* (soy bean curd) and *kōri-tōfu* (*frozen tōfu*). Recently, Tadokoro and Yoshimura[77] compared oryzenin with frozen oryzenin in respect to the distribution of nitrogen, free amino nitrogen, the specific rotation of alkali solution, and the turbidity of alkali solution after the titration of HCl.

The author undertook the following experiments to determine the influence of freezing upon the alkali and acid solutions of the denatured glycinin.

(i) Preparation of sample

Soy bean meal, pulverized and sieved through 0.2 mm. mesh, was treated with water, 5% saline solution and 7% $MgCl_2$ solution[52] successively to separate all the soluble proteins. The residue was treated with 0.2% NaOH as previously described. The filtrate was again filtered through a thick pulp layer. This alkaline solution was used as a sample. Nitrogen was determined by the Kjeldahl method with 25 cc. 10 cc. of this sample contained 0.03488 gram of nitrogen.

Acid solutions of protein were prepared as follows: From the above alkaline solution, the protein was precipitated by the addition of dilute acetic acid and washed perfectly with distilled water. A small amount of the protein was poured with water into a volumetric flask of 250 cc. capacity. 125 cc. of $\frac{1}{5}$ N citric acid and water were added to the mark, shaken and allowed to stand in an ice-box until next day resulting in an opalescent solution. In the same way, a protein solution of 1/10 N HCl was prepared. The undissolved protein was

separated by centrifuging. 20 cc. of each sample contained the following number of grams of nitrogen.

Citric acid solution 0.005324
Hydrochloric acid solution 0.005975

(ii) Process and degree of freezing

Alkali solution of protein

Let the sample as above described be denoted as a concentrated sample, a part of which was diluted with water to three times its volume. Let this latter sample be called the dilute one. 30 cc. of each solution was taken in a hard glass bottle of 400 cc. capacity, and 2 cc. of toluene was added and stoppered. One set of several bottles was allowed to freeze spontaneously at temperatures as shown in TABLE XXIII. A control set was kept in an ice-box during the same periods at −3°—0° c. For convenience' sake, let the sample kept in the ice-box be called the control and the frozen sample, F. Let the concentrated and dilute samples be represented by c and d respectively. For instance, " control c " means a concentrated solution kept in the ice-box.

During the time of freezing, the maximum and minimum temperatures of the atmosphere*1) and of the ice-box*2) were as follows:

TABLE XXIII

Maximum and minimum temperatures during the time of freezing

Date	1/28	29	30	31	3/1	2	3	4	5	6	7
Ice-box											
max.	—	+1	+1	−1	−2	−2	+3	+1	+1	+1	—
min.	—	0	−1	−3	−4	−3	−5	−4	−2	−3	—
Atomsph.											
max.	−2.7	−2.3	+2.7	−1.8	−4.8	−3.9	−2.0	−0.3	−0.0	−0.1	−2.1
min.	−6.5	−9.1	−11.3	−15.8	−14.5	−13.1	−11.8 (p.m.)	−19.5 (a.m.)	−10.5	−14.0	−3.1

*1) Observation of the Sapporo Meteorological Observatory.

*2) Observation during 24 hours i. e. from 9 a. m. of one day to the same hour of the following day.

On the last day, the bottles were put in water until the sample melted and were kept in an ice-box for 24 hours, after which they were used for various experiments.

Different degrees of freezing may result in different degrees of influence upon the protein solutions. Therefore two kinds of samples were prepared which were frozen at different degrees. Let the two kinds be known as " lightly frozen " and " severely frozen " respectively. The former samples were obtained at $-15.8°$—$+2.7°$C. (during the time from Jan. 28th to Feb. 1st) and the latter ones at $-19.5°$—$+2.7°$C. The difference of both minimum temperatures is slight, but as it could not be expected on 7th Feb. that the temperature would decrease further, no more freezing was continued.

Acid solution of protein

20 cc. of each acid solution of protein was taken respectively is an Erlenmeyer's flask of 50 cc. capacity and then was diluted with water or with the same acid solution to twice the volume. To each flask, 1 cc. of toluene was added, and stoppered. The procedure was the same as in the case of the alkali solutions. The temperature in the ice-box was kept so as not to fall below 0°C. Four different frozen samples were obtained at the minimum temperature as follows:

F_1 $-14.1°$C. (from Feb. 15 to Feb. 17)
F_2 $-16.2°$,, (,, ,, ,, ,, ,, 20)
F_3 $-16.2°$,, (,, ,, ,, ,, ,, 25)
F_4 $-16.2°$,, (,, ,, ,, ,, Mar. 4)

The atmospheric temperatures of these periods* were as follows:

TABLE XXIV

Maximum and minimum temperatures during the time of freezing

Date	2/15	16	17	18	19	20	21	22	23	24
Max.	−3.2	−3.8	−4.0	−5.8	−5.0	−0.9	+4.5	+5.2	+5.2	+2.0
Min.	−8.8	−14.1	−15.7	−16.2	−16.0	−15.9	−12.9	−9.4	−8.3	−7.0

* Observation of the Sappro Meteorological Observatory.

Date	25	26	27	28	29	3/1	2	3	4
Max.	−7.8	−6.2	−4.5	−2.3	+1.7	+0.6	+1.6	+5.0	−2.2
Min.	−9.3	−13.1	−11.7	−9.3	−7.0	−3.6	−9.6	−3.8	−4.4

(iii) State of frozen samples

Samples that had been kept in the ice-box did not change in appearance within two weeks or so, while those that had been allowed to freeze froze completely within the next day. Fine coagulated precipitates collected arround the central vertical axis and bound themselves in the form of a stick. When the solution was severely frozen, an upheaval was caused on the central part of its surface. When the degree of freezing was not so great, the state of the solution, when melted, was almost the same as before freezing. But if it had been severely frozen, the sample appeared turbid and viscous when melted.

The gelatination of the fluid was observed in F. c. and F. d. of the "severely frozen" sample.

Samples which had been gelatinized by freezing, seemed to decrease their viscosity changing gradually into sol state with time after they were diluted with water or diluted alkali solution.

The samples which had been melted and kept at −2°C. in the ice-box, showed no change apparently, but after they were cooled unconciously at −3°C., some of them became entirely frozen and the others were beginning to gelatinize as shown below.

F.c. F.d.: Frozen solid.

F.c.+2 times its volume of water+3 times its volume of 1/20 N NaOH.: Fine precipitates were seen. The fluid was just about to change into gel state.

Filtrate of F.d.+2 times its volume of 1/20 N NaOH.: Frozen solid.

Control c. and d.: No change.

From the above observation, it was recognized that the alkali solution of the denatured glycinin which had once been frozen and changed once into gel state, had a tendency to change again into gel state easily even if it was kept at comparatively high temperatures. The transition point seemed to be about −3°C.

Acid solution of the protein did not become viscous by freezing and the precipitated protein almost failed to dissolve again after melting.

These phenomena could not be seen in the cases of alkali solutions of the denatured glycinin.

(iv) Quantity of nitrogen of precipitated protein which resulted from freezing

Sodium hydroxide solution of protein

The frozen sample was kept for a definite period in water at 15° c., shaking occasionally and then it was filtered through a dry filter of Sweden No^{00}. 15 cc. of each filtrate was used to determine the quantity of nitrogen by the Kjeldahl method. As a "severely frozen" sample was very viscous, centrifugal filtration was utilized before practices. The insoluble precipitates of the fluids remained still insoluble even by the addition of water, but when the equal volume of 1/20 N NaOH was added instead of water, the precipitates were almost dissolved in a short time.

At the instance of dilution and at 2 hours after, nitrogen was determined with following results:

TABLE XXV

The result of nitrogen determination of protein sodium hydroxide solutions

	Hours after melting	N in 10 cc. g.	Ratio
Control d.	0	0.011628	100.00
Lightly frozen F. d.	2	0.011245	96.71
Control c.	0	0.034883	100.00
Severely frozen F. c.	2	0.031017	88.91
Filtrate of F. c. diluted to 2 times its volume with 1/20 N NaOH	0	0.016684 (×2)	95.65
,, ,,	2	0.016799 (×2)	96.30

As above tabulated, the percentage of protein of concentrated NaOH solution which was precipitated by freezing was much more than that of the diluted solution. The greater part of the precipitated

protein dissolved quickly and the remaining part gradually dissolved when it was diluted with NaOH solution*.

Acid solution of protein

10 cc. of filtrate of each melted sample was used to determine the nitrogen content by the Kjeldahl method. The result was as follows:

TABLE XXVI

The result of nitrogen determination of acid solutions of protein

		Sample taken cc.	N in 10 cc. g.	Ratio
Hydrochloric acid solution	Control 1/10 N	15	0.002986	100.00
	F_1 1/10	15	0.002741	91.78
	F_3 1/10	15	0.001347	45.09
	F_4 1/10	20	0.000615	20.85
	Control 1/20	15	0.002812	100.00
	F_2 1/20	15	0.001991	70.81
	F_3 1/20	15	0.001441	51.25
	F_4 1/20	20	0.000791	28.13
Citric acid solution	Control 1/10	10	0.002630	100.00
	F_4 1/10	,,	0.002284	86.84
	Control 1/20	20	0.001669	100.00
	F_4 1/20	,,	0.001312	78.61

As shown above, some part of protein of acid solution was changed into insoluble form by freezing. This tendency was remarkable in HCl solution. From 15 to 20% of the nitrogen became insoluble in the case of F_4 of citric acid solution, while it was from 75% to 80% of the nitrogen in all cases of HCl solution.

* Denatured glycinin, which had been prepared by washing with alcohol and ether as in the ordinary method was easily soluble in an alkaline solution, while frozen protein, even though it was in a state of fine precipitate, redissolved much more slowly than the former protein.

(v) Viscosity of the frozen solution of protein

The viscosity of frozen acid solution of protein could not be determined with an Ostwald's viscosimeter, for the precipitate resulted from freezing was hard to dissolve. But in the case of alkali solution, it was possible because the precipitate dissolved well. After the frozen sample melted, with 5 cc. of the filtrate or of the solution resulted from the dilution of the filtrate with water or with dilute alkali solution, the viscosity was determined with the viscosimeter at 15°C. Control c. was treated in the same manner. The determination was repeated until a constant result was obtained.

TABLE XXVII

The result of determination of specific viscosity for various dilutions

	Original solution*		Orig. sol.+the same quantity of water			
			20–30 mins. after dil.		20 hours after dil.	
	Control c.	F. c.	Control c.	F. c.	Control c.	F. c.
Time of flow (secs.)	147.1	283.9	111.9	175.4	107.1	143.8
Specific viscosity	1.73	3.35	1.32	2.07	1.29	1.73
Ratio	1.00 : 1.94		1.00 : 1.57		1.00 : 1.34	

Org. sol.+4 times H_2O				Orig. sol.+the same quant. of 1/20 N NaOH after 20 mins.		Orig. sol.+the same quant. of 1/20 N NaOH after 1 hr.	
after 20–30 mins.		after 19 hrs.					
Control c.	F. c.	Control c.	F. c.	Control c.	F. c.	Control c.	F. c.
98.0	126.5	96.8	117.1	122.9	135.3	123.2	132.3
1.16	1.49	1.16	1.41	1.45	1.60	1.48	1.59
1.00 : 1.28		1.00 : 1.20		1.00 : 1.00		1.00 : 1.07	

* The solution which was obtained by centrifuging from the melted sample which had been kept in an ice-box the previous day.

The difference of viscosity between control c. and F. c. was pretty large, but it was not so large when they had been diluted with NaOH.

After the frozen samples were melted, they returned gradually to the sol state as previously stated. Two different solutions were made, one of which had been diluted with water, and other with NaOH. Both were kept in an ice-box for 5 days. The viscosity was measured as given in TABLE XXVIII where (a) denotes the dilution with four times the volume of water (not filtered before dilution). (b) denotes the dilution with two times the volume of water and three times the volume of 1/20 N NaOH (filtered before dilution). (c) denotes the dilution with the same volume of 1/20 N NaOH. As the melted sample of F. c. became very viscous in the ice-box, it was perfectly melted again and diluted to the same concentration of NaOH as (c). The sample thus obtained is denoted by (d)

TABLE XXVIII

The result of determination of specific viscosity of various samples for various dilutions

	(a)		(b)		(c)	(d)	
	Cont. c.	F. c.	Cont. c.	F. c.	F. d.	Cont. c.	F. c.
Flow time (secs.)	153.5	165.0	152.1	150.9	155.0	151.0	182.2
Specific viscosity	1.08	1.17	1.07	1.07	1.09	1.07	1.70
Ratio	1.00 : 1.08		1.00 : 1.00		1.02	1.00 : 1.20	

All the concentrations of NaOH of (b), (c) and (d) were the same. Each 10 cc. of (b), (c) and (d) contained the following quantity of nitrogen.

TABLE XXIX

The quantity of nitrogen of the samples described in TABLE XXVIII

(b) Cont. c.	(b) F. c.	(c) F. d.	(d) F. c.
0.005813 g.	0.005791 g.	0.005623 g.	0.005004 g.

As above shown, the viscosity of a frozen sample was higher than that of the control, even though the soluble nitrogen had been decreased by freezing. After melting, the viscosity of a frozen sample decreased gradually and became close to that of the control. But at below the transition point (−3°C.) the viscosity increased remarkably. From the results of the above experiments, the influence of freezing upon the viscosity of NaOH solutions of the denatured protein, was recognized to be as follows:

(1) The frozen samples showed remarkably high viscosity just at the time when it was melted.

(2) After melting, the viscosity of a frozen sample decreased gradually and became nearer to that of the control. The viscosity of a frozen sample or of the solution which had been diluted with water decreased very slightly, but the viscosity of the solution which had been diluted with NaOH decreased very quickly.

(3) The protein sodium hydroxide solution which had been once frozen became gelatinous at comparatively high temperature if it was cooled again. The transition point was about −3°C. The precipitate of protein from acid solution which had resulted from freezing was not easily dissolvable, therefore the viscosity could not be measured by an Ostwald's viscosimeter.

(vi) Turbidity of the sodium hydroxide solution of protein resulting from the addition of HCl solution

Tadokoro and Yoshimura[77] made a study on the influence of freezing upon oryzenin. The frozen protein was treated with alcohol and ether in the ordinary way. They dissolved the protein in NaOH solution. Comparison of the normal oryzenin with the frozen one was made in respect to the quantity of HCl necessary to bring the maximum turbidity of NaOH solution of each protein. The quantity of HCl for the frozen oryzenin was less than that for the normal. The NaOH solution of the denatured glycinin, prepared as previously stated, was tested in the same way. To 10 cc. of each solution were added different volumes in cc. of 1/20 N HCl. In TABLE XXX, E means emulsoid, S means suspensoid and $\times 25(H_2O)$ means the dilution with water to 25 times the volume of the solution. The degree of turbidity of each solution did not change within twenty four hours. From the data in TABLE XXX, it was observed that the maximum turbidity of sodium hydroxide solution of protein occurred at the addition of less

TABLE XXX

Comparison of turbidity of frozen sample for different quantities of HCl with that of control

	N g in 10 cc. before dilution	Dilution	1/50 N HCl cc.								Observation after hours given below
			0.40	0.60	0.65	0.70	0.75	0.80	0.85	0.90	
Control c.	0.0034883	$\times 25(H_2O)$	+	E	S+E	S+E	S	S+E	S+E	+	1
Filtrate of "severley frozen" c.	0.0031017	,,	+	E	S+E	S	S+E	S+E	E	+	1
			1/50 N HCl cc.								
			1.70	1.75	1.80	1.85	1.90	1.95	2.00		
Control c.	0.034883	$\times 3(H_2O) \times 2$(1/20 N NaOH) $\times 11(H_2O)$	+	++	++ ++	++ +++	+++ +++	++ ++	++		2
"Severely frozen"	0.034883	,,		+	+ ++	++ +++	++ ++	+ ++	+		2

quantity of HCl in the frozen sample than in the ordinary one.* This tendency agrees with the result of Tadokoro.

The influence of freezing was examined with the sodium hydroxide, hydrochloric and citric acid solutions of the denatured glycinin. The results are summarized as follows:

(1) Influence of freezing upon NaOH solution of the protein

(a) The influence of spontaneous freezing for several days at $+7°$——$16°$C. did not give the ultimate influence of freezing. The continuance of freezing resulted in further influence.

(b) Some part of nitrogen became very insoluble.

(c) When frozen, the maximum point of turbidity resulting from titration with HCl receded upon the addition of the acid in small quantity.

(d) The sol state changed into gel state.

(e) The gel state of melted "frozen solution" recovered sol state very slowly even at low temperatures. The viscosity decreased and approached that of the control.

(f) The alkali solution of protein which had been once frozen and melted, changed easily into gel state when it was cooled again. This transition point of NaOH solution such as 10 cc. of it containing 0.05 g. of nitrogen was about $-3°$C.

(2) Influence of freezing upon hydrochloric and citric acid solutions of the protein

The insoluble nitrogen which had been resulted by freezing was much more in hydrochloric acid solution than in citric acid. The

* The two samples under experiment were kept at different temperatures. As F had been kept at lower temperatures and "control" near at 0°C., the difference between the two samples in influence of NaOH had to be examined. At the same time of preparation of protein solutions, "the concentrated solution" was diluted to 25 times its volume, to each 10 cc. of which a different amount of 1/50 N HCl was added. On the other hand, the same diluted solutions of the sample that had been kept in the ice-box were made. The turbidities of the above two kinds of protein solutions were compared. No difference could be seen between the two samples which had been kept for the period at different temperatures. Next, examination was made of the influence of different quantity of nitrogen in the two samples under observation. Each 0.1, 0.2, 0.3 gram of the denatured glycinin was dissolved in 25 cc. of 1/40 N NaOH respectively. Next day, 10 cc. of which was titrated with 1/50 N HCl to compare the degrees of turbidity. The appearances of turbidity of the three sets were not equal, but the maximum point of turbidity of each set was seen at the addition of 1.15 cc. of HCl. Therefore the data shown in TABLE XXX are considered to have resulted from the sole influence of freezing.

protein acid solution was not changed into gel state by freezing. The resultant precipitate was not easily redissovable. These were characteristic phenomena in acid solution of the denatured glycinin.

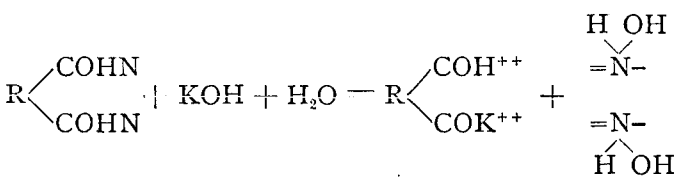
IV. Differences of physical and chemical properties between the normal and denatured glycinins

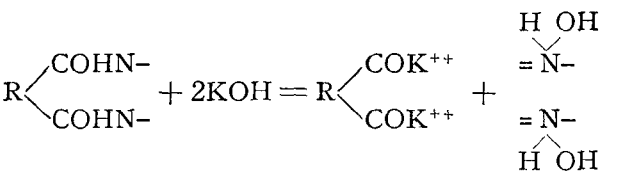
From the results given in Chapter I, it was concluded that the protein of soy bean meal soluble in sodium hydroxide solution was the denatured protein that had originated from glycinin. The comparisons of some physical and chemical properties of the denatured glycinin with those of the normal glycinin in soy beans were studied. The results were described in Chapter II. The influence of freezing upon the alkali solution of protein, which had been once denatured by steaming was studied in Chapter III. Next, in this chapter, some physical and chemical properties of the normal glycinin will be compared with those of the denatured glycinin, from the results of which some discussion on the mode of denaturation of protein will be essayed.

(i) Solubility in various acid solutions

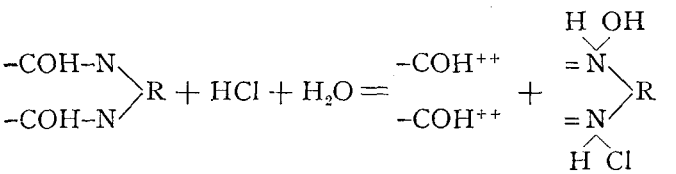
The combining capacity of protein with acids or bases has more intimate relation to its COHN group than to the NH_2 or COOH group. The COHN group has two types which are known as keto and enol forms. These two forms can not be determined quantitatively, but it is generally considered that the keto group is easily neutralized with acid, but not to be combined with bases and it is generally more stable than enol form, while the latter is considered to be unstable as combine either with acids or bases. Robertson[68] suspected that diamino and dicarboxylic radicles in protein molecules played a predominant part in accomplishing the neutralization of acids and bases, and the following combinations in respect to COHN group were considered possible in many instances.

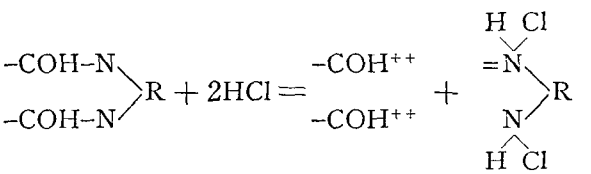
In combination with bases:



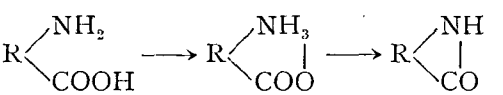


In combination with acids:

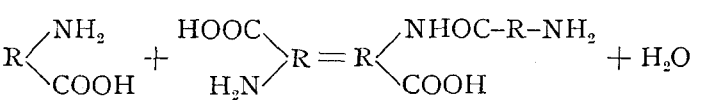


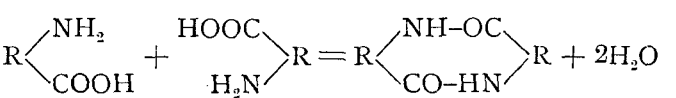


Winkelblech[82] supposed so called internal salts such as



which has almost no ability to combine with acids or bases. There is a theory of poly-amino acid structure. Two or more molecules of an amino acid unite with one another in different ways as follows:





It is considered that the two types of keto and enol forms exist also in diketopiperazin. As above cited, there are many theories in

regard to the characters or modes of combination of various atomic groups in protein molecules. The summation of various compon.nts of each protein, quantitatively and qualitatively, defines the specific character of the protein. The difference of solubility between the normal and denatured proteins in various acid solutions, may also be due to the difference of molecular structures between the two proteins.

Experiment

Hydrochloric, acetic, formic, tartaric and citric acid solutions were used as solvents. Some quantity of a solvent at a definite concentration was taken in a 50 cc. conical flask to which some dry protein was added little by little. The flask was stoppered, shaken well and kept in an ice-box for several hours. When no trace of undissolved protein was observed, more protein was added and the whole shaken well. Such a treatment was continued until undissolved protein was observed for two days. With 1 cc. of the filtrate through dry filter paper, Toyo No. 3, or with 1 cc. of the 5 to 6 times diluted solution, nitrogen was measured by the micro-method of Kjeldahl.

The nitrogen percentage of each protein and the coefficients are as follows:

	Nitrogen %	Coefficient
Glycinin	17.106	5.846
Denatured glycinin	15.558	6.428

In TABLES XXXI—XXXVII, gram numbers of nitrogen in 100 cc. of filtrate will be given and the impossibility of measurement owing to the difficulty of filtration will be denoted by ∞.

As shown in the tables the denatured glycinin was always less soluble than the normal glycinin in acid solutions except 1/5 N and 1/10 N of tataric acid. The experimental results of the solubility of both proteins are summarized as follows:

(1) Although the solubility of the denatured glycinin was less than that of the normal, the difference at high concentrations of an acid was comparatively not so large as it was at low concentrations.

(2) The solubility of both the proteins in acetic, formic, tartaric

and citric acids increased according to the increase of acid concentration. Hydrochloric and oxalic acids, unlike the other acids, had the maximum solubility power at $^1/_{25}$ N.

(3) The concentration of protein, at which the filtration was difficult, was lower in the denatured glycinin than in the normal.

(ii) Refractive index of an alkali or acid solution

The refractive index is generally recognized to increase with the increase of the degree of dissociation. Therefore the difference of refractive index between the two proteins makes one infer the difference of dissociation degree between them.

The solutions of sodium hydroxide, hydrochloric, acetic, tartaric and citric acids were used as solvents. The concentration of each solvent given in TABLE XXXVIII was taken considering each solubility power from the results of the preceding experiment. To 20 cc. of each solvent, 0.1 g. dry protein was added and shaken. After 15 hours, the refractive index was measured at 20°C. with a Goertz's refractometer, using sun light. The degree of refraction of water was 1.3400.

TABLE XXXI

Solubility of the normal and denatured glycinins in hydrochloric acid solutions

Conc. (N)	N g. in 100 cc.		Protein g. in 100 cc.	
	Glycinin	Denat. glycinin	Glycinin	Denat. glycinin
2	0.0047	—	0.0275	—
1 1/2	0.0069	—	0.0403	—
1	0.0173	—	0.1011	—
1/2	0.0381	0.0032	0.2227	0.0206
1/5	0.1300	0.0045	0.7600	0.0289
1/10	∞	0.0186	∞	0.1196
1/15	∞	0.0945	∞	0.6075
1/25	1.0040	∞	5.8694	∞
1/50	0.7889	0.2154	4.6119	1.3846
1/100	0.3580	0.0526	2.0929	0.3381
1/200	0.1976	—	1.1552	—
1/250	—	0.0099	—	0.0617
1/400	0.0775	0.0063	0.4531	0.0405

TABLE XXXII

Solubility of the normal and denatured glycinins in acetic acid solutions

Conc. (N)	N g. in 100 cc.		Protein g. in 100 cc.	
	Glycinin	Denat. glycinin	Glycinin	Denat. glycinin
1/5	∞	0.0114	∞	0.0733
1/10	1.0668	0.0111	6.2363	0.0714
1/15	0.9785	0.0084	3.9665	0.0540
1/20	—	0.0058	—	0.0373
1/25	0.0498	0.0042	2.8534	0.0270
1/50	0.1709	0.0019	0.9991	0.0116
1/100	0.0168	0.0003	0.0982	0.0019
1/200	0.0034	—	0.0198	—

TABLE XXXIII

Solubility of the normal and denatured glycinins in formic acid solutions

Conc. (N)	N g. in 100 cc.		Protein g. in 100 cc.	
	Glycinin	Denat. glycinin	Glycinin	Denat. glycinin
1/5	0.6834	—	3.9952	—
1/10	0.3700	∞	2.1630	∞
1/25	0.1935	0.0396	1.1312	0.1729
1/50	0.1160	0.0125	0.6781	0.0804
1/100	0.0504	0.0088	0.2946	0.0566
1/200	0.0220	0.0037	0.1286	0.0238
1/400	0.0093	0.0032	0.0544	0.0206
1/800	0.0018	0.0019	0.0222	0.0122

TABLE XXXIV

Solubility of the normal and denatured glycinins in oxalic acid solutions

Conc. (N)	N g. in 100 cc.		Protein g. in 100 cc.	
	Glycinin	Denat. glycinin	Glycinin	Denat. glycinin
1	0.0853	—	0.4987	—
1/2	0.1644	0.0385	0.9611	0.2475
1/5	0.3922	0.0699	2.2928	0.4493
1/10	0.6589	0.0855	3.8519	0.5496
1/15	∞	0.2221	∞	1.4277
1/20	∞	0.1189	∞	0.7643
1/25	0.3821	0.0588	2.2338	0.3780
1/50	0.1901	0.0268	1.1107	0.1723
1/200	0.1389	0.0055	0.8120	0.0354
1/200	0.0350	—	0.2046	—
1/800	0.0035	—	0.0205	—

TABLE XXXV

Solubility of the normal and denatured glycinins in malonic acid solutions

Conc. (N)	N g. in 100 cc.		Protein g. in 100 cc.	
	Glycinin	Denat. glycinin	Glycinin	Denat. glycinin
1 1/2	∞	∞	∞	∞
1	0.8362	∞	4.8884	∞
1/2	0.7273	∞	4.2518	∞
1/5	0.5177	∞	3.0265	∞
1/10	0.4079	∞	2.3845	∞
1/25	0.2157	∞	1.2610	∞
1/50	0.1034	0.0332	0.6045	0.2134
1/100	0.0381	0.0081	0.2227	0.0521
1/200	0.0170	0.0037	0.0994	0.0238
1/400	0.0056	0.0035	0.0327	0.0225
1/800	0.0011	0.0010	0.0065	0.0064

TABLE XXXVI

Solubility of the normal and denatured glycinins in tartaric acid solutions

Conc. (N)	N g. in 100 cc.		Protein g. in 100 cc.	
	Glycinin	Denat. glycinin	Glycinin	Denat. glycinin
1/2	∞	∞	∞	∞
1/5	0.6544	1.2865	3.8256	8.2696
1/10	0.6234	0.8168	3.6443	5.2504
1/15	—	0.1735	—	1.1153
1/20	—	0.0612	—	0.3934
1/25	0.4057	0.0490	2.3717	0.3150
1/50	0.1983	0.0183	1.1593	0.1176
1/100	0.1025	0.0122	0.5992	0.0784
1/200	0.0423	—	0.2473	—
1/400	0.0157	—	0.0918	—
1/800	0.0046	—	0.0269	—

TABLE XXXVII

Solubility of the normal and denatured glycinins in citric acid solutions

Conc. (N)	N g. in 100 cc.		Protein g. in 100 cc.	
	Glycinin	Denat. glycinin	Glycinin	Denat. glycinin
1/2	1.1242	∞	6.5721	∞
1/5	0.7807	0.5718	4.5640	3.6755
1/10	0.6287	0.1225	3.6754	0.7874
1/15	—	0.0816	—	0.5245
1/25	0.4130	0.0245	2.4144	0.1575
1/50	0.0885	0.0041	0.5174	0.0264
1/100	0.0461	—	0.2695	—
1/200	0.0161	—	0.0941	—
1/400	0.0147	—	0.0859	—
1/800	0.0028	—	0.0164	—

TABLE XXXVIII

The refractive index of the alkali and acid solutions of normal and denatured glycinins

Solvent	Conc. (N)	Glycinin	Denat. glycinin
Sodium hydroxide	1/10	1.3419	1.3422
Hydrochloric acid	1/50	1.3412	1.3414
Acetic acid	1	1.3445	1.3447
Tartaric acid	1	1.3488	1.3491
Citric acid	1	1.3497	1.3498

The refractivity of acid solution of each protein increased with the increase of concentration of acid the data of which was omitted. Comparing the solutions of different acids, the refractivity increased with the increase of molecular weight of solvent. The refractive index of the denatured glycinin solution was distinctly higher than that of glycinin in all cases. The ionization degree of the former is inferred surely to be higher than the latter protein.

(iii) Specific rotatory power

The comparison of specific rotatory powers of the two kinds of protein is interesting if it is made in connection with that of other experimental results. 0.1 g. of dry protein was dissolved in 15 cc. of 1/10 N NaOH and allowed to stand in a room of 20° C. At the end of each hour given in TABLE XXXIX the rotatory power was measured, using a 100 mm. tube. The specific rotatory power was computed by the formula,

$$[\alpha]_D^{20^\circ} = \frac{a \times 0.346 \times cc.}{l \times g},$$

where a means reading.

As shown in the table, the rotatory power of both protein solutions increased with time during some definite time and then decreased gradually. In all cases the rotatory power of the normal glycinin was less than that of the denatured.

TABLE XXXIX

The rotatory power of sodium hydroxide solutions of normal and denatured glycinins

Hours		5	24	48	72	96	120	144
Glycinin	Rot. power	−1.70	−1.8c	−1.60	−1.50	−1.40	−1.35	−1.35
	$[\alpha]_D^{20°}$	−88.23	−93.42	−83.04	−77.85	−72.66	−70.65	−67.47
Denat. glycinin	Rot. power	−1.90	−2.co	−2.00	−1.60	−1.6c	−1.50	−1.4c
	$[\alpha]_D^{20°}$	−98.61	−103.80	−103.80	−83.04	−83.04	−77.85	−72.66

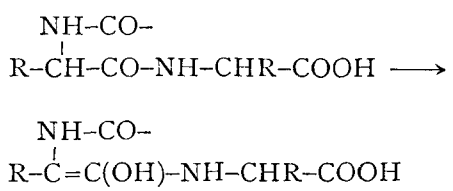
To examine further the rotatory power of an alkali solution of a denatured protein is higher or not than that of the normal protein, the author examined further using casein of the highest quality for industrial use produced in Australia, and a denatured product of the same. Both the caseins were prepared as follows: Casein was dissolved in 0.2% NaOH. The solution was filtered through clean thick pulp. Dilute acid was titrated to precipitate casein. After the precipitate was repeatedly washed with water, alcohol and ether and dried in the ordinary way, it was used as normal casein. To prepare a denatured casein, normal casein was taken in a beaker and wet with water. Superheated steam of about 130°C. was passed thoroughly for 30 minutes, during which time the sample was well mixed continuously with a glass rod. Then the sample was dissolved in 0.2 % NaOH and treated as in the case of the normal casein. The specific rotatory power of the normal and denatured caseins were as follows:

TABLE XL

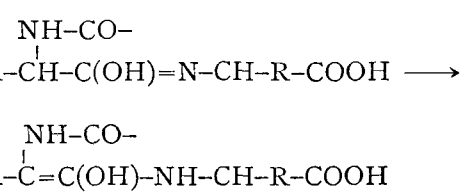
The rotatory power of sodium hydroxide solutions of the normal and denatured caseins

Hours		5	24	48	72	96	120	144
Casein	Rot. power	−2.2	−2.5	−2.5	−2.3	−2.10	−1.90	−1.90
	$[\alpha]_D^{20°}$	−114.18	−129.75	−129.75	−119.37	−108.99	−98.61	−98.61
Denat. Casein	Rot. power	−2.40	−2.90	−2.60	−2.30	−2.30	−2.20	−2.20
	$[\alpha]_D^{20°}$	−124.56	−145.32	−134.94	−119.37	−119.37	−114.18	−114.18

The difference of the rotatory power between the two kinds of casein has the same tendency as in the case of the normal and denatured glycinins. Progressive diminution of the rotatory power of protein solutions was observed by Kossel[44] and Dakin,[14, 15] when the protein was treated with rather concentrated alkali. Dakin recognized the enolization in protein molecule as analogous to the change which occurred in hydantoins as in the following way:



It is supposed that the group given first is optically active and that of the second inactive. That is to say, the diminution of optical activity means the change in the forward direction. Kober[42] objected to Dakin's explanation of the progressive less of optical activity of protein alkali solutions. At the present time, the racemization of protein is attributed to the change in enol form such as:



The action of rather concentrated alkali solution upon protein, is not probably to be attributed to a mare change such as depicted above; for in alkali solution the rotatory power of the normal and denatured glycinins increased with time during a certain period of time. Anyhow, the fact that the protein in alkali solution changes unceasingly with time makes one infer some unceasing change or changes in the protein molecules. It is a characteristic point that the rotatory power of alkali solutions of the denatured glycinin is always higher than that of the normal.

(iv) Free amino nitrogen

By the formol titration method of Sörensen, the difference of quantity of free amino nitrogen between the normal and denatured giycinins was examined. One tenth gram of dry protein was dissolved in 12 cc. of 1/5 N NaOH. After 5 hours, 10 cc. of neutralized formaldehyde solution was added, and titrated with 1/10 N HCl. One cc. of alcohol solution of 0.1% phenolphthalein was used as indicator. The normal and denatured caseins were compared with each other in parallel. The free amino nitrogen of each denatured protein was determined as more than that of the respective normal protein with following results:

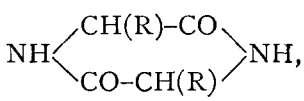
TABEL XLI

The result obtained in the determination of free amino nitrogen with glycinins and caseins

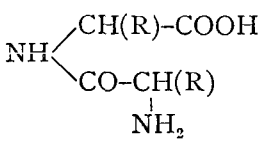
	Free amino nitrogen %	% of total nitrogen
Glycinin...	1.4373	8.5893
Denat. glycinin	1.9583	12.5874
Casein	2.3993	15.4943
Denat. casein	2.5363	17.1401

(v) Free carboxyl group

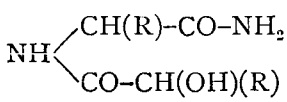
From the preceding experiments, the normal and denatured proteins are known to be evidently characterized by the difference of quantity of free amino nitrogen. As one of the chemisms of the formation of free amino nitrogen, in an atomic group such as



two modes of hydration are supposed with following products:



(1)



(2)

In the formation of (1), a NH_2 group and a COOH group occur. These formations are given merely as examples. Many types of changes in relation to the formation or destruction of NH_2 or COOH may actually occur in protein molecules when protein is denatured. Therefore it is interesting to make a comparison of COOH as well as NH_2 groups between the normal and denatured proteins. From the results of such a comparison some supposition may be deduced in regard to the chemisms of protein denaturation. For the determination of COOH, Woodman's modified method[84] of Foreman's method was applied. The procedure is as follows: Protein is taken in alcohol, mixed well and titrated with NaOH solution in the presence of thymol blue as indicator. As it is very difficult to determine the end point of titration, the result obtained by this method may not mean the absolute quantity of COOH. But, to determine the comparative quantity of COOH, the method may be applicable. As the medium, 80, 85, 90 and 95% ethyl alcohol was used. One tenth gram of protein and 25 cc. of alcohol were taken in a 25 cc. conical flask and mixed well. After 6 hours, three drops of 0.05% thymol blue was added and titrated with 1/100 N NaOH. In such case when the NaOH solution is added drop by drop, it is difficult to determine the end point. Therefore 0.2 cc. of NaOH was added at once and lightly shaken. When the blue colour did not disappear within 30 seconds, the titration was stopped. To make sure, the comparison of the normal casein with the denatured was examined in the same way.

TABLE XLII

Titration number (cc.) for free carboxyl group obtained with glycinins and caseins

Alcohol %	Blank test	Glycinin	Denat. glycinin	Casein	Denat. casein
80	0.25	0.80	1.40	1.80	3.00
85	0.35	1.00	1.80	1.80	2.80
90	0.35	1.00	1.40	1.60	2.60
95	0.40	0.80	1.20	1.20	2.20

Waldshmidt-Leitz recommended 85% ethyl alcohol for the determination of COOH. From the data of above table, it is clearly recognized that the number of COOH group is larger in the denatured

protein than in the normal. As the reason for this phenomenon must be discussed considering other phenomena, it will be discussed in (x).

(vi) Turbidity and surface tension of alkali solutions of protein when they are titrated with HCl

Turbidity

The quantity of HCl which is necessary to bring the maximum turbidity or surface tension of alkali solution of a protein gives some conception of the atomic groups in protein molecules which combine with acids or bases. Experiments were undertaken with both glycinins and caseins. 0.15 gram of each protein was dissolved in 15 cc. of $^1/_{50}$ N NaOH and allowed to stand in an ice-box for 20 hours. 13 cc. of the solution was diluted with water to 65 cc., 5 cc. of which was pipetted into each of several test tubes and titrated with different cc. of $^1/_{250}$ N HCl. In TABLE XLIII, the number of " +'s " indicates the degree of turbidity.

TABLE XLIII

The degree of turbidity for different quantities of HCl

1/250 N HCl cc.	4.00	4.25	4.50	4.75	5.00	5.20	5.25	5.50	5.75
Glycinin	+	+	+	++	++	+ ++	++	+	+
Denat. glycinin	+	+	++	+ ++	+ ++		++	+	+
Casein	−	+	+	+	++		+ ++	+	+
Denat. casein	+	+	+	+	+ ++		++	+	+

From the above table, it was recognized that the maximum turbidity of the alkali solution of each denatured protein was resulted by the addition of smaller quantity of HCl than that in case of the respective normal protein. Such a property may be noticed as a characteristic of a denatured protein. The maximum turbidity obtained as such, indicates an apparent isoelectric point of the protein. The hydrogen ion concentration of the solution at the maximum turbidity was determined by a potentiometer with quinhydron electrodes. The results are given in TABLE XLIV. P_H value was computed by the formula,

$$P_H = \frac{0.4541 - 0.00033\,(t-18) - E_1}{0.0577 + 0.0002\,(t-18)}.$$

TABLE XLIV

Apparent isoelectric points of glycinins and caseins

Glycinin...	5.32	Casein	4.75
Denat. glycinin ...	4.86	Denat. casin ...	4.61

Although the P_H value thus obtained does not mean a true isoelectric point, it is inferred that a distinct remove of isoelectric point towards more acidic side occurs through denaturing process.

Surface tension

The same solutions as had been used in the previous experiment were used to determine surface tension. A Nouy's apparatus was used at 18°C. 73 dynes per unit square centimetre were measured by the apparatus as the surface tension of water. This number was used as a unit but not the true surface tension of water. The results are given in the following table.

TABLE XLV

Surface tension for different quantities of HCl

1/250 N HCl	4.25	4.50	4.75	5.00	5.20	5.25	5.50	5.70
Glycinin	—	59.34	59.39	61.30	**61.95**	61.90	60.65	57.30
Denat. glycinin	60.00	63.26	**64.56**	63.27	—	61.30	—	60.97
Casein	—	56.41	56.41	55.75	—	**58.47**	58.07	59.13
Dent. Casein	—	56.08	56.08	**58.47**	—	57.08	55.75	55.09

As in the case of maximum turbidity, the quantity of HCl which is necessary to bring the maximum surface tension of protein alkali solution is smaller for each denatured protein than for the respective normal protein.

(vii) Viscosity of protein alkali and acid solutions

In colloid chemistry, the viscosity of a solution indicates the degree of ionization of the solute. Therefore the viscosity of a protein solution may be considered as an index of the ionization degree of the protein. The difference of the viscosity of alkali and acid solutions between both the normal and denatured proteins was examined.

Alkali solution

The equivalent of 0.1 gram of dry protein was dissolved in a solution of 20 cc. of each $^1/_{10}$, $^1/_{20}$, $^1/_{50}$, $^1/_{100}$ and $^1/_{200}$ N NaOH. After 24 or 48 hours, using 5 cc. of each sample, the viscosity was determined with an Ostwald's viscosimeter at 15°C. The specific viscosity was calculated by taking the flow time of water, 80.6 secs., as a unit. The results are given in TABLE XCVI. Viscosities in the absence of protein are shown in TABLE XLVII.

As given in the table the viscosity was always measured higher with the denatured protein than the respective normal proteins. The viscosity change for the lapse of time after the dissolution of proteins, was next examined. The results are given in TABLE XLVIII. As given in this table the viscosity decreased with the lapse of time with few exceptions. This decreasing tendency may be due to the influences of temperature or treatment such as shaking. Owing probably to the same reason, the maximum viscosity might not have been observed always at the same concentration of NaOH as shown in the above two tables. The concentration of NaOH showing the maximum viscosity was found further several times as shown in TABLE XLIX. In this experiment the concentration of protein was the same as in the previous case except in the sole case of the fifth test taking 0.1 gram of protein in 10cc. From this examination, it was recognized that the concentration of NaOH giving the maximum viscosity was not always constant. This inconsistency was remarkable in glycinin solution. At any rate, the existence of a concentration of NaOH at which the protein showed the maximum viscosity was proven. Next, the viscosity was determined at different temperatures. For convenience of comparison, the flow time of water at each temperature was taken as the unit of measurement. As given in TABLE L, in the result obtained with a sodium hydroxide solution of protein, the ratio of flow time of solution to that of water was almost constant at any temperature. The viscosity of the denatured protein was higher than that of the normal.

TABLE XLVI

The viscosities of protein alkali solutions

		Conc. of NaOH (N)				
		1/10	1/20	1/50	1/100	1/200
After 24 hours	Glycinin {Flow time (secs.)	87.25	90.5	92.0	91.0	91.0
	Glycinin {Spec. visc.	1.08	1.12	1.14	1.13	1.13
	Denat. glycinin {Flow time (secs.)	89.0	94.0	110.25	106.0	113.0
	Denat. glycinin {Spec. visc.	1.10	1.17	1.37	1.32	1.40
	Casein {Flow time (secs.)	92.0	97.0	107.0	100.0	94.0
	Casein {Spec. visc.	1.14	1.20	1.33	1.24	1.17
	Denat. casein {Flow time (secs.)	95.0	99.0	112.0	109.0	99.0
	Denat. casein {Spec. visc.	1.18	1.23	1.39	1.35	1.23
After 48 hours	Glycinin {Flow time (secs.)	88.00	89.5	92.0	90.0	88.00
	Glycinin {Spec. visc.	1.09	1.11	1.14	1.12	1.09
	Denat. glycinin {Flow time (secs.)	91.0	93.5	103.0	104.0	106.0
	Denat. glycinin {Spec. visc.	1.13	1.16	1.28	1.29	1.32
	Casein {Flow time (secs.)	90.0	96.0	103.0	98.0	92.0
	Casein {Spec. visc.	1.12	1.19	1.28	1.22	1.14
	Denat. casein {Flow time (secs.)	92.0	19.5	106.0	107.0	97.0
	Denat. casein {Spec. visc.	1.14	1.23	1.32	1.33	1.20

TABLE XLVII

Viscosities obtained in the control test

Conc. of NaOH (N)	1/10	1/20	1/50	1/100	1/200	0
Flow time (secs.)	82.6	82.45	81.6	81.0	80.6	80.6
Spec. viscosity	1.02	1.02	1.01	1.00	2.00	1.00

TABLE XLVIII

The viscosity change for different lapse of time after the dissolution of proteins

NaOH N	Mins. required to dissolve	Hours after dissolution	Glycinin 1	Glycinin 3	Glycinin 5	Glycinin 24	Denat. glycinin 1	Denat. glycinin 3	Denat. glycinin 5	Denat. glycinin 24
1/10	30	Flow time (secs.)	94.7	—	92.3	91.7	94.8	—	93.8	93.5
		Spec. visc.	1.17	—	1.15	1.14	1.18	—	1.16	1.16
1/20	40	Flow time (secs.)	—	94.9	94.9	94.0	—	95.6	97.2	95.5
		Spec. visc.	—	1.18	1.18	1.17	—	1.19	1.21	1.18
1/50	60	Flow time (secs.)	—	104.8	101.0	100.0	—	102.8	102.5	102.5
		Spec. visc.	—	1.36	1.25	1.24	—	1.28	1.27	1.27
1/100	60	Flow time (secs.)	—	107.6	107.3	107.3	—	108.0	104.8	104.3
		Spec. visc.	—	1.33	1.33	1.33	—	1.34	1.30	1.29
1/200	over 180	Flow time (secs.)	—	—	121.6	111.0	—	—	93.8	93.7
		Spec. visc.	—	—	1.51	1.39	—	—	1.16	1.18

NaOH N	Mins. required to dissolve	Hours after dissolution	Casein 1	Casein 3	Casein 5	Casein 24	Denat. Casein 1	Denat. Casein 3	Denat. Casein 5	Denat. Casein 24
1/10	30	Flow time (secs.)	95.2	—	94.2	94.3	89.5	—	89.0	88.2
		Spec. visc.	1.18	—	1.17	1.17	1.11	—	1.10	1.09
1/20	40	Flow time (secs.)	—	96.4	96.1	96.8	—	88.0	88.3	92.0
		Spec. visc.	—	1.20	1.19	1.20	—	1.09	1.10	1.14
1/50	60	Flow time (secs.)	—	104.3	103.5	102.4	—	91.0	89.3	92.0
		Spec. visc.	—	1.29	1.28	1.27	—	1.23	1.10	1.14
1/100	60	Flow time (secs.)	—	108.5	107.0	103.9	—	97.5	90.3	94.5
		Spec. visc.	—	1.35	1.33	1.29	—	1.21	1.12	1.17
1/200	over 180	Flow time (secs.)	—	—	94.5	94.5	—	—	89.1	89.4
		Spec. visc.	—	—	1.17	1.17	—	—	1.11	1.11

TABLE XLIX

The concentration of NaOH for equal quantity of protein showing the maximum viscosity

	Glycinin	Denat. glycinin	Casein	Denat. caseinin
1st. test	1/50 N	1/200 N	1/100 N	1/100 N
2nd. ,,	1/20	,,	,,	,,
3rd. ,,	1/100	,,	,,	,,
4th. ,,	1/20	,,	,,	,,
5th. ,,	1/20	,,	1/50	1/50

Hydrochloric acid solution

The method of determination was the same with that in the previous experiment. The equivalent of 0.1 gram of dry protein was taken in 20 cc. or 1 cc. of each of 1/10, 1/50, 1/100 and 1/200 N HCl and shaken well. The flow time (secs.) of 1/50 and 1/100 N HCl was 81.25 and 81.30 respectively. The data in TABLE LI were obtained by dividing each number of measured secs. by each number of secs. in the control test.

TABLE L

The specific viscosity of alkali solution of proten for different temperatures

	Glycinin					Denat. Glycinin				
N / C.	1/10	1/20	1/50	1/100	1/200	1/10	1/20	1/50	1/100	1/200
5	1.10	1.11	1.13	1.14	1.16	1.13	1.17	1.22	1.29	1.14
15	1.07	1.14	1.11	1.13	1.14	1.14	1.17	1.19	1.31	1.38
25	1.11	1.12	1.11	1.12	1.15	1.12	1.22	1.22	1.31	1.34
35	1.11	1.11	1.12	1.14	1.14	1.12	1.21	1.21	1.29	1.31
45	1.12	1.06	1.08	1.10	1.12	1.25	1.25	1.19	1.25	1.25
55	1.08	1.10	1.08	1.10	1.08	1.12	1.17	1.19	1.23	1.25

	Casein					Denat. casein				
C. \ N	1/10	1/20	1/50	1/100	1/200	1/10	1/20	1/50	1/100	1/200
5	1.14	1.23	1.26	1.32	1.23	1.16	1.17	1.29	1.38	1.22
15	1.15	1.16	1.24	1.31	1.22	1.16	1.19	1.25	1.35	1.23
25	1.15	1.13	1.26	1.30	1.22	1.16	1.18	1.30	1.32	1.21
35	1.15	1.17	1.31	1.26	1.25	1.15	1.18	1.31	1.29	0.97
45	1.14	1.16	1.21	1.25	1.19	1.16	1.16	1.23	1.27	1.14
55	1.10	1.17	1.19	1.21	1.04	1.12	1.19	1.21	1.23	1.10

TABLE LI

The viscosity of hydrochloric acid solution of protein (15°C.)

Conc. of protein		0.1 g. in 20 cc.		0.1 g. in 15 cc.	
Conc. of acid (N)		1/50	1/100	1/50	1/100
Glycinin	Flow time (secs.)	90.0	90.4	96.5	103.3
	Spec. visc.	1.11	1.11	1.19	1.27
Denat. glycinin	Flow time (secs.)	163.0	288.0	245.0	604.7
	Spec. visc.	2.00	3.54	3.02	8.18
Casein	Flow time (secs.)	102.4	96.0	110.4	102.8
	Spec. visc.	1.26	1.18	1.36	1.26
Denat. casein	Flow time (secs.)	102.6	96.4	109.8	102.5
	Spec. visc.	1.26	1.19	1.36	1.26

As given in this table, the difference of viscosity between the normal and denatured glycinins was remarkable, while the difference between the normal and denatured caseins was very slight. In case of NaOH solution the viscosity of the denatured casein was always remarkably higher than that of the normal as already demonstrated before.

The difference of viscosity of protein solution as given in the above tables, may surely be due to the specific property of each protein. As in the case of the alkali solutions, the ionization degree of the denatured glycinin or casein in acid solutions is inferred to be higher than that

of the respective normal protein. Such a phenomenon agrees with the results of difference of free NH_2 or COOH group between the two respective proteins.

Oxalic acid solution

The treatment was run as in case of the previous experiment. The protein was dissolved and after 20 hours the viscosity was determined. As 0.1 gram of both the normal and denatured caseins did not dissolve entirely in 20 cc. of oxalic acid solution, they could not be determined. The value of specific viscosity was obtained by dividing the mean number of measured secs. by the number of secs. in the blank test.

TABLE LII

The viscosity of oxalic acid solution of protein (15°C.)

Conc. of acid (N)		1/5	1/10	1/15	1/20	0
Blank test (secs.)		82.5	81.9	81.5	81.9	81.8
Glycinin	Flow time (secs.)	87.9	87.7	87.5	88.5	—
	Spec. visc.	1.07	1.07	1.07	1.08	—
Denat. glycinin	Flow time (secs.)	—	140.0	165.9	158.5	—
	Spec. visc.	—	1.71	2.04	2.43	—

The viscosity of the denatured protein was remarkably higher than that of the normal as in the case of HCl solution.

Acetic acid solution

The results are shown in TABLE LIII. The viscosity of the denatured glycinin was, remarkably higher than that of the normal, while the difference of viscosity between the normal and denatured caseins was not noticeable.

Formic acid solution

The results are given in TABLE LIV. The viscosity of the denatured casein was higher than that of the normal, but the difference was not large, while the difference between the normal and denatured glycinins was noticeable.

TABLE LIII

The viscosity of acetic acid solution of protein (15°C.)

	After 20 hours			After 48 hours			
Conc. of acid. (N)	2	1	1/2	2	1	1/2	1/5
Blank test (secs.)	*	90.6	86.0	—	—	—	83.7
Glycinin Flow time (secs.)	126.5	105.0	99.0	118.0	108.8	101.7	94.8
Glycinin Spec. visc.	—	1.16	1.15	—	1.20	1.18	1.13
Denat. glycinin Flow time (secs.)	404.0	459.0	452.0	437.9	459.0	452.0	557.0
Denat. glycinin Spec. visc.	—	5.07	5.26	—	5.07	5.26	6.65
Casein Flow time (secs.)	119.5	109.0	137.0	114.8	104.9	100.8	114.3
Casein Spec. visc.	—	1.20	1.59	—	1.16	1.17	1.37
Denat. Casein Flow time (secs.)	121.0	108.0	111.7	120.9	110.1	112.4	150.2
Denat. Casein Spec. visc.	—	1.19	1.30	—	1.22	1.31	1.79

Malonic acid solution

The viscosity was determined at the end of 20 hours after the proteins were dissolved. The results are given in TABLE LV. The difference between the normal and denatured glycinins was remarkable, but not between the two caseins.

Tartaric acid solution

The viscosity was measured after 20 hours of dissolution with results given in TABLE LVI. In tartaric acid, a dibasic acid, the viscosity of the denatured glycinin was remarkably higher than that of the normal. The difference was recognized also distinctly between the normal and denatured caseins although it was not observed so distinctly in the case of other acids previously used.

* The mean datum could not be gained, because the variation in each measurement was very large.

TABLE LIV

The viscosity of formic acid solution of protein (15°C.)

	Conc. of acid (N)	1 N	1/5	1/10	1/15	1/20	1/25
After 20 hours	Blank test (secs.)	84.0	82.7	81.7	82.0	82.2	82.9
	Glycinin {Flow time (secs.)	90.9	93.3	94.1	94.2	93.4	93.3
	Glycinin {Spec. vics.	1.08	1.13	1.15	1.15	1.14	1.12
	Denat. glycinin {Flow time (secs.)	108.1	259.0	443.4	495.0	543.0	596.0
	Denat. glycinin {Spec. visc.	1.29	3.13	4.69	6.04	6.61	7.19
	Casein {Flow time (secs.)	93.0	93.7	94.6	96.5	96.6	97.0
	Casein {Spec. visc.	1.11	1.13	1.16	1.18	1.18	1.16
	Denat. casein {Flow time (secs.)	97.0	95.9	98.0	101.0	119.0	108.0
	Denat. casein {Spec. visc.	1.15	1.16	1.20	1.23	1.45	1.30
After 72 hours	Glycinin {Flow time (secs.)	93.3	92.4	94.2	94.5	82.7	94.1
	Glycinin {Spec. visc.	1.11	1.12	1.15	1.1	1.15	1.14
	Denat. glycinin {Flow time (secs.)	151.3	234.4	329.0	402.0	522.0	585.0
	Denat. glycinin {Spec. visc.	1.80	2.83	4.3	4.90	6.35	7.6
	Cesein {Flow time (secs.)	92.8	92.8	93.8	93.8	93.6	96.1
	Cesein {Spec. visc.	1.10	1.12	1.15	1.17	1.14	1.16
	Denat. cesein {Flow time (secs.)	95.2	95.0	97.3	98.5	103.5	101.0
	Denat. cesein {Spec. visc.	1.13	1.15	1.19	1.26	1.20	1.22

TABLE LV

The viscosity of malonic acid solution of protein (15°C)

Conc. of acid (N)	1	1/5	1/10	1/15	1/20	1/25	0
Blank test (secs.)	87.4	85.3	82.4	82.0	81.8	82.3	81.6
Glycinin {Flow time (secs.)	94.8	92.7	92.6	93.8	94.2	95.0	—
Glycinin {Spec. visc.	1.09	1.09	1.12	1.14	1.15	1.15	—
Denat. glycinin {Flow time (secs.)	97.3	193.0	409.0	373.0	499.4	576.5	—
Denat. glycinin {Spec. visc.	1.57	2.26	3.49	4.55	6.11	7.01	—
Casein {Flow time (secs.)	97.4	93.2	92.9	93.8	93.9	97.2	—
Casein {Spec. visc.	1.12	1.09	1 12	1.14	1.15	1.18	—
Denat. casein {Flow time (secs.)	111.9	106.3	98.8	101.8	103.5	116.8	—
Denat. casein {Spec. visc.	1.28	1.25	1.19	1.24	1.27	1.42	—

TABLE LVI

The viscosity of tartaric acid solution of protein (15° C.)

Conc. of acid (N)		2	1	1/2	1/5	1/10	0
Blank test (sec.)		107.8	93.0	88.9	84.0	81.9	81.3
Glycinin	Flow time (secs.)	116.4	104.3	96.1	92.8	95.3	—
	Spec. visc.	1.08	1.13	1.09	1.10	1.16	—
Denat. glycinin	Flow time (secs.)	163.8	158.5	167.0	248.0	285.0	—
	Spec. visc.	1.52	1.70	1.90	2.95	3.48	—
Casein	Flow time (secs.)	117.5	108.4	98.2	94.7	96.7	—
	Spec. visc.	1.09	1.17	1.12	1.13	1.18	—
Denat. casein	Flow time (secs.)	131.9	121.2	109.0	107.9	123.0	—
	Spec. visc.	1.22	1.30	1.24	1.28	1.50	—

Citric acid solution

The viscosity was determined after 20 hours of dissolution with results given in TABLE LVII. The viscosity of the denatured glycinin was remarkably higher than that of the normal. The difference between the normal and denatured caseins was also recognized.

TABLE LVII

The viscosity of citric acid solution of protein (15° C.)

Conc. of acid (N)		2	1	1/2	1/5	1/10	0
Blank test (secs.)		105.4	91.7	86.0	84.6	82.4	81.8
Glycinin	Flow time (secs.)	117.1	102.5	97.1	95 0	93.6	—
	Spec. visc.	1.11	1.12	1.13	1.12	1.14	—
Denat. glycinin	Flow time (secs.)	164.3	169.5	204.8	327.1	390.3	—
	Spec. visc.	1.56	1.85	2.38	3.87	4.74	—
Casein	Flow time (secs.)	116.2	103.1	102.0	97.1	102.0	—
	Spec. visc.	1.10	1.12	1.19	1.15	1.24	—
Denat. casein	Flow time (secs.)	121.1	109.5	109.9	117.4	133.5	—
	Spec. visc.	1.15	1.19	1.25	1.39	1.62	

As demonstrated above, it was made clear that the viscosity of the denatured glycinin was always higher than that of the normal, when they were dissolved either in NaOH or in various acid solutions. About the same tendency was observed between the normal and denatured caseins. Such a tendency of viscosity is also recognized to indicate the higher degree of ionization of denatured protein than that of the normal in the solutions of acids or bases if the tendency is considered in connection with the difference of the number of NH_2 and COOH groups between the two proteins which were previously described.

(viii) Decomposition by acid and alkali

Mashino[47] decomposed soy bean meal with HCl, H_2SO_4 and NaOH, and determined ammonia and free amino nitrogen. The author compared the results of decomposition of the normal glycinins with those of the when they were decomposed by these reagents.

Hydrochloric acid

5, 10 and 20% HCl were obtained with a hydrometer. 15 cc. of each solution was taken in 100 cc. Erlenmeyer's flask to which 0.5 gram equivalent of dry protein was added and decomposed on an asbestos plate for some definite time with a reflective condenser, after which HCl was eliminated under reduced pressure at 38°—40°C. Ammonia was determined after the sample was made faintly alkaline by the addition of 10% suspension of $Ca(OH)_2$. Then it was filtered, made neutral to litmus paper and taken into a 100 cc. volumetric flask with water to the mark. Using 2 cc. of it, free amino nitrogen was determined by

TABLE LVIII

Nitrogen quantity in the filtrate for different quantity of basic lead acetate

Hours	cc. of basic lead acetate		
	1.5 cc.	2.0 cc.	2.5 cc.
1	10.231	9.335	9.335
2	10.829	9.783	9.820
10	11.127	11.202	11.214

TABLE LIX

The results of decomposition of glycinins obtained with 5% HCl

	Decomposition time (hours)	Ammonia N		Free amino N		Digested protein	
		%	% of total N	%	% of total N	%	% of total N
Glycinin	1	1.967	11.784	0.395	2.364	6.973	41.768
	2	1.997	11.946	0.535	3.203	11.030	66.069
	5	2.058	12.327	0.816	4.888	11.408	68.334
	10	2.119	12.690	0.922	5.525	12.038	72.107
	15	2.157	12.918	1.002	6.001	12.255	73.407
	20	2.172	13.008	1.027	6.155	12.466	74.671
	30	2.202	13.191	1.036	6.020	12.614	75.557
	40	2.217	13 282	1.068	6.398	12.678	75.941
	50	—	—	1.120	6.706	—	—
Denat. glycinin	1	1.686	10.520	0.480	2.995	7.868	49.096
	2	1.730	10.803	0.615	3.839	10.155	63.367
	5	1.762	10.992	0.794	4.956	11.197	69.869
	10	1.777	11.088	0.689	5.421	11.664	72.783
	15	1.807	11.277	0.960	5.987	11.876	74.106
	20	1.822	11.372	0.993	6.194	12.003	74.899
	30	1.868	11.656	1.108	6.915	12.088	75.424
	40	1.914	11.941	1.134	7.073	12.215	76.221
	50	—	—	1.138	7.101	—	—

the Van Slyke method. To the above same solution, a proper quantity of basic lead acetate was added, with the residue of which, the nitrogen of undigested protein was determined. The proper quantity of basic lead acetate was determined as follows: 0.5 gram of the denatured glycinin was decomposed with 15 cc. of 20% HCl for 1, 2 or 10 hours. After the elimination of ammonia the filtrate was neutralized and made

TABLE LX

The results of decomposition of glycinins obtained with 10% HCl

	Decomposition time (hours)	Ammonia N		Free amino N		Digested protein	
		%	% of total N	%	% of total N	%	% of total N
Glycinin	1	1.801	10.786	0.652	3.903	10.248	61.385
	2	1.968	11.790	0.848	5.080	11.199	67.082
	5	2.058	12.327	0.983	5.888	12.045	72.149
	10	2.103	12.598	1.029	6.166	12.578	75.342
	15	2.119	12.690	1.121	6.714	12.678	75.941
	20	2.157	12.918	1.138	6.819	12.720	76.192
	30	2.187	13.100	1.168	6.997	—	—
	40	—	—	—	—	12.763	76.456
Denat. glycinin	1	1.337	8.340	0.663	4.137	10.180	63.523
	2	1.458	9.097	0.815	5.087	11.027	68.808
	5	1.549	9.666	0.976	6.092	11.961	74.635
	10	1.822	11.372	1.026	6.405	12.300	76.752
	15	1.914	11.940	1.080	6.741	12.343	77.020
	20	1.929	12.034	1.098	6.848	12.512	78.075
	30	1.944	12.130	1.105	6.896	—	—
	40	2.005	12.773	1.144	7.135	—	—

to 100 cc. as above described, to each 10 cc. of which each different quantity of basic lead acetate was added, diluted to 25 cc. and filtered. The nitrogen in each filterate was determined with results given in TABLE LVIII. As it was recognized from the data that 2 cc. of basic lead acetate was proper for determing undigested protein it was used through the experiment.

The results obtained in the decomposition of proteins by hydrochloric acid will be given in TABLES LIX—LXI. In the results of decomposition of both normal and denatured glycinins with 5, 10 and

TABLE LXI

The results of decomposition of glycinins obtained with 20% HCl

	Decomposition time (hours)	Anmonia N		Free amino N		Digested protein	
		%	% of total N	%	% of total N	%	% of total N
Glycinin	1/2	2.005	12.008	0.760	4.561	11.431	64.586
	1	2.020	12.099	0.928	5.568	11.938	71.628
	2	2.035	12.190	1.056	6.333	12.255	73.530
	5	2.065	12.372	1.110	6.659	12.424	74.544
	8	2.126	12.736	1.129	6.776	12.467	74.802
	15	2.211	13.266	1.144	6.866	12.530	75.180
	20	2.226	13.357	1.144	6.866	12.551	75.306
Denat. glycinin	1/2	1.701	10.614	0.833	5.195	10.900	68.076
	1	1.716	10.709	0.892	5.566	11.154	69.662
	2	1.731	10.803	1.028	6.416	11.494	71.786
	5	1.792	11.183	1.054	6.579	11.918	74.434
	8	1.822	11.372	1.090	6.802	12.003	74.964
	15	1.862	11.619	1.114	6.949	12.087	75.489
	20	1.914	11.941	1.131	7.056	—	—

20% HCl, the resulting ammonia, free amino nitrogen and digested protein increased, as a matter of course, according to the increase of concentration of HCl. In the comparison of the normal glycinin with the denatured, it was observed as follows:

(1) The resulting ammonia of the denatured glycinin was always more than that of the normal.

(2) In the result of decomposition with 5% HCl, both the absolute amount of amino nitrogen and its precentage in total nitrogen, were predominant in the denatured glycinin. In the case of 10% HCl, they were predominant also in the denatured protein in the first half of the decomposition, but it was the contrary in the latter half of the decomposition. The percentage of free amino nitrogen in total nitrogen

TABLE LXII

The results of decomposition of glycinins obtained with 10% H_2SO_4

	Decomposition time (hours)	Ammonia N		Free amino N		Digested protein	
		%	% of total N	%	% of total N	%	% of total N
Glycinin	1	1.938	11.630	0.331	1.984	7.396	44.376
	2	2.026	12.176	0.431	2.588	9.466	56.796
	5	2.120	12.721	0.739	4.435	11.579	69.474
	10	2.151	12.903	0.901	5.407	11.875	71.250
	20	2.211	13.266	0.979	5.875	12.445	74.670
	30	2.241	13.448	1.005	6.027	12.741	76.446
Denat. glycinin	1	1.701	10.623	0.396	2.475	7.210	45.030
	2	1.762	11.003	0.485	3.028	9.013	56.291
	5	1.792	11.193	0.655	4.091	11.028	68.875
	10	1.838	11.477	0.763	4.763	11.770	73.509
	20	1.883	11.762	0.851	5.314	12.088	75.495
	30	1.914	11.951	0.851	5.314	12.406	77.481

was generally higher in the denatured glycinin. In the results obtained with 20% HCl, the difference of free amino nitrogen was large in the former half of decomposition, but in the latter stage of decomposition it became very small with slight predominance in the denatured protein.

(3) In most cases the nitrogen of undigested protein of the denatured glycinin was generally more than that of the normal glycinin. But the percentage in total nitrogen of the normal glycinin was lower.

Sulphuric acid

The results of decomposition by H_2SO_4 are given in TABLES LXII and LXIII.

In the comparison of both proteins in respect to the quantity of ammonia nitrogen, of free amino nitrogen and of digested protein obtained in the decomposition by 10% and 20% H_2SO_4, the same tendency as in the case of HCl, was recognized.

TABLE LXIII

The results of decomposition of proteins obtained with 20% H_2SO_4

	Decomposition time (hours)	Ammonia N		Free amino N		Digested protein	
		%	% of total N	%	% of total N	%	% of total N
Glycinin	1/2	1.908	11.449	0.389	2.334	8.029	48.174
	1	1.938	11.630	0.556	3.335	10.692	64.152
	2	1.999	11.994	0.693	4.155	11.199	67.194
	5	2.105	12.630	0.936	5.615	12.213	73.278
	8	2.196	13.175	1.019	6.115	12.889	77.334
	15	2.196	13.175	1.091	6.545	13.185	79.110
	20	2.211	13.266	1.091	6.545	13.206	79.236
Denat. glycinin	1/2	1.671	10.433	0.524	3.275	9.395	58.052
	1	1.701	10.623	0.624	3.898	11.049	68.007
	2	1.747	10.908	0.881	5.501	12.661	79.074
	5	1.792	11.192	0.934	5.831	12.851	80.261
	8	1.807	11.287	0.971	6.065	12.894	80.529
	15	1.914	11.951	1.063	6.636	12.936	80.792
	20	1.914	11.951	1.067	6.663	12.936	80.792

Sodium hydroxide

The results of decomposition obtained with NaOH are tabulated in TABLE LXIV and LXV.

From the above tables it is clear that ammonia, free amino nitrogen and nitrogen in the form of digested protein obtained from the normal glycinin were more than those of the denatured glycinin respectively in the decomposition by 5% NaOH. In the results of decomposition obtained with 10% NaOH, the same difference was observed between the two proteins as in the case of 5% NaOH except that the difference of ammonia in percentage of total nitrogen was noticeable.

TABLE LXIV

The results of decomposition of glycinin obtained with 5% NaOH

	Decomposition time (hours)	Ammonia N		Free amino N		Digested protein	
		%	% of total N	%	% of total N	%	% of total N
Glycinin	1	3.089	18.536	0 457	2.744	7.015	42.091
	2	3.120	18.718	0.519	3.113	7.734	46.404
	5	3.695	22.171	0.672	4.034	8.790	52.740
	10	3.816	22.898	0.786	4.717	9.001	54.006
	20	4.028	24.170	0.914	5.482	9.297	55.780
	30	4.240	25.442	0.940	5.641	9.509	57.054
	40	4.256	25.533	0.940	5.641	9.593	57.558
Denat. glycinin	1	2.640	16.504	0.389	2.431	6.489	40.527
	2	3.554	22.195	0.424	2.648	7.634	47.678
	5	3.584	22.384	0.668	4.171	8.271	51.656
	10	3.675	22.954	0.776	4.843	8.695	54.304
	20	3.827	23.904	0.833	5.201	8.822	55.088
	30	3.873	24.187	0 866	5.407	9.034	56.422
	40	4.878	24.187	0.908	5.668	9.119	56.953

From the above data obtained in the decomposition of both proteins by HCl, H_2SO_4 and NaOH, the results are compared with one another for the same concentration (%) of each reagent as follows:

(1) In respect to ammonia, the decomposition by NaOH was most rapid, that by H_2SO_4 next and that by HCl was least powerful. The fact that ammonia was liberated more in the decomposition by alkali than in the decomposition by acid was due to the liberation of the one half arginine nitrogen in addition to the amide nitrogen which had been already made clear by Osborne.[60]

(2) Free amino nitrogen was obtained most with HCl, moderately and noticeably least with NaOH and H_2SO_4 respectively.

(3) Digested protein decreased in the order of HCl, H_2SO_4 and NaOH.

TABLE LXV

The results of decomposition of glycinins obtained with 10% NaOH

	Decomposition time (hours)	Ammonia N		Free amino N		Digested protein	
		%	% of total N	%	% of total N	%	% of total N
Glycinin	1	3.180	19.081	0.582	3.489	8.663	51.978
	2	3.574	21.443	0.699	4.196	9.423	56.538
	5	4.028	24.170	0.802	4.811	9.593	57.558
	10	4.080	24.478	0.896	5.375	9.720	58.320
	20	4.080	24.478	0.940	5.642	10.037	60.222
	30	4.128	24.770	0.954	5.722	10.058	60.348
Denat. glycinin	1	2.582	16.125	0.552	3.404	8.059	50.332
	2	3.007	18.780	0.657	4.105	9.543	59.601
	5	3.432	21.436	0.769	4.804	9.628	60.132
	10	3.797	23.712	0.911	5.687	9.680	60.456
	20	3.842	23.997	0.919	5.737	9.691	60.525
	30	4.131	25.799	0.919	5.737	9.691	60.525

(ix) Discussion on the denaturation of glycinin

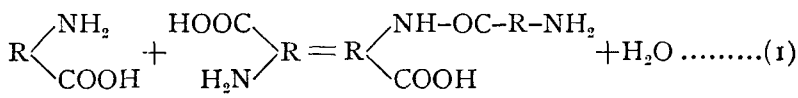
From the results of experiments given in paragraphs (i)—(viii), the difference of some properties between the normal and denatured glycinins were summarized at the end of each paragraph. Now, the changes of atomic groups at the denaturation of glycinin will be discussed from author's results of experiments refering some theories of preceding investigators which have been hitherto made and widely accepted.

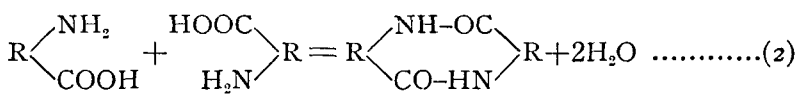
1. *Free NH_2 group and free COOH group*

The denatured glycinin was richer in both free amino nitrogen and free carboxyl group as shown in TABLES XLI and XLII. The same differences were observed between the normal and denatured caseins. Such a tendency is to be recognized as a characteristic pro-

perty of a protein that is denatured by steaming. In the results of decomposition with acids (TABLES LIII—LVII), free amino nitrogen obtained from the denatured glycinin was more than that from the normal in the earlier time of decomposition. But this difference between both proteins became gradually small according to the progress of decomposition. It is rather to be considered that this free form of nitrogen is richer in the denatured protein itself than the normal even before the decomposition by acids, than to consider that the decomposition itself of the former is more speedier than that of the latter.

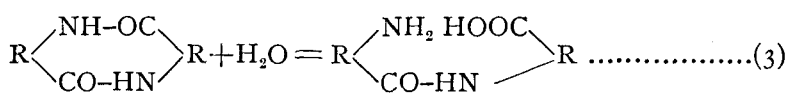
Next, the author wishes to discuss the mode of occurrence of such free amino nitrogen and free carboxyl groups of the denatured glycinin which are produced at the denaturation of the normal glycinin. It is recognized that polyamino acid structure is resulted by dehydration as follows:



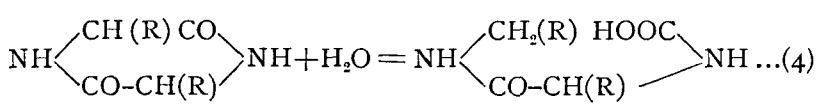


In the proceeding of reaction from left to right in these equations both NH_2 and COOH groups decrease or entirely disappear. Such reactions are possible as some of the modes of denaturation of protein molecule by steaming. In these cases, a protein molecule loses one or two keto groups and increases newly terminal NH_2 and COOH groups.

Such a following reaction may also be possible.



In this reaction a keto group disappears and a free amino group and a free carboxyl group appear. And in such a reaction as



a keto group is lost and a COOH group appears. As the mode of com-

bination of C with N, the following types are considered to be possible.

$$-CH_2-NH-CH_2- \quad \ldots\ldots(5)$$

$$-CH_2-NH-CO- \quad \ldots\ldots(6)$$

$$-CH_2-NH-C(NH)- \quad \ldots\ldots(7)$$

The backward changes of (1) and (2) are considered to be some modes of the decomposition of the group -NH-CO- of (6) and a free amino group is possible to appear in the hydration of the group of (7) as shown below.

$$-CH_2-NH-C(NH)-+H_2O = -CH_2-NH-(OH)\overset{|}{C}-NH_2 \quad \ldots\ldots(8)$$

In this case a NH_2 group as well as an enol group appear through hydration process.

The rotatory power of alkali solution of denatured either glycinin or casein is always higher than that of respective normal protein (TABLES XXXIX and XL). In the reaction from right to left in equation (8) a racemic carbon appears possibly. Therefore such a reaction may be surely possible as one of denaturation processes of glycinin.

Amide nitrogen is recognized to exist in protein molecules in the following forms:

$$-CONH_2 \quad \ldots\ldots(9) \qquad -CONHOC- \quad \ldots\ldots(10)$$

In (9) one group of NH_2 combines with one group of COOH, and in (10) the same NH_2 group combines with two groups of COOH. It is very naturally supposed that a pretty large quantiy of nitrogen exists in the form of (9) or (10) in a protein such as glycinin which is rich in nitrogen content and also in dicarboxyl acids, glutamic and aspartic acids. Moreover, from the facts that the denatured glycinin is richer than the normal glycinin in ammonia (TABLES LIV—LV) and in free carboxyl group, it is considered to be very possible that the atomic group such as (9) or (10) evolutes ammonia and results newly the formation of free COOH through hydrolytic process as in the following ways:

$$-CONH_2+H_2O = -COOH+NH_3 \quad \ldots\ldots(11)$$

$$-CONHOC-+H_2O = -CONH_2+-COOH \quad \ldots\ldots(12)$$

$$-CONHOC-+2H_2O = 2-COOH+NH_3 \quad \ldots\ldots(13)$$

Such a supposition agrees not only with the results of comparative determination of ammonia and free carboxyl group but also with the results of refractive index, turbidity, surface tension and the viscosity of acid and alkali solutions of both proteins. That is, the supposition is most possible in regard to the changes of molecular structure of glycinin when it is denatured.

He is going furthermore to discuss the difference of quantity of both keto and enol groups between both proteins.

2. Keto and enol groups

To examine various properties of protein, the two types of $-COHN-$, keto and enol forms, such as

$$=CH-CO-HN-CH_2- \quad \ldots\ldots(14)$$

$$=CH-C(OH)=N-CH_2- \quad \ldots\ldots(15)$$

as well as terminal NH_2 and COOH groups are very important and must be especially attended to among various types of molecular structure. In protein molecules these types of $-COHN-$ have more part to combine with acids or bases than terminal NH_2 and COOH as already shown by Blasal and Matula,[10] and Pauli and Hirschfeld.[64]

The surface tension and turbidity of alkali solution of protein resulted by the titration with HCl (TABLES XLIII and XLV) and apparent isoelectric point (TABLE XLIV) are remarkably different between the normal and denatured proteins. What have the relation to such properties are terminal NH_2 and COOH groups as well as keto and enol forms of -COHN-, the summation of which in each protein, quantitatively and qualitatively, brings its special values in the test of such properties as surface tension, turbidity etc., though the direct determination of keto and enol groups is impossible. At the same time one must attend to the increase of terminal NH_2 and COOH through denaturation. From the discussion of the changes (11)—(13), COOH group is considered to appear pretty much when glycinin is denatured.

It is necessary to discuss again the mode of appearance of terminal NH_2 and COOH groups. In the reaction (5), the decrease of one keto group and the increase of one COOH group are considered, however, merely by such reactions so large difference of free amino nitrogen between the two proteins as shown in TABLE XLI should not be resulted. The reaction such as (1) or (2) means the incease of NH_2 and COOH and at the same time the decrease of keto group, and the reaction such as (8) means the increases of free amino and enol groups in a denatured protein, if they are supposed as the changes in the denaturation. These three reactions as some of the modes of denaturation of glycinin are very possible if the experimental results are examined. As such reactions as (11)—(13) are most possible, by the increase of COOH group the isoelectric point of the denatured glycinin must have been observed at a remarkably more acidic point than that of the original glycinin. The author proved that the denatured glycinin dissolves in acid solutions of any concentration less than the normal glycinin (TABLES XXXI—XXXVII). If the solubility of protein in acid solution are due chiefly to terminal NH_2, the denatured glycinin must have dissolved more than the normal. The actual result was contrary to this assumption. Such a consideration makes one also infer that the normal glycinin is richer than the denatured in keto group which is possible to combine with acids.

It is necessary to consider some other types of reaction for the conclusive discussion on the increase or decrease of keto and enol groups which will be stated at the end of (ix).

3. Hydration and dehydration at the denaturation of glycinin

In regard to the changes of atomic groups shown in paragraphs 1 and 2 the author discussed the occurence and vanishing of free NH_2, free COOH, amide nitrogen as well as of keto and enol groups. These changes mean, at the same time, the process of hydration. Chick and Martin[13] proved that heat coagulation means a hydration process. But the denaturation have been asserted to be a dehydration process by some other investigators. Robertson[68] maintained that heat coagulation meant dehydration but not hydration. He proposed four types of reaction. Some or all of them were said to be possible.

$$H_2.N.R.COH^{++} + = \overset{H}{\underset{Cl}{N}}.R.COOH + 2NaNO_3$$

$$= H_2N.R.CONa = \overset{Na}{\underset{Cl}{N}}.R.COOH + 2HNO_3 \quad \text{.....(16)}$$

$$H_2N.R.CONa^{++} + = \overset{H}{\underset{OH}{N}}.R.COOH + NaCl$$

$$= H_2N.R.CONa = \overset{Na}{\underset{Cl}{N}}.R.COOH + H_2O \quad \text{.....(17)}$$

$$H_2N.R.CONa^{++} + = \overset{Na}{\underset{Cl}{N}}.R.COOH$$

$$= H_3N.R.CONa = \overset{Na}{\underset{Cl}{N}}.R.COO \quad \text{.....(18)}$$

$$H_2N.R.CONa = \overset{Na}{\underset{Cl}{N}}.R.COOH + H_2O$$

$$= H_2N.R.CONa = \overset{H}{\underset{Cl}{N}}.R.COOH + NaOH \quad \text{.....(19)}$$

On the other hand, he depicted that two types of hydration were possible in heat denaturation as follows:

$$H_2N.R.COH.N.R.COOH = HN.R.COH.N.R.CO + H_2O \quad \text{.....(20)}$$

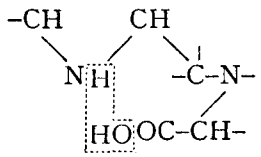
$$2H_2N.R.COH.N.R.COOH = H_2N.R.COH.N.R.COH.N.R.COOH + H_2O \quad \text{.....(21)}$$

His assertion according to heat denaturation is characterized by taking such reactions as to derive H_2O from terminal NH_2 and COOH. If it be true, and if the contrary reaction, hydration, does not occur

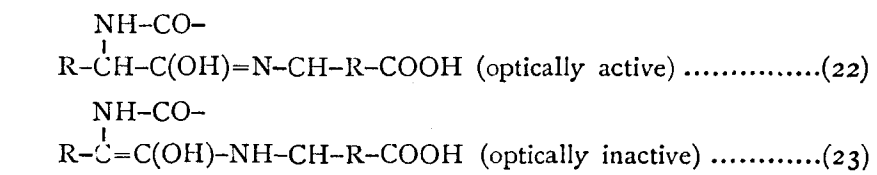
in the denaturation of glycinin, one must recognize the decrease of free NH_2 and free COOH and the increase of keto group in the denatured glycinin. Moreover, in elemental components, the decrease of H and O, and the increase of C must result. However, one observes the remarkable increase of free NH_2 and COOH in the denatured glycinin and casein as often stated. In the comparison of free amino nitrogen quantity of the normal oryzenin with that of the denatured the same tendency of difference was observed by Tadokoro and Yoshimura. From the difference between the two glycinins in the degree of turbidity of protein alkali solution when titrated with HCl one may infer that the normal glycinin is richer than the denatured in keto group. In short, two contrary processes of hydration and dehydration, the former agrees well with experimental results while the latter is quite contrary, are not supposed possibly to occur at the same time when glycinin changes to denatured glycinin.

4. Imino group

Fodor[21] depicted that, in the decomposition of gelatine by acetic acid anhydride, a dehydration process occurred between NH and COOH as given below.



On the other hand, in racemization such a change of molecular formula from (22) to (23) as shown below is recognized which was previously described.



The supposition of Fodor means the isolation of H from imino group, and the change of (22) to (23) means the formation of imino group. From these two theories, a dehydration process between NH and COOH is not surely impossible at heat denaturation as shown below.

$$-NH-CH-R-COOH \longrightarrow -N-CH-R-CO \quad \text{.....(24)}$$

However, the process means the decrease of COOH in the product. In protein molecule, pretty large quantities of –NH– and COOH may possibly exist, but from the distinct increase of COOH in the denatured glycinin, the reaction (24) may not occur or may occur negligibly in the denaturation of glycinin.

5. Amide nitrogen

The evolution of ammonia was always more vigorous in the normal glycinin than in the denatured, when they were decomposed with HCl, H_2SO_4 or NaOH of various concentrations for different periods of time (TABLES LIX—LXV). This fact means the decrease of amide nitrogen of glycinin through the heat denaturation, and agrees with the reactions (11), (12) and (13).

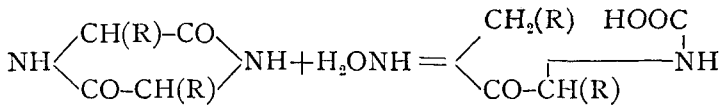
The author has discussed various types of reaction, some of which are supposed to be possible as the denaturation processes of glycinin. These possible types do not mean at all the increase of keto group, but many of them mean the decrease of that group in the products. It is rather to say that they are agreeable with the chemical and physical differences between both proteins observed in actual experiments. Therefore, the author recognizes that glycinin is richer in keto group and that the denatured glycinin is richer in enol group than the other protein respectively.

From the above discussion based upon experimental results the possible types of denaturation process, which are considered for glycinin at the preparation of soy bean meal by extraction method, are summarized and classified into four types of chemical change according to the kind of atomic group.

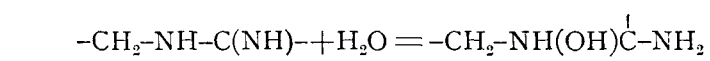
(1) For the increase in free NH_2 and free COOH groups and decrease in keto group:

$$R\begin{cases}NH\text{-}OC\text{-}R\text{-}NH_2\\ COOH\end{cases} + H_2O = R\begin{cases}NH_2\\ COOH\end{cases} + \begin{cases}HOOC\\ H_2N\end{cases}R$$

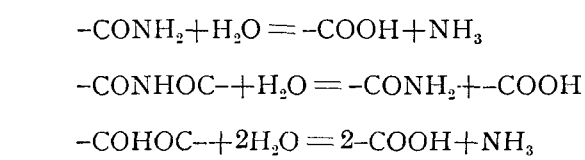
(2) For the increase in free COOH group only and decrease in keto group:



(3) For the increase in free NH_2:



(4) Hydration process at amide linkage:



These possible changes increase terminal NH_2, COOH or enol groups and decrease keto group in the denatured glycinin.

V. Decomposition of the normal and denatured glycinins by enzymes

Many studies on the specificity of a protein splitting enzyme in regard to the kind or to the molecular structure of protein have been undertaken by many investigators. Recently, Abderhalden[2),3)(4)] decomposed some peptides and amino acids with erepsin and trypsin, after the elimination of COOH by aniline condensation. The substrates were splitted by the former enzyme but not by the latter. He proved that peptides which contained alanine were not decomposed by enzymes and that ureido derivatives were slightly splitted by pancreas extract but not entirely by erepsin or trypsin kinase. Such results show the specificity of enzyme in regard to the molecular structure of protein. The activity of enzyme to denatured protein has been studied by some investigators. Yong[86)] did not recognize any difference of splitting velocity in peptic digestion between normal and coagulated egg-whites which had been boiled at 100°C. for 20–30 mins. Kuo-Hao-Lin and his coworkers[45)] recognized the difference of optimum acidity in peptic and tryptic digestion between crystalline egg albumin and its denatured products which had been obtained by heating in dilute acid or alkali, or by the treatment with alcohol or by shaking. The digestibilities of

the normal and denatured proteins by trypsin were about equal, while the digestion of the normal protein by pepsin was always more speedier than that of the denatured. From this result they supposed that the cleavage linkage for peptic action was not changed by such treatments, while, as the change of molecular structure at the first stage of tryptic digestion and the change caused by such a treatment were equal under their supposition, the tryptic decomposition of the denatured protein was consequently always more progressive than that of the normal. Farlanc[23] reported that the peptic decomposition of acid meta protein was more slowly than that of the original egg albumin.

As the author found already the differences of physical and chemical properties between the normal and denatured glycinins, he expected some difference between both proteins also in enzyme activity. To ascertain it, pepsin, pancreatin and papain were used.

(i) Studies on enzymic decomposition taking time as a variable

A. Experiment

1. Decomposition by pepsin

About 0.6 g. equivalent of dry protein was dissolved in 40 cc. of 0.025 N NaOH. After one hour, 80 cc. of water was added to it to make the concentration of solution 1/100 N of NaOH. Each 10 cc. of the solution was taken in a 50 cc. conical flask to which 6.5 cc. of water and 12.5 cc. of a Clark and Lubs's buffer solution of P_H 1.2 were added. After keeping it for 30 mins. in a thermostat at 40°C., 1 cc. of 1/200 water solution of Merck's pepsin was added. The flask was allowed to stand for each definite time at 40°C. At the end of time, after cooling the falsk in running water, 3 cc. of 1/5 N NaOH was added and the solution was made faintly alkaline to make the enzyme inactive. Then 7 cc. of 20% trichloracetic acid was added to precipitate undigested protein. With 5 cc. of the filtrate, nitrogen in the form of digested protein was determined by micro Kjeldahl's method. As control, two samples in the absence of pepsin were allowed to stand for the shortest (15 mins.) and the longest (360 mins.) periods of time and the treatments were run uuder the same conditions as demonstrated above. Nitrogen in 10 cc. of each protein solution and in the blank test was determined as follows:

TABLE LXVI

The result of preliminary test of decomposition by pepsin

	N g. in 10cc. of original solution (a)	N g. obtained in		Diff. (b)	$\frac{b}{a} \times 100$
		360 mins.	15 mins.		
Glycinin	0.0085191	0.0002391	0.0001793	0.0000598	0.70
Denat. Glycinin	0.0074816	0.0003585	0.0002615	0.0000970	1.29

The difference of nitrogen % between two extreme periods was so small as to be only 0.7% in glycinin and 1.29% in the denatured. These data are negligible for the calculation of digested protein. Therefore the quantity of substrate decomposed by enzyme was calculated such as to deduce, equally in every case, the quantity obtained in the shortest time in the blank test from each apparent quantity of nitrogen of decomposed protein. The results are given below.

TABLE LXVII

The results obtained with pepsin, 1/200 solution

Decomposition time (mins.)	Glycinin		Denat. glycinin	
	Protein decomposed g.	%	Protein decomposed g.	%
15	0.0048925	9.82	0.0030250	6.29
30	0.0092150	18.50	0.0058103	12.08
60	0.0145869	29.29	0.0102764	21.37
90	0.0177167	35.57	0.0142624	29.66
120	0.0226198	45.41	0.0184882	38.44
180	0.0275428	55.30	0.0237719	49.43
240	0.0315912	63.43	0.0275163	57.22
300	0.0344965	69.26	0.0292455	60.81

As given in this table, the quantity of glycinin decomposed by pepsin, was always larger than that of the denatured glycinin.

2. Decomposition by pancreatin

The experimental method and condition were about the same as in the case of pepsin. They are briefly described below.

10cc. of protein solution (ca. 0.5 g. in 100cc. of $^1/_{100}$ N NaOH) + 6.5 cc. of H_2O + 12.5 cc. of Clark and Lubs's buffer solution of P_H 7.4 + 1 cc. of enzyme solution (the filtrate of $^1/_{200}$ or $^1/_{800}$ water solution); 40°C.; + 3 cc. of $^1/_{10}$ N HCl + 7 cc. of 20% CCl_3COOH; N in 5 cc. of the filtrate was determined.

The results are as follows:

TABLE LXVIII

The results obtained with pancreatin, $^1/_{200}$ solution

Decomposition time (mins.)	Glycinin		Denat. glycinin	
	Protein decomposed g.	%	Protein decomposed g.	%
15	0.0240645	50.08	0.0250720	51.80
30	0.0297251	61.85	0.2322209	66.57
45	0.0331924	69.07	0.0349564	72.22
60	0.0354683	73.80	0.0402698	83.20
90	0.0390448	81.25	0.0435982	90.08
120	0.0417527	86.88	0.0458053	94.64
180	0.0435872	90.70	0.0463924	95.85
240	0.0457707	95.24	0.0465961	96.27
300	—	—	0.0468672	96.83

As shown in this table, the quantity of the denatured glycinin decomposed was always larger than that of the normal. This tendency was contrary to that in the case of peptic degestion. This characteristic tendency in each enzymic decomposition is agreeable with that found by Kuo-Hao-Lin in the digestive experiment with egg-white and its denatured products. As a comparatively large amount was splitted in

a short time in above experiment, a lesser quantity of enzyme, 1 cc. of the filtrate of 1/800 water solution, was used. 10 cc. of each normal and denatured glycinin solutions under this experiment contained 0,048056 and 0.048405 gram nitrogen respectively. The results are as follows:

TABLE LXIX

The results obtained with pancreatin, 1/800 solution

Decomposition time (mins.)	Glycinin		Denat. glycinin	
	Protein decomposed g.	%	Protein decomposed g.	%
15	0.0063733	13.26	0.0064961	12.42
30	0.0095220	19.81	0.0097262	20.09
45	0.0124936	26.00	0.0120620	24.92
60	0.0144665	30.10	0.0157165	32.47
90	0.0190515	39.64	0.0208151	43.00
120	0.0215688	44.88	0.0257345	53.16
180	0.0264787	54.97	0.0313645	64.79
240	0.0301414	62.72	0.0369931	76.42
300	0.0321781	66.96	0.0396807	81.98
360	0.0364098	75.76	0.0417441	86 23

In the above data, the same tendency as previously mentioned was also recognized between the normal and denatured glycinins.

3. Decomposition by papain

Merck's "Saccus Carricae Papayae" was used. Ten times or twenty times water solution of papain was shaken well. One cc. of the filtrate was used as an enzyme solution in each case. A control test in the absence of protein was operated for each period of time. The experimental method was as follows:

10 cc. of protein solution (ca. 0.5 g. in 100 cc. of 1/100 N NaOH) +6.5 cc. H_2O + 12.5 cc. of Clark and Lubs's buffer solution of P_H 6.2 + 1 cc. of enzyme solution; 40°C.; 3 cc. of 1/10 N HCl + 7cc. of 20% CCl_3COOH; N in 5 cc. of the filtrate was determined.

The results are as follows:

TABLE LXX

The results obtained with papain, 1/10 water solution

Decomposition time (mins.)	Glycinin		Denat. glycinin	
	Protein decomposed g.	%	Protein decomposed g.	%
15	0.0237240	39.54	0.0410464	68.41
30	0.0255120	42.52	0.0430080	71.68
45	0.0301144	50.19	0.0457136	76.19
60	0.0310984	51.83	0.0462736	77.12
90	0.0328680	54.78	0.0475744	79.29
120	0.0369600	61.60	0.0485104	80.85
180	0.0382024	63.67	0.0494456	82.41
240	0.0417544	69.59	0.0511200	85.20
300	0.0428224	71.37	0.0513000	85.50
360	0.0442440	73.74	—	—

TABLE LXXI

The results obtained with papain, 1/20 water solution

Decomposition time (mins.)	Glycinin		Denat. glycinin	
	Protein decomposed g.	%	Protein decomposed g.	%
15	0.0221224	36.87	0.0345184	57.53
30	0.0259440	43.24	0.0377824	62.97
45	0.0279840	46.64	0.0397440	66.24
60	0.0306544	51.09	0.0422584	70 43
90	0.0322504	53.75	0.0448504	74.75
120	0.0326040	54.34	0.0468304	78.05
180	0.0352680	58.78	0.0487920	81.32
240	0.0359824	59.79	0.0489784	81.63
300	0.0386464	64.41	0.0506584	84.43
360	0.0395344	65.89	0.0515880	85.98

From the above two tables, it is distinct that the quantity of the denatured glycinin decomposed by papain was always larger than that of the normal.

In a test, one cc. of the filtrate of either 1/100 or 1/200 water solution of papain was used to decompose the denatured glycinin. The results are as follows:

TABLE LXXII

The results of decomposition of the denatured glycinin obtained with papain, 1/100 and 1/200 water solutions

Decomposition time (mins.)	1/100 water solution		1/200 water solution	
	Protein decomposed g.	%	Protein decomposed g.	%
15	0.0191224	31.87	0.0120360	20.06
30	0.0237904	39.65	0.0166080	27.68
45	0.0261240	43.54	0.0195904	32.65
60	0.0312544	52.09	0.0218280	36.38
90	0.0321840	53.64	0.0264000	44.00
120	0.0338280	56.38	0.0270544	45.09
180	0.0396480	66.08	0.0342360	57.06
240	0.0424504	70.75	0.0362880	60.48
300	0.0434760	72.46	0.0369424	61.57
360	0.0441240	73.54	0.0381544	63.59

The degree of decomposition of the normal glycinin by pepsin, pancreatin and papain was compared with that of the denatured. Peptic digestion of the former protein was more progressive than that of the latter, but the digestion by the other two enzymes was contrary. As the action of tryptic enzyme such as pancreatin is recognized to break the anhydride structure of protein, the denatured glycinin may be supposed to be on the way of destruction.

B. Application of equations to experimental data

There have been many studies on the reaction velocity of enzyme, and although there are many equations which were formulated by many investigators from different experimental data, they are usually applicable only within narrow range of decomposition. One can not see an equation which had been proved to be generally applicable. The author expected some equation that could express the reaction velocity of each enzyme used in his experiments. At first, the Schütz

law, one of the most generally accepted equations until recently, was examined, but the result was not satisfactory at all. The author examined further the reasons why the Schütz law was not applicable well and reached to a conclusion[53] that the Schütz law, $\frac{x}{\sqrt{t}}=K$, had no worth in the kinetics of enzyme. At the same time $\frac{x}{\sqrt{E}}=K$, one of the equations of the Schütz law, was examined. The author tried further to apply some other equations. The results were all unsatisfactory. The endeavour to find some satisfactory equation for his results was continued.

1. Application of Nakamura's equation

Rona and Kleinmann[69] applied the equation of the second order, $\frac{1}{t}\frac{x}{a(a-x)}=K$, to their data of digestion experiments with casein and trypsin and considered this equation to be satisfactorily applicable to such experimental data. Nakamura, obtaining a suggestion from this equation and the Schütz law, $\frac{1}{\sqrt{t}}=K$, made a new equation, $\frac{1}{t^{K'}}\frac{x}{a(a-x)}=K$, which he applied to his experimental data of peptic and tryptic digestion. He took the proteins of various barleys as substrates. He attempted a discussion of the differences between proteins of varieties of barley from the results obtained in applying his equation. He considered also his equation to be applicable to the data obtained with proteolytic enzymes. Although Nakamura showed that his equation was applicable to his data, the range of the decomposition applied was, in most cases, within 20%. Therefore, it is necessary to study whether or not his equation is applicable to the data in further proceeding of decomposition, or whether there may be another equation which is more satisfactorily applicable to the data obtained with any enzyme or protein. The present author applied Nakamura's equation to his data above described. In applying this equation, the method of least square is used to find the values of K and K'. The logarithmic form of his equation, is first given.

$$\log K+K' \log t=\log x-\log (a-x) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

In this equation, x denotes the quantity of substrate decomposed, a the quantity of substrate taken and if x be denoted in percentage then

a is equal to 100. The value of each term of the right side is known. Let the total value of the right side terms be denoted by c, an equation, $\log K+K' \log t=c$, is formed according to each different value of t. If the number of experimental results of one group be n, the normal equations are given as follows:

$$n \log K+[K' \log t]=[c]$$

$$[\log t] \log K+[(\log t)^2] K'=[c \log t]$$

From these simultaneous equations, the values of K and K' can be calculated. Insert a value of t and that of a as well as the values of $\log K$ and K' in equation (1), then the calculated value of x can be found. Thus all the calculated values of x can be found. By the method above demonstrated, the author obtained the calculated results as given in TABLES LXXIII — LXXIX. Each probable error was calculated by the formula, $\pm 0.6745\sqrt{\frac{\Sigma \Delta^2}{n-1}}$. In TABLE LXXX, P. E. denotes probable error. Each summation of differences is also given.

The author proposes next to study the applicability of Nakamura's equation, $\frac{1}{t^{K'}} \frac{x}{a(a-x)}=K$, from the results of its application above shown. Let an experiment be run accurately under proper conditions. If an equation be applied to the data thus obtained, one can surely recognize the perfect applicability of the equation through the ascertainment that the sum of differences in each pair of experimental and calculated values is equal to or near zero and that either a positive or a negative difference occurs irregularly. In the result of application given above to the data of peptic or papain decomposition, the sum of all differences is seen to be below 1 and the probable error proves to be satisfactorily small. Therefore, one may accept the perfect applicability of the equation, $\frac{1}{t^{K'}} \frac{x}{a(a-x)}=K$, for these two enzymes. But in the applied results for the data of pancreatic decomposition, as the probable error is remarkably larger than that in cases of the former two enzymes and the sum of negative differences is much larger than that of positive, one can not accept the perfect applicability of the equation. Therefore, the author considers that there may be some equation which is better applicable to the data of pancreatic decomposition of normal and denatured glycinins.

TABLE LXXIII

The values of K and K' in application of the equation, $\frac{1}{t'}\frac{x}{a(a-x)}=K$ to the data obtained in proteolytic decomposition

	Pepsin, $\frac{1}{200}$ sol.			Pancratin, $\frac{1}{200}$ sol.			Pancreatin, $\frac{1}{800}$ sol.			Papain, $\frac{1}{10}$ sol.		
	Range of decomp.	K	K'	Range of decomp.	K	K'	Range of decomp.	K	K'	Range of decomp.	K	K'
Glycinin	9.82—69.26	7.035×10^{-5}	0.99974	50.08—95.24	4.726×10^{-4}	1.04126	13.26—75.76	1.0772×10^{-4}	0.92365	39.54—73.74	1.59066×10^{-3}	0.43712
Denat. glycinin	6.29—60.81	3.560×10^{-5}	1.07287	51.80—96.27	2.8311×10^{-4}	1.27443	13.42—86.23	4.4891×10^{-5}	1.18015	68.41—85.50	8.3226×10^{-3}	0.34238

	Papain, $\frac{1}{20}$ sol.			Papain, $\frac{1}{100}$ sol.			Papain, $\frac{1}{200}$ sol.		
	Range of decomp.	K	K'	Range of decomp.	K	K'	Range of decomp.	K	K'
Glycinin	76.87—65.89	2.2353×10^{-3}	0.36031	—	—	—	—	—	—
Denat. glycinin	57.53—85.98	5.53—85.98	0.48560	31.87—73.54	8.9972×10^{-4}	0.58531	20.06—63.59	4.4188×10^{-4}	0.63361

TABLE LXXIV

The calculated results obtained by using the values of K and K' in TABLE LXXIII for the data obtained with pepsin, $^1/_{200}$ solution

	Time (mins.)	15	30	60	90	120	180	240	300	Summation
Glycinin	Exp. value	9.82	18.50	29 29	35.57	45.41	55.30	63.43	69.26	
	Calc. value	9.54	17.08	29.66	38.74	45.74	55.84	62.77	67.82	
	Diff. Δ	+0.28	+1.42	−0.37	−3.17	−0.33	−0.54	+0.66	+1.44	+3.80−4.41=−0.61
	Δ^2	0.0784	2.0164	0.1369	10.0489	0.1085	0.2916	0.4356	2.0736	15.1903
Denat. glycinin	Exp. value	6.29	12.08	21.37	29.66	38.44	49.43	57.22	60.81	
	Calc. value	6.11	12.04	22.21	30.78	37.72	47.69	56.02	61.81	
	Diff. Δ	+0.18	+0.04	−0.84	−1.12	+0.72	+1.74	+1.20	−1.00	+3.88−2.96=+0.92
	Δ^2	0.0324	0.0016	0.7056	1.2544	0.5184	3.0276	1.4400	1.0000	7.9800

TABLE LXXV

The result of calculation using the values of K and K' in TABLE LXXIII for the data obtained with pancreatin, $^1/_{200}$ solution

	Time (mins.)	15	30	60	90	120	180	240	300	Summation
Clyinin	Exp. value	50.08	61.85	69.07	73.80	81.25	86.88	90.70	95.24	
	Calc. value	43.63	62.00	71.33	77.05	83.66	87.36	91.33	93.43	
	Diff. Δ	+6.45	−0.15	−2.26	−3.25	−2.41	−0.48	−0.63	+1.81	+8.26−9.18=−0.92
	Δ^2	41.6025	0.0225	5.1076	10.5625	5.8081	0.2304	0.3969	3.2761	67.0066
Denat. glycinin	Exp. value	51.80	66.57	72.22	83 20	90.08	94.64	95.85	96.27	
	Calc. value	47.19	68.37	78.37	83.95	89.76	92.67	95.39	96.84	
	Diff. Δ	+4.61	−1.80	−6.15	−0.75	+0.32	+1.97	+0.46	−0.57	+7.36−9.27=−1.19
	Δ^2	21.2521	3.2400	37.8225	0.5625	0.1024	3.8009	0.2116	0.3249	67.3169

TABLE LXXVI

The result of calculation using the values of K and K' in TABLE LXXIII for the data obtained with pancreatin $^1/_{800}$ solution

	Time (mins.)	15	30	45	60	90	120	180	240	300	360	Summation
Glycinin	Exp. value	13.26	19.81	26.00	30.10	39.64	44.88	54.97	62.72	66.96	75.76	
	Calc. value	11.59	19.92	26.56	32.05	40.69	47.28	56.60	62.98	67.64	71.22	
	Diff. Δ	+ 1.67	− 0.11	− 0.56	− 1.95	− 1.05	− 2.40	− 1.63	− 0.26	− 0.68	+ 4.54	+6.21−8.64=−2.43
	Δ^2	2.7889	0.0121	0.3136	3.8025	1.1025	5.7600	2.6569	0.0676	0.4624	20.6116	37.5781
Denat. glycinin	Exp. value	13.42	20.09	24.92	32.47	43.00	53.16	64.79	76.42	81.98	86.23	
	Calc. value	9.88	19.91	28.63	36.03	47.61	56.07	67.31	74.30	79.00	82.35	
	Diff. Δ	+ 3.54	+ 0.18	− 3.71	− 3.56	− 4.61	− 2.91	− 2.52	+ 2.12	+ 2.98	+ 3.88	+12.70−17.31=−4.61
	Δ^2	12.5316	0.0324	13.7641	12.6736	21.2521	8.4681	6.3504	4.4944	8.8804	15.0554	103.5025

TABLE LXXVII

The results of calculation using the values of K and K' in TABLE LXXIII for the data obtained with papain, $^1/_{10}$ solution

	Time (mins.)	15	30	45	60	90	120	180	240	300	360	Summation
Glycinin	Exp. value	39.54	42.52	50.19	51.83	54.78	61.60	63.67	69.59	71.37	73.74	
	Calc. value	36.67	44.63	49.45	52.87	57.60	60.96	65.46	68.49	70.74	72.52	
	Diff. Δ	+ 2.87	− 2.11	+ 0.74	− 1.04	− 2.82	+ 0.64	− 1.79	+ 1.10	+ 0.63	+ 1.22	+7.20−7.76=−0.56
	Δ^2	8.2369	4.4521	0.5476	1.0816	7.9524	0.4096	3.2041	1.2100	0.3969	1.4884	28.9796
Denat. glycinin	Exp. value	68.41	71.68	76.19	77.12	79.29	80.85	82.41	85.20	85.50	—	
	Calc. value	67.77	72.73	75.39	77.17	79.52	81.08	83.12	84.46	85.44	—	
	Diff. Δ	+ 0.64	− 1.05	+ 0.80	− 0.05	− 0.23	− 0.23	− 0.71	+ 0.74	+ 0.06	—	+2.24−2.27=−0.03
	Δ^2	0.4096	1.1025	0.6400	0.0025	0.0529	0.0529	0.5041	0.5476	0.0036	—	3.3157

TABLE LXXVIII

The results of calculation obtained by using the values of K and K' in TABLE LXXIII for the data obtained with papain, 1/20 solution

	Time (mins.)	15	30	45	60	90	120	180	240	300	360	Summation
Glycinin	Exp. value	36.87	43.24	46.64	51.09	53.75	54.34	58.78	59.79	64.41	65.89	
	Calc. value	37.23	43.22	46.84	49.43	53.07	55.65	59.21	61.69	63.57	65.08	
	Diff. Δ	− 0.36	+ 0.02	− 0.20	+ 1.66	+ 0.68	− 1.31	− 0.43	− 1.90	+ 0.84	+ 0.81	+4.01−4.20=−0.19
	Δ^2	0.1296	0.0004	0.0400	2.7556	0.4624	1.6161	0.1849	3.6100	0.7056	0.6561	10.1247
Denat. Glycinin	Exp. value	57.53	62.97	66.24	70.43	74.75	78.05	81.32	81.63	84.43	85.98	
	Calc. value	55.53	53.62	68.04	71.00	74.88	77.42	80.52	82.76	84.25	85.39	
	Diff. Δ	+ 2.00	− 0.65	− 1.80	− 0.57	− 0.13	+ 0.63	+ 0.80	− 1.13	+ 0.18	+ 0.59	+4.20−4.28=−0.08
	Δ^2	4.0000	0.4225	3.2400	0.3249	0.0169	0.3969	0.6400	1.2769	0.0324	0.3481	10.6986

TABLE LXXIX

The results of calculation obtained by using the values of K and K' in TABLE LXXIII for the data obtained in decomposition of denatured glycinin with papain, 1/100 and 1/200 solutions

	Time (mins.)	15	30	45	60	90	120	180	240	300	360	Summation
1/100 sol.	Exp. value	31.87	39.65	43.54	52.09	53.64	56.38	66.08	70.75	72.46	73.54	
	Calc. value	30.51	39.66	45.51	49.71	55.61	59.72	65.28	68.99	71.71	73.83	
	Diff. Δ	+ 1.36	− 0.01	− 1.97	+ 2.38	− 1.97	− 3.34	+ 0.80	+ 1.76	+ 0.75	− 0.29	+7.05−7.58=−0.53
	Δ^2	1.8497	0.0001	3.8809	5.6644	3.8809	11.1556	0.6400	3.0976	0.5625	0.0841	30.8157
1/200 sol.	Exp. value	20.06	27.68	32.65	36.38	44.00	45.09	57.06	60.48	61.57	63.59	
	Calc. value	19.72	27.60	33.02	37.17	43.34	47.85	54.26	58.74	62.12	64.80	
	Diff. Δ	+ 0.34	+ 0.08	− 0.37	− 0.79	+ 0.66	− 2.76	+ 2.80	+ 1.74	− 0.55	− 1.21	+5.62−5.68=−0.06
	$\Sigma\Delta$	0.1156	0.0064	0.1369	0.6241	0.4356	7.6176	7.8400	3.0276	0.3025	1.4641	21.5704

TABLE LXXX

The summation of differences and probable error in application of the equation, $\frac{1}{t^{K'}} \frac{x}{a(a-x)} = K$

	Pepsin, 1/200 sol.		Pancreatin, 1/200 sol.		Pancreatin, 1/800 sol.		Papain, 1/10 sol.	
	Summation of diffs.	P.E.	Summation of diffs.	P.E.	Summation of diffs.	P.E.	Summation of diffs.	P.E.
Glycinin	$+3.80-4.41=-0.61$	± 1.84	$+8.26-9.18=-0.92$	± 2.09	$+6.21-8.64=-2.43$	± 1.38	$+7.20-7.76=-0.56$	± 1.20
Denat. glycinin	$+3.88-2.92=-0.92$	± 0.68	$+7.36-9.27=-1.91$	± 2.04	$+12.70-17.31=-4.61$	± 2.29	$+2.24-2.27=-0.03$	± 0.47

	Papain, 1/20 sol.		Papain, 1/100 sol.		Papain, 1/200 sol.	
	Summation of diffs.	P. E.	Summation of diffs.	P. E.	Summation of diffs.	P. E.
Glycinin	$+4.01-4.20=-0.19$	± 0.72	—	—	—	—
Denat. glycinin	$+4.20-4.28=-0.08$	± 0.74	$+7.05-7.58=-0.53$	± 1.25	$+5.62-5.68=-0.06$	± 1.04

2. Examination of applicable experimental equation

It can not be explained here, why the above equation is not perfectly applicable to the author's data of pancreatic decomposition while it was satisfactory for the data of peptic or papain decomposition. Anyhow, the writer endeavoured further to find some experimental equation which might express the velocity of pancreatic decomposition in his experiment.

In the equation, $\frac{1}{t^{K'}}\frac{x}{a(a-x)}=K$, if $K'=1$ then the equation, $\frac{1}{t^{K}}\frac{x}{a(a-x)}=K$, results. Therefore it is not necessary to examine the applicability of the latter equation. Such equations as Arrhenius's $\frac{x}{t}=K$, Matsuyama and Nakamura's $\frac{x}{t^{\frac{1}{3}}}=K$, have already been refuted[58] together with the Schütz law, $\frac{x}{\sqrt{t}}=K$. The author undertook to find some equation which would be generally applicable at least to the data of pancreatic decomposition. Various equations were formulated, some of which was expected to show satisfactory application. Among these formulated equations, three ones, $\frac{1}{t}\log t.\log\frac{a}{a-x}=K$, $\frac{1}{\sqrt{t}}\log\frac{a}{a-x}=K$ and $\frac{1}{\sqrt{t}\cdot\log t}\log\frac{a}{a-x}=K$ gave comparatively near values of K as shown in TABLES LXXXI and LXXXII. The values of $\frac{1}{\sqrt{t}}\log\frac{a}{a-x}$ are pretty uniform for the data of glycinin obtained with pancreatin, 1/200 solution. It was nearly the same, for the data of the denatured glycinin. But, for the data gained by pancreatin, 1/800 solution, the value of $\frac{1}{\sqrt{t}}\log\frac{a}{a-x}$ was

One has many reports which evaluate mistakenly the applicability of some equation judging only the nearness of the values of K. But in some cases, each difference between the experimental and calculated values is unexpectedly large, even when the values of K of an equation are comparatively near one another, while in the case of some other equation, each difference is comparatively small for the same data which is obtained by the mean value of K averaged from the values of K which are distinctly not near one another. Therefore, in order to evaluate the applicability of an equation, or to determine the equation of the better applicability, the uniformity of the values of K or the comparison of the uniformities of the values of K of different equations has not so important meaning. It is necessary to see or to make comparison regarding 1) the magnitude of each difference between the experimental and calculated values, or 2) the value of each probable error.

not very constant, while the value of $\frac{1}{\sqrt{t}\cdot\log t}\log\frac{a}{a-x}$ was almost constant except in the case of $t=15$. Substituting the mean value of K, the values of t and a (100) in the two respective equations, then the calculated value of x can be found. Each calculated value of x here obtained is compared with the experimental value in TABLES LXXXIII and LXXXIV.

TABLE LXXXI

The values of K of $\frac{1}{t}\log t\cdot\log\frac{a}{a-x}=K$, $\frac{1}{\sqrt{t}}\log\frac{a}{a-x}=K$ and $\frac{1}{\sqrt{t}\cdot\log t}\log\frac{a}{a-x}=K$ for the data obtained with pancreatin, 1/200 solution

Time (mins.)	Glycinin			
	x	$\frac{1}{t}\log t\cdot\log\frac{a}{a-x}$	$\frac{1}{\sqrt{t}}\log\frac{a}{a-x}$	$\frac{1}{\sqrt{t}\cdot\log t}\log\frac{a}{a-x}$
15	50.08	0.02366	0.07791	0.06624
30	50.62	0.02061	0.07641	0.05173
45	69.07	0.01872	0.07606	0.04601
60	72.52	0.01724	0.07510	0.04223
90	81.25	0.01579	0.07663	0.03921
120	86.88	0.01528	0.08052	0.03873
180	90.70	0.01292	0.07688	0.03409
240	95.24	0.01311	0.08536	0.03586
			mean 0.07811	

Time (mins.)	Denat. glycinin			
	x	$\frac{1}{t}\log t\cdot\log\frac{a}{a-x}$	$\frac{1}{\sqrt{t}}\log\frac{a}{a-x}$	$\frac{1}{\sqrt{t}\cdot\log t}\log\frac{a}{a-x}$
15	51.80	0.02485	0.08183	0.06958
30	66.57	0.02342	0.08688	0.05882
45	72.22	0.02044	0.08293	0.05016
60	83.20	0.02296	0.10001	0.05624
90	90.08	0.02179	0.10578	0.05413
120	94.64	0.02202	0.11601	0.05580
180	95.85	0.01731	0.10300	0.04567
240	96.27	0.01417	0.09220	0.03874
300	96.83	0.01238	0.08654	0.03494
			mean 0.09502	

TABLE LXXXII

The values of K of $\frac{1}{t}\log t\cdot\log\frac{a}{a-x}=K$, $\frac{1}{\sqrt{t}}\log\frac{a}{a-x}=K$ and $\frac{1}{\sqrt{t}\cdot\log t}\log\frac{a}{a-x}=K$ for the data obtained with pancreatin, 1/800 solution

Time (mins.)	Glycinin				Denat. glycinin			
	x	$\frac{1}{t}\log t\cdot\log\frac{a}{a-x}$	$\frac{1}{\sqrt{t}}\log\frac{a}{a-x}$	$\frac{1}{\sqrt{t}\log t}\log\frac{a}{a-x}$	x	$\frac{1}{t}\log t\cdot\log\frac{a}{a-x}$	$\frac{1}{\sqrt{t}}\log\frac{a}{a-x}$	$\frac{1}{\sqrt{t}\log t}\log\frac{a}{a-x}$
15	13.26	0.04844	0.01595	0.01356	13.42	0.04907	0.01616	0.01374
30	19.81	0.04721	0.01751	0.01185	20.09	0.04796	0.01778	0.01204
55	26.00	0.04804	0.01949	0.01179	24.92	0.04573	0.01856	0.01123
60	30.10	0.04609	0.02008	0.01130	32.47	0.05053	0.02201	0.01238
90	39.64	0.04761	0.02311	0.01183	43.00	0.05301	0.02573	0.01317
120	44.88	0.04482	0.02361	0.01136	53.16	0.05707	0.03007	0.01446
180	54.97	0.04341	0.02583	0.01145	64.79	0.05680	0.03379	0.01498
240	62.72	0.04250	0.02766	0.01162	76.42	0.06223	0.04050	0.01702
300	66.96	0.03971	0.02777	0.01121	81.98	0.06145	0.04297	0.01735
360	75.76	0.04367	0.03241	0.01268	86.23	0.06114	0.04538	0.01775
				mean 0.01187				mean 0.01441

TABLE LXXXIII

The calculated values of x obtained by using each mean value of K in application of equation, $\frac{1}{\sqrt{t}} \log \frac{a}{a-x} = K$, for the data obtained with pancreatin, $^1/_{200}$ solution

	Time (mins)	15	30	45	60	90	120	180	240	300	mean
Glycinin	Exp. value	50.08	61.85	69.07	73.80	81.25	86.88	90.70	95.24	—	—
	Calc. value	50.17	62.66	70.07	75.17	81.85	86.06	91.05	93.84	—	—
	Diff.	− 0.09	− 0.81	− 1.00	− 1.37	− 0.60	+ 0.82	− 0.35	+ 1.40	—	0.81
Denat. glycinin	Exp. value	51.80	66.57	72.22	83.20	90.08	94.64	95.85	96.27	96.83	—
	Calc. value	57.15	69.83	76.95	81.64	87.45	90.90	94.69	96.63	97.74	—
	Diff.	− 5.35	− 3.26	− 4.73	+ 1.56	+ 2.63	+ 3.74	+ 1.16	+ 0.36	− 0.91	2.63

TABLE LXXXIV

The calculated values of x obtained by using the mean value of K in application of equation $\frac{1}{\sqrt{t} \cdot \log t} \frac{a}{a-x} = K$, for the data obtained with pancreatin, $^1/_{800}$ solution

	Time (mins.)	15	30	45	60	90	120	180	240	300	360	mean
Glycinin	Exp. value	13.26	19.81	26.00	30.10	39.64	44.88	54.97	62.72	66.96	75.76	—
	Calc. value	11.71	19.84	26.14	31.37	39.75	44.61	56.27	63.50	69.05	73.44	—
	Diff.	+ 1.55	− 0.03	− 0.14	− 1.27	− 0.11	+ 0.27	− 1.30	− 0.78	− 2.09	+ 2.32	0.99
Denat. glycinin	Exp. value	13.42	20.09	24.47	32.47	43.00	53.16	64.79	76.42	81.98	86.23	—
	Calc. value	14.03	23.54	30.78	36.68	45.95	53.03	63.36	70.58	75.91	80.00	—
	Diff.	− 0.61	− 3.45	− 6.31	− 4.21	− 2.95	+ 0.13	+ 1.43	+ 5.84	+ 6.07	+ 6.23	3.72

Each mean difference in the above two tables will be extracted and compared in the following table with the value of probable error which was already given in the application of the equation, $\frac{1}{t^{K'}} \frac{x}{a(a-x)} = K$. Any of the above three experimental equations will

be denoted by I, and $\frac{1}{t^{K'}} \frac{x}{a(a-x)} = K$ by II. The sigin ± is omitted.

TABLE LXXXV

The values of mean difference and probable error in application of equations I and II

	Pancreatin, 1/200 sol.		Pancreatin, 1/800 sol.	
	I	II	I	II
Glycinin	0.81	2.09	0.99	1.38
Denat. glycinin	2.63	2.04	3.63	2.29

As clearly seen in TABLES LXXXI and LXXXII, the experimental equations, $\frac{1}{\sqrt{t}} \log \frac{a}{a-x} = K$ and $\frac{1}{\sqrt{t} \cdot \log t} \log \frac{a}{a-x} = K$, show pretty high applicability, however, if each mean difference in the applied results of these equations is compared with the probable error in case of the equation, $\frac{1}{t^{K'}} \frac{x}{a(a-x)} = K$, the superiority of applicability is not decided between I and II, viz., equation I is better for the data obtained with glycinin, but worse for the data obtained with the denatured glycinin.

3. Application of a newly formulated equation

As above demonstrated, the author was able to find some experimental equations with pretty high applicability to the data of pancreatic decomposition of the normal and denatured glycinins. But for different results obtained with each different amount of pancreatin, the best applicable equation was not constant. In short, each above experimental equation can be recognized as applicable well to some special data. Such an equation has almost no value, even if the applicability is very high in some special case.

One has great many kinds of equation which have been hitherto proposed. But most of them seem to be not generally applicable. To find an equation which is applicable well at least to the data obtained

by the author, he examined the applicability of many equations. The result was not satisfactory. He turned his mind on that each equation which was applied and gave comparatively uniform values of K has a form closely resembling that of the equation of the first order, $\frac{1}{t}\log\frac{a}{a-x}=K$. Here the author considered that, if there exists a satisfactory equation for his data, it must have a close relation to $\frac{1}{t}\log\frac{a}{a-x}=K$ which is a theoretical equation. As Nakamura's equation becomes the equation of the second order if $K'=1$, the present author considered his equation as a general form of the equation of the second order. As some equations which resembled the equation of the first order had been far better applicable than $\frac{1}{t^{K'}}\frac{x}{a(a-x)}=K$ in some cases, the author formulated an equation, $\frac{1}{t^{K'}}\log\frac{a}{a-x}=K$ as a general form of the equation of the first order. The new equation will be examined below. In applying this equation, a logarithmic form of the equation,

$$\log K+K'\log t=\log\left(\log\frac{a}{a-x}\right)$$

is made, in which t denotes decomposition time, x the quantity of substrate decomposed, a the quantity of substrate taken, and if x be denoted in percentage, a is 100. From the normal equations, the values of K and K' are calculated. The calculated values of x are found as in the case of equation, $\frac{1}{t^{K'}}\frac{x}{a(a-x)}=K$. The calculated results will be given in TABLES LXXXVI—LXXXVIII. By the formula, $\pm 0.6745\sqrt{\frac{\Sigma\Delta^2}{n-1}}$, each probable error was calculated which will be compared with that obtained in applying the equation, $\frac{1}{t^{K'}}\frac{x}{a(a-x)}=K$. The results will be given in TABLE LXXXIX where the new equation is represented by I and $\frac{1}{t^{K'}}\frac{x}{a(a-x)}=K$ by II, and the sign of each probable error is omitted. As shown in the following tables, $\frac{1}{t^{K'}}\log\frac{a}{a-x}=K$ was surely more satisfactory than $\frac{1}{t^{K'}}\frac{x}{a(a-x)}=K$ in all cases.

TABLE LXXXVI

The values of K and K' in application of equation, $\frac{1}{t^{K'}}\log\frac{a}{a-x}=K$, for the data obtained with pancreatin

	Pancreatin, 1/200 sol.			Pancratin, 1/800 sol.		
	Range of decomp.	K	K'	Range of decomp.	K	K'
Glycinin	50.08—95.24	0.091598	0.46364	13.26—75.76	0.0087704	0.70963
Denat. glycinin	51.80—96.27	0.066604	0.58398	13.42—86.23	0.0054136	0.85531

It can be sure that the pancreatic decomposition of the normal and denatured glycinins follows the equation, $\frac{1}{t^{K'}}\log\frac{a}{a-x}=K$, by the fact that the probable error is satisfactorily small and that the occurrence of a positive or negative difference between the experimental and calculated values is satisfactory. It is an interesting problem that the best applicable equation are not the same in the cases of decomposition by pancreatin and of that by both pepsin and papain.

From the above examination, it was ascertained that, to the data of both results of decomposition of the normal and denatured glycinins by either pepsin or papain, Nakamura's equation, $\frac{1}{t^{K'}}\frac{x}{a(a-x)}=K$, was applicable well while to the data obtained with pancreatin a newly formulated equation, $\frac{1}{t^{K'}}\log\frac{a}{a-x}=K$, was satisfactorily applicable. The difference between both proteins observed from the results of application of equations will be discussed later on.

(ii) Studies on the enzymic decomposition taking the relative quantity of enzyme as a variable

The author undertook further a study of enzymic decomposition of the normal and denatured glycinins taking the relative quantity of enzyme as a variable. From the results of this kind of experiment, he proposed to compare the decomposition degrees of both the proteins

TABLE LXXXVII

The calculated values for pancreatin, 1/200 solution obtained by the values of K and K' given in TABLE LXXXVI

	Time (mins.)	15	30	45	60	90	120	180	240	Summetion
Glycinin	Exp. value	50.08	61.85	69.07	73.80	81.25	86.88	90.70	95.24	
	Calc. value	52.30	63.97	70.83	75.53	81.71	85.65	90.39	93.17	
	Diff. Δ	− 2.22	− 2.12	− 1.76	− 1.73	− 0.46	+ 1.23	+ 0.31	+ 2.07	+3.61−8.29=−4.68
	Δ^2	4.9284	4.4944	3.0976	2.9929	0.2116	1.5129	0.0961	4.2849	21.6188
Denat. glycinin	Exp. value	51.80	66.57	72.22	83.20	90.08	94.64	95.85	96.27	
	Calc. value	52.56	67.22	75.74	81.89	88.03	91.88	95.85	97.68	
	Diff. Δ	− 0.76	− 0.65	− 3.52	+ 1.31	+2.05	+2.76	0.00	− 1.41	+6.12−6.34=−0.22
	Δ^2	0.5776	0.4225	12.3904	1.7161	4.2025	7.6176	0.0000	1.9881	28.9148

TABLE LXXXVIII

The results of calculation obtained by using the values of K and K' in TABLE LXXXVI for the data obtained with pancreatin, 1/800 solution

	Time (mins.)	15	30	45	60	90	120	180	240	300	360	Summation
Glycinin	Exp. value	13.26	19.81	26.00	30.10	39.64	44.88	54.97	62.72	66.96	75.76	
	Calc. value	12.86	20.16	25.93	30.80	38.80	45.25	55.20	62.64	68.45	73.18	
	Diff. Δ	+ 0.40	− 0.35	+0.07	− 0.70	+ 0.84	− 0.37	− 0.23	+ 0.08	− 1.49	+2.58	+3.97−3.14=+0.83
	Δ^2	0.1600	0.1225	0.0049	0.4900	0.7056	0.1369	0.0529	0.0064	2.2201	6.6564	10.5557
Denat. glycinin	Exp. value	13.42	20.09	24.92	32.47	43.00	53.16	64.79	76.42	81.98	86.23	
	Calc. value	11.87	20.43	27.63	33.87	44.29	52.68	65.30	74.17	80.57	87.16	
	Diff. Δ	+1.55	− 0.34	− 2.71	− 1.40	− 1.29	+0.48	− 0.51	+2.25	+1.41	− 0.93	+5.69−7.18=−1.49
	Δ^2	2.4025	0.1156	7.3441	1.9600	1.6641	0.2304	0.2601	5.0625	1.6881	0.8649	21.5923

TABLE LXXXIX

The values of probable error in the application of equations I and II

	Pancreatin, 1/200 sol.		Pancreatin, 1/800 sol.	
	I	II	I	II
Glycinin	1.18	2.09	0.73	1.38
Denat. glycinin	1.37	2.04	1.02	2.29

and to try moreover to find some equation that might be generally applicable to the data of such experiment.

A. Experiment

The methods or conditions in this experiment were almost the same as previously described under (i).

(1) Decomposition by pepsin

Quantities of 0.1 to 7 cc. of 1/200 water solution of pepsin were taken. Buffer solutions of P_H 1.2 and 1.8 were used. The process is briefly written as follows:

10 cc. of protein solution (0.6 g. in 100 cc. of 1/100 N NaOH)+ 12.5 cc. of Clark and Lubs's buffer solution+7.5 cc. (H_2O and pepsin solution); digested for 2 hours at 40°C.; + 3 cc. of 1/5 N NaOH + 7 cc. of 20% CCl_3 COOH; N was determined with 5 cc. of the filtrate.

The results are given in TABLE XC.

TABLE XC

The results of decomposition of proteins by pepsin

E	0.1	0.25	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0
x (%) for P_H 1.2 Glycinin	8.16	17.23	25.76	39.90	54.86	64.66	75.57	80.45	82.22	84.43
x (%) for P_H 1.2 Denat. glycinin	4.20	7.12	11.14	23.03	35.21	43.18	50.12	55.15	63.70	70.52
x (%) for P_H 1.8 Glycinin	7.56	18.14	27.81	40.58	57.43	66.50	73.15	78.08	82.52	84.63
x (%) for P_H 1.8 Denat. glycinin	2.58	7.58	11.36	22.73	37.64	42.64	52.12	59.09	65.15	69.50

The normal glycinin was decomposed always more rapidly than the denatured. Between the mediums of P_H 1.2 and 1.8, no remarkable difference was observed.

(2) Decomposition by pancreatin

Quantities of 0.1 to 7.0 cc. of the filtrate of either $^1/_{1000}$ or $^1/_{500}$ water solution of pancreatin were used. The process is briefly written as follows:

10 cc. of protein solution (0.6 g. in 100 cc. of $^1/_{100}$ N NaOH)+ 12.5 cc. of Clark and Lubs's buffer solution of P_H 7.4+7.5 cc. of water and pancreatin solution; digested for 2 hours at 40°C.; + 3 cc. of $^1/_{10}$ N HCl + 7 cc. of 20% CCl_3COOH; N in 5 cc. of the filtrate was determined.

The results will be given in TABLE XCI. The decomposition of the denatured glycinin was remarkably more rapid than that of the normal in all cases.

TABLE XCI

The results of decomposition of proteins by pancreatin, $^1/_{1000}$ and $^1/_{500}$ solutions

E		0.1	0.25	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0
$^1/_{1000}$ sol. x (%)	Glycinin	1.79	2.86	5.00	10.00	16.43	22.32	28.04	33.93	38.57	42.14
	Denat. glycinin	5.39	14.15	23.94	38.21	60.61	74.75	80.81	85.85	91.25	92.25
$^1/_{500}$ sol. x (%)	Glycinin	3.11	5.00	8.93	15.36	25.54	35.00	43.93	49.64	55.00	60.03
	Denat. glycinin	9.09	22.56	39.39	56.90	77.44	87.54	94.28	97.64	—	—

(3) Decomposition by papain

Quantities of 0.1 to 7.0 cc. of $^1/_{200}$ or $^1/_{100}$ water solution of papain were used. The process is briefly written as follows:

10 cc. of protein solution (0.6 g. in 100 cc. of $^1/_{100}$ N NaOH)+ 12.5 cc. of Clark and Lub's buffer solution of P_H 6.2+1 cc. of enzyme solution ($^1/_{200}$ or $^1/_{100}$ water solution); digested for 2 hours at 40°C.; + 3 cc. of $^1/_{10}$ N HCl + 7 cc. of 20% CCl_3COOH; N in 5 cc. of the filtrate was determined.

The results are given in TABLE XCII.

TABLE XCII

The results of decomposition of proteins by papain, 1/200 and 1/100 solutions

E		0.1	0.25	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0
1/200 sol. x(%)	Glycinin	10.00	15.71	23.75	33.57	46.07	53.21	56.79	60.36	65.36	68.93
	Denat. glycinin	12.13	18.52	27.66	38.80	52.04	58.35	65.36	71.05	74.75	78.45
1/100 sol. x(%)	Glycinin	10.71	21.43	32.86	47.86	61.07	69.96	73.04	77.14	80.89	84.50
	Denat. glycinin	13.47	23.23	37.71	—	69 36	76.77	83.50	87.20	91.75	94.28

The decomposition of the denatured glycinin was more rapid than that of the normal.

From the results of above experiment, it becomes clear that, in the cases where the quantity of enzyme be taken as a variable, the decomposition by pepsin is more rapid in the normal glycinin, but that by either pancreatin or papain is speedier in the denatured glycinin.

B. Application of equations to experimental data

Schütz may be the first proposer of an equation in the study of enzymic decomposition. He made a study on peptic digestion with egg-white taking the relative quantity of enzyme as a variable, from the data of which he formulated "the Schütz law," $\frac{x}{\sqrt{E}}=K$. Until recently, the equation had been one of widely accepted equations. Besides this, many different equations have been proposed by many other investigators. The present author examined the principal equations and came to the conclusion[53] that each of them was usually applicable to the data of some special result and was not generally applicable to the data of decomposition even by an enzyme except the Schütz equation, $\frac{x}{\sqrt{E}}=K$, for the data obtained with dilute solution of pepsin. The present author examined the applicability of this equation which had ever been accepted by the present author for the data in Schütz's original paper. Recently he ascertained from the examination of other published data that the Schütz equation was not satisfactory even in the case of dilute peptic solution. This will be made clear later in another paper. Therfore he endeavoured to find some other equation that would be satisfactorily applicable to the data of at least peptic

digestion. The author proved already that either $\frac{1}{t^{K'}} \log \frac{a}{a-x} = K$ or $\frac{1}{t^{K'}} \frac{x}{a(a-x)} = K$ was satisfactorily applicable to the data of any result obtained with pepsin, pancreatin or papain in the experiment with time as a variable. He tried the application of these two equations to the data obtained in the experiment with the relative quantity of enzyme as a variable. Substitute E to t in the above equations, then the equations,

$$\frac{1}{E^{K'}} \log \frac{a}{a-x} = K \quad \text{I}$$

and

$$\frac{1}{E^{K'}} \frac{x}{a(a-x)} = K \quad \text{II}$$

result, where E denotes the relative quantity of enzyme. The meaning of a and x are the same with that in the original equations. The author applied the above two equations to his data and examined their applicability.

1. Applications to the data of peptic decomposition

The values of K and K' of equations I and II for the data of peptic decomposition are given in the following table.

TABLE XCIII

The values of K and K' of equations I and II to the data obtained in decomposition by pepsin

		P_H 1.2			P_H 1.8		
		Range of decomposition	K	K'	Range of decomposition	K	K'
Glycinin	I	8.16—84.43	0.21125	0.72447	7.56—84.63	0.213395	0.72076
	II	,,	0.0074358	0.98223	,,	0.0075342	0.96567
Denat. glycinin	I	4.20—70.52	0.11644	0.79490	2.58—69.50	0.09899	0.86919
	II	,,	0.0043023	0.60826	,,	0.0028623	1.01402

By the substitution of the values of K, K' and E in the original equations, the calculated values of x can be found as given in TABLES

TABLE XCIV

The results of application of equations I and II to the data obtained in decomposition by pepsin, P_H. 1.2

		E	0.1	0.25	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	Summation
Glycinin	I	Exp. value	8.16	17.23	25.76	39.90	54.86	64.66	75.57	80.45	82.22	84.43	
		Calc. value	8.76	16.32	25.50	38.52	55.24	65.98	73.50	78.52	83.16	86.36	
		Diff. Δ	−0.60	+0.91	+0.26	+1.38	−0.38	−1.32	+2.07	+1.93	−0.94	−1.93	+6.55−5.17=+1.38
		Δ^2	0.3600	0.8281	0.0676	1.9044	0.1444	1.7424	4.2849	3.7249	0.8836	3.7249	17.6652
	II	Exp. value	8.16	17.23	25.76	39.90	54.86	64.66	75.57	80.45	82.22	84.43	
		Calc. value	7.19	16.00	27.35	42.65	59.50	68.63	74.37	78.32	81.21	83.40	
		Diff. Δ	+0.97	+1.23	−1.59	−2.75	−4.64	−3.97	+1.20	+2.13	+1.01	+1.03	+7.57−12.95=−5.38
		Δ^2	0.9409	1.5129	2.5281	7.5625	21.5296	15.7609	1.4400	4.5369	1.0201	1.0609	57.8928
Denat. glycinin	I	Exp. value	4.20	7.81	11.14	23.03	35.21	43.18	50.12	55.15	63.70	70.52	
		Calc. value	3.76	7.12	12.88	21.28	33.97	43.61	51.33	57.68	62.99	67.49	
		Diff. Δ	+0.44	−0.69	−1.74	+1.75	+1.24	−0.43	−1.21	−2.53	+0.71	+3.03	+7.17−6.60=+0.57
		Δ^2	0.1936	0.4761	3.0276	3.0625	1.5376	0.1849	1.4641	6.4009	0.5041	9.1809	26.0323
	II	Exp. value	4.20	7.12	11.14	23.03	35.21	43.18	50.12	55.15	63.70	70.52	
		Calc. value	9.59	15.62	22.01	30.08	39.55	45.63	49.99	53.38	56.13	58.42	
		Diff. Δ	−5.39	−8.50	−10.87	−7.05	−4.34	−2.45	+0.13	+1.77	+7.57	+12.10	+21.57−38.60=−17.03
		Δ^2	29.0521	72.2500	118.1569	49.7025	18.8356	6.0025	0.0169	3.1329	57.3049	146.4100	500.8643

TABLE XCV

The results of application of equations I and II to the data obtained in decomposition by pepsin, P_H 1.8

		E	0.1	0.25	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	Summation
Glycinin	I	Exp. value	7.56	18.14	27.81	40.58	57.43	66.50	73.15	78.08	82.52	84.63	
		Calc. value	8.93	16.55	25.74	38.82	55.57	66.20	73.67	79.14	83.26	86.43	
		Diff. Δ	− 1.37	+ 1.59	+ 2.07	+ 1.76	+ 1.86	+ 0.30	− 0.52	− 1.06	− 0.74	− 1.80	+7.58−5.49=−2.09
		Δ^2	1.8769	2.5281	4.2849	3.0976	3.4596	0.0900	0.2704	1.1236	0.5476	3.2400	20.5187
	II	Exp. value	7.56	18.14	27.81	40.58	57.43	66.50	73.15	78.08	82.52	84.63	
		Calc. value	7.54	16.50	27.84	42.97	59.54	68.52	4.187	78.09	80.96	83.15	
		Diff. Δ	+ 0.02	+ 1.64	− 0.03	− 2.39	− 2.11	− 2.02	− 1.03	− 0.01	+ 1.56	+ 1.48	+4.70−7.59=−2.89
		Δ^2	0.0004	2.6896	0.0009	5.7121	4.4521	4.0804	1.0609	0.0001	2.4336	2.1904	22.6205
Denat. glycinin	I	Exp. value	2.58	7.58	11.36	22.73	37.64	42.67	52.12	59.09	65.15	69.50	
		Calc. value	3.04	6.60	11.74	20.38	34.05	44.69	53.26	60.28	66.10	70.97	
		Diff. Δ	− 0.46	+ 0.98	− 0.38	+ 2.35	+ 3.59	− 2.02	− 1.14	− 1.19	− 0.95	− 1.47	+6.92−7.61=−0.69
		Δ^2	0.2116	0.9604	0.1444	5.5225	12.8881	4.0804	1.2996	1.4161	0.9025	2.1609	29.5865
	II	Exp. value	2.58	7.58	11.36	22.73	37.64	42.67	52.12	59.09	65.15	69.50	
		Calc. value	2.70	6.56	12.41	22.25	36.63	46.58	53.86	59.41	63.78	67.31	
		Diff. Δ	− 0.12	+ 1.02	− 1.05	+ 0.48	+ 1.01	− 3.91	− 1.74	− 0.32	+ 1.37	+ 2.19	+6.07−7.14=−1.07
		Δ^2	0.0144	1.0404	1.1025	0.2304	1.0201	15.2881	3.0276	0.1024	1.8769	4 796	28.4989

TABLE XCVI

The values of probable errors calculated from the data given in TABLES XCIV and XCV

	P_H 1.2		P_H 1.8	
	I	II	I	II
Glycinin	±0.95	±1.71	±1.02	±1.07
Denat. glycinin	±1.15	±5.03	±5.03	±1.20

XCIV and XCV. To compare the applicabilities of the two equations, each probable value was calculated with results as shown in TABLE XCVI.

As seen in these tables it is recognized that to the data obtained at P_H 1.2 equation I is better applicable than II, while to the data at P_H 1.8 it is the contrary. According to the present author's consideration, if the decomposition was run between one definite substrate and one definite enzyme with several results, only one equation must be sufficiently applicable to the data of any of the results. In other words, only one equation shall be constantly best applicable to the data of any result obtained with an enzyme and the substrate of one kind. The hydrogen ion concentration gives much influence on the activity of pepsin or the velocity of decomposition. However, it is considered that the fundamental mechanism between a certain substrate and pepsin may not be changed by the difference of hydrogen ion concentration. In other words, if the velocity of peptic decomposition of a protein be expressed by either equation I or II, only one of them must be fundamentally sufficient and the other apparently or not sufficient. From this point of view, only one of them, I or II, can be generally applicable to the data of any result obtained in both cases of P_H 1.2 and 1.8. By the examination of these equations for the data of peptic decomposition obtained by several investigators it was ascertained that equation I was applicable well in any case. The author considered that the satisfactory equation must be I also for the data obtained at P_H 1.8. He has often such experiences that the applicabilities of $\frac{1}{t^{K'}}\log\frac{a}{a-x}=K$ and $\frac{1}{t^{K'}}\frac{x}{a(a-x)}=K$ are almost

the same for the data of some special result. In Fig. I, let curve A be fundamentally expressed by equation I and curve B by II. Such a curve as C which is situated between A and B is often expressed by either of the two equations, I or II, with negligible difference of applicability. Yet the fundamental nature of curve C must belong to only one of them. Consider that curve C represents the data obtained at P_H 1.8, then it may be possible that although the probable error calculated in applying equation II is smaller than that calculated in applying I, the latter can be always not so large in the calculation for different ranges of E. The range of the relative quantities of pepsin was between 0.1 and 7. If an equation be fundamentally applicable, the value of either K or K' must be about constant in the calculated results for different ranges of E. From this point of view, the author calculated the values of K and K' omitting the datum for the least quantity of E, 0.1, and compared the calcaluted result with that for the whole data in both cases of P_H 1.2 and P_H 1.8. The values of constants for limitted data will be given in TABLE XCVII. The result will be compared with that obtained from the whole data, E (0.1—7.0), in TABLE XCVIII. From this table, a remarkable point can be observed, that is, in the

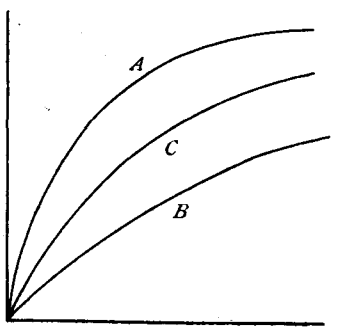


Fig. I

TABLE XCVII

The values of K and K' of equations I and II to the data obtained in decomposition by pepsin omitting the datum of the lowest value of E

	P_H 1.2			P_H 1.8		
	Range of decomposition	K	K'	Range of decomposition	K	K'
Glycinin I	17.23—84.43	0.216185	0.70390	18.14—84.63	0.225075	0.6732c
Glycinin II	,,	0.0073375	0.97952	,,	0.007408	0.9667c
Denat. glycinin I	7.12—70.52	0.10039	0.82559	7.58—69.50	0.10413	0.82401
Denat. glycinin II	,,	0.0027788	0.99842	,,	c.0028955	1.00155

TABLE XCVIII

The values of K and K' of equations I and II for the data of different range of E in petpic decomposition

		P_H 1.2			P_H 1.8		
		Range of E	K	K'	Range of E	K	K'
Glycinin	I	0.1—7.0	0.21125	0.72447	0.1—7.0	0.21339	0.72078
	I	0.25—7.0	0.216185	0.70390	0.25—7.0	0.225075	0.67320
	II	0.1—7.0	0.0073897	0.95989	0.1—7.0	0.0075342	0.96567
	II	0.25—7.0	0.0073375	0.97952	0.25—7.0	0.0075273	0.96649
Denat. glycinin	I	0.1—7.0	0.11644	0.79490	0.1—7.0	0.09890	0.86919
	I	0.25—7.0	0.10039	0.82559	0.25—7.0	0.10413	0.82401
	II	0.1—7.0	0.0043023	0.60826	0.1—7.0	0.0028623	1.01402
	II	0.25—7.0	0.0027788	0.99842	0.25—7.0	0.0028955	1.00155

cases of both proteins decomposed at P_H 1.2, the values of either K or K' of equation I in the two results for E (0.1—7.0) and E (0.25—7.0), are almost equal, while those of equation II differ pretty largely from eath other. In the cases of P_H 1.8, the result is the contrary, i. e., the values of each constant of equation II are almost equal (0.0075342 : 0.0075273, 0.96567:0.96649 for glycinin), while the values of constant of equation I differ remarkably from each other in both calculated results. By using the values of K and K' which had been found in the case of E (0.25—7.0), the calculated values of x were found as given in TABLE XCIX and C. The probable error was found and tabulated with that in the case of E (0.1–7.0) in TABLE CI. The sign $\pm$ is not written. From the results given in this table the author considers that equation I is applicable fundamentally also to the data obtained at P_H 1.8.

By the above examination, it is made certain that the peptic decomposition of both normal and denatured glycinins taking the relative quantity of enzyme as a variable, follows the equation, $\frac{1}{E^{K'}}\log\frac{a}{a-x}=K$. At the same time, it may be sure that only one equation is funda-

TABEE XCIX

The results of application of equations I and II obtained by using the values of K and K' for P_H 1.2 given in TABLE XCVII

		E	0.25	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	Summation
Glycinin	I	Exp. value	17.23	25.76	39.90	54.86	64.66	75.57	80.45	82.22	84.43	
		Calc. value	17.10	26.33	39.23	55.56	65.99	73.31	78.68	82.74	85.89	
		Diff. Δ	+0.13	−0.57	+0.67	−0.70	−1.33	+2.26	+1.77	−0.52	−1.46	+4.83−4.58=+0.25
		Δ^2	0.0169	0.3249	0.4489	0.4900	1.7689	5.1076	3.1325	0.2704	2.1316	13.7021
	II	Exp. value	17.23	25.76	39.90	54.86	64.66	75.57	80.45	82.22	84.43	
		Calc. value	15.87	27.12	42.32	59.13	68.38	74.05	78.02	80.93	83.15	
		Diff. Δ	+1.36	−1.36	−2.42	−4.27	−3.72	+1.52	+2.43	+1.29	+1.28	+7.88−11.77=−3.89
		Δ^2	1.8496	1.8496	5.8564	18.2329	13.8384	2.3104	5.9049	1.6641	1.6384	53.1447
Denat. glycinin	I	Exp. value	7.12	11.14	23.03	35.21	43.18	50.12	55.15	63.70	70.52	
		Calc. value	7.10	12.23	20.63	33.61	43.59	51.62	58.23	63.75	68.41	
		Diff. Δ	+0.02	−1.09	+2.40	+1.60	−0.41	−1.50	−3.08	−0.05	+2.11	+6.13−6.13=0
		Δ^2	0.0004	1.1881	5.7600	2.5600	0.1681	2.2500	9.4864	0.0025	4.4521	25.8676
	II	Exp. value	7.12	11.14	23.03	35.21	43.18	50.12	55.15	63.70	70.52	
		Calc. value	6.51	12.21	21.75	35.70	45.42	52.59	58.09	62.44	65.98	
		Diff. Δ	+0.61	−1.07	+1.28	−0.49	−2.24	−2.47	−2.94	+1.26	+4.54	+7.69−9.21=−1.52
		Δ^2	0.3721	1.1449	1.6384	0.2401	5.0176	6.1009	8.6436	1.5876	20.6116	45.3568

TABLE C

The results of application of equations I and II obtained by using the values of K and K' for P_H 1.8 given in TABLE XCVIII

		E	0.25	0.5	1.0	2.0	3.0	4.0	5.0	6.0	0.7	Summation
Glycinin	I	Exp. value	18.14	27.81	40.58	57.43	66.50	73.15	78.08	82.52	84.63	
		Calc. value	18.43	28.26	40.49	56.24	66.24	73.23	78.38	82.34	85.35	
		Diff. Δ	−0.29	−0.45	+0.09	+1.19	+0.26	−0.08	−0.30	+0.18	−0.72	+1.72−1.84=−0.12
		Δ^2	0.0841	0.2025	0.0081	1.4161	0.0676	0.0064	0.0900	0.0324	0.5184	2.4256
	II	Exp. value	18.14	27.81	40.58	57.43	66.50	73.15	78.08	82.52	84.63	
		Calc. value	16.47	27.84	42.95	59.49	68.52	74.19	78.10	80.96	83.15	
		Diff. Δ	+1.67	−0.03	−2.37	−2.06	−2.02	−1.04	−0.02	+1.56	+1.48	+4.71−7.54=−2.83
		Δ^2	2.7889	0.0009	5.6169	4.2436	4.0804	1.0816	0.0004	2.4336	2.1904	22.4367
Denat. glycinin	I	Exp. value	7.58	11.36	22.73	37.64	42.67	52.12	59.09	65.15	69.50	
		Calc. value	7.37	12.66	21.32	34.59	44.72	52.83	59.47	64.99	69.63	
		Diff. Δ	+0.21	−1.30	+1.41	+3.05	−2.05	−0.71	−0.38	+0.16	−0.13	+4.83−4.57=+0.26
		Δ^2	0.0441	1.6900	1.9881	9.3025	4.2025	0.5041	0.1444	0.0256	0.0169	17.9182
	II	Exp. value	7.58	11.36	22.73	37.64	42.67	52.12	59.09	65.15	69.50	
		Calc. value	6.75	12.66	22.50	36.75	46.56	53.78	59.21	63.59	67.08	
		Diff. Δ	+0.83	−1.30	+0.23	+0.89	−3.92	−1.66	−0.12	+1.56	+2.42	+5.93−7.00=−1.07
		Δ^2	0.6889	1.6900	0.0529	0.7921	15.3664	2.7556	0.0144	2.4336	5.8564	29.6503

TABLE CI

The values of probable errors in application of equations I and II for different ranges of E

	P_H 1.2		P_H 1.8	
E	0.1–7.0	0.25–7.0	0.1–7.0	0.25–7.0
Glycinin I	0.95	0.88	1.02	0.37
Glycinin II	1.71	1.74	1.07	1.12
Denat. glycinin I	1.15	1.22	1.22	1.01
Denat. glycinin II	5.03	1.61	1.20	1.30

mentally applicable to the data obtained with the substrate of one kind and an enzyme.

From the calculated results of peptic digestion of both normal and denatured glycinins, the values of K and K' of equation I in the case of E (0.25–7.0) for the two proteins are compared with each other as follows:

TABLE CII

The values of K and K' of the applicable equation for the data of range of E: 0.25–7.0 obtained in decomposition of proteins by pepsin

	P_H 1.2			P_H 1.8		
	Range of decomp.	K	K'	Range of decomp.	K	K'
Glycinin	17.23–84.43	0.216185	0.70390	18.14–84.63	0.225075	0.67320
Danat. glycinin	7.13–7.052	0.10039	0.82559	7.58–69.50	0.10413	0.82401

As already shown, the percentage of decomposition is always greater in the normal glycinin than in the denatured. Here in the applied results of $\frac{1}{E^{K'}} \log \frac{a}{a-x} = K$, K is always larger, and K' is always smaller in the former protein than in the latter.

2. Application to the data of pancreatic decomposition

The results of application of equations I and II to the data of pancreatic decomposition are given in TABLES CIII and CIV.

TABLE CIII

The results of application of equations I and II to the data obtained in decomposition by pancreatin, 1/1000 solution

		E	0.1	0.25	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	Summation
Glycinin	I	Exp. value	1.79	2.86	5.00	10.00	16.43	22.33	28.04	33.93	38.57	42.14	
		Calc. value	1.50	3.20	5.66	9.91	17.02	23.07	28.38	33.15	37.46	41.37	
	$K = 0.045296$	Diff. Δ	+0.29	−0.34	−0.66	+0.09	−0.59	−0.74	−0.34	+0.78	+1.12	+0.77	+3.05−2.67=+0.38
	$K' = 0.83922$	Δ^2	0.0841	0.1156	0.4356	0.0081	0.3481	0.5476	0.1156	0.6084	1.2544	0.5929	41.104
	II	Exp. value	1.79	2.86	5.00	10.00	16.43	22.33	28.04	33.93	38.57	42.14	
		Calc. value	1.42	3.19	5.79	10.30	17.65	23.60	28.58	32.85	36.57	39.85	
	$K = 0.0011148$	Diff. Δ	+0.37	−0.33	−0.79	−0.30	−1.22	−1.27	−0.54	+1.08	+2.00	+2.29	+5.74−4.45=+1.29
	$K' = 0.90081$	Δ^2	0.1369	0.1089	0.6241	0.0900	1.4884	1.6129	0.2916	1.1664	4.0000	5.2441	14.7633
Denat. glycinin	I	Exp. value	5.39	14.15	23.94	38.21	60.61	74.76	80.81	85.85	91.25	92.25	
		Calc. value	6.01	13.10	22.95	38.39	59.32	72.53	81.19	86.99	90.93	93.63	
	$K = 0.210315$	Diff. Δ	−0.62	+1.05	+0.99	−0.18	+1.29	+2.22	−0.38	−1.14	+0.32	−1.38	+5.87−3.70=−2.17
	$K' = 0.89325$	Δ^2	0.3844	1.1025	0.9801	0.0324	1.6641	4.9284	0.1444	1.2996	0.1024	1.9044	12.5427
	II	Exp. value	5.39	14.15	23.94	38.21	60.61	74.75	80.81	85.85	91.25	92.25	
		Calc. value	4.46	12.78	25.82	45.27	66.27	76.52	82.36	86.05	88.56	90.39	
	$K = 0.0082706$	Diff. Δ	+0.93	+1.37	−1.88	−7.06	−5.66	−1.77	−1.55	−0.20	+2.69	+1.86	+6.85−18.12=−11.27
	$K' = 1.124833$	Δ^2	0.8649	1.8769	3.5344	49.8436	32.0356	3.1329	2.4025	0.0400	7.2361	3.4596	104.4265

TABLE CIV

The results of application of equations I and II to the data obtained in decomposition by pancreatin, 1/500 solution

		E	0.1	0.25	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	Summation
Glycinin	I	Exp. value	3.11	5.00	8.93	15.36	25.54	35.00	43.93	49.64	55.00	60.03	
		Calc. value	2.64	5.53	9.56	16.26	26.93	35.45	42.56	48.63	53.86	58.43	
	$K = 0.07087$	Diff. Δ	+0.47	−0.53	−0.63	−0.90	−1.39	−0.45	+1.37	+1.01	+1.14	+1.60	+5.59−3.90=+1.69
	$K' = 0.82146$	Δ^2	0.2209	0.2809	0.3969	0.8100	1.9321	0.2025	1.8769	1.0201	1.2996	2.5600	10.5999
	II	Exp. value	3.11	5.00	8.93	15.36	25.54	35.00	43.93	49.64	55.00	60.03	
		Calc. value	2.41	5.47	9.93	17.36	28.58	36.85	42.27	48.42	52.66	56.21	
	$K = 0.002100$	Diff. Δ	+0.70	−0.47	−1.00	−2.00	−3.04	−1.85	+0.66	+1.22	+2.34	+3.82	+8.74−8.36=+0.38
	$K' = 0.93030$	Δ^2	0.4900	0.2209	1.0000	4.0000	9.2416	3.4225	0.4356	1.4884	5.4756	14 5924	40.3670
Denat. glycinin	I	Exp. value	9.09	22.56	39.39	56.90	77.44	87.54	94.28	97.67	—	—	
		Calc. value	9.97	21.32	36.07	56.62	78.97	89.42	94.56	97.15	—	—	
	$K = 0.36275$	Diff. Δ	−0.88	+1.24	+3.32	+0.28	−1.53	−1.88	−0.28	+0.49	—	—	+5.33−4.57=+0.76
	$K' = 0.90054$	Δ^2	0.7744	1.5376	11.0224	0.0784	2.3409	3.5344	0.0784	0.2401	—	—	19.6066
	II	Exp. value	9.09	22.56	39.39	56.90	77.44	87.54	94.28	97.64	—	—	
		Calc. value	6.81	21.34	42.25	66.37	85.94	90.49	93.49	95.18	—	—	
	$K = 0.19735$	Diff. Δ	+2.28	+1.22	−2.86	−9.47	−8.50	−2.95	+0.79	+2.46	—	—	+6.75−23.78=−17.03
	$K' = 1.43145$	Δ^2	5.1984	1.4884	8.1796	89.6809	72.2500	8.7025	0.6241	6.0516	—	—	192.1755

From the above two results, each probable error is calculated and tabulated with the values of K and K' in TABLE CV.

TABLE CV

The values of K and K' and probable errors in application of equations I and II to the data obtained in decomposition by pancreatin

	Pancrecin, 1/1000 sol.			
	Range of decomp.	K	K'	P. E.
Glycinin I	1.79—42.14	0.045296	0.83922	0.46
Glycinin II	,,	0.001148	0.90081	0.86
Denat. glycinin I	5.39—92.29	0.210315	0.89325	0.80
Denat. glycinin II	,,	0.0082706	1.124833	2.30

	Pancreatin, 1/500 sol.			
	Range of decomp.	K	K'	P. E.
Glycinin I	3.11—60.03	0.077089	0.82146	0.73
Glycinin II	,,	0.002100	0.93030	1.43
Denat. glycinin I	9.09—97.64	0.36275	0.90054	1.13
Denat. glycinin II	,,	0.19735	1.43145	3.53

As shown above, the pancreatic decomposition of both normal and denatured glycinins, taking the relative quantity of enzyme as a variable follows the equation, $\frac{1}{E^{K'}} \log \frac{a}{a-x} = K$. The value of either K' or K for the normal glycinin is smaller than that for the denatured.

3. Application to the data of decomposition by papain

The results of application of equations I and II to the data of decomposition by 1/200 and 1/100 water solutions of papain are given in TABLES CVI and CVII. From these two tables, probable errors were calculated which are tabulated with the values of K and K' in TABLE CVIII.

TABLE CVI

The results of application of equations I and II to the data obtained in decomposition by papain, 1/200 solution

		E	0.1	0.25	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	Summation
Glycinin	I	Exp. value	10.00	15.71	23.75	33.57	46.07	53.21	56.79	60.30	65.36	68.93	
		Calc. value	13.13	16.40	23.30	32.46	44.05	51.82	57.68	62.27	66.06	69.25	
	$K = 0.17043$	Diff. Δ	−0.13	−0.69	+0.45	+1.11	+2.02	+1.39	−0.89	−1.91	−0.70	−0.32	+4.97−4.64=+0.33
	$K' = 0.56528$	Δ^2	0.0169	0.4761	0.2025	1.2321	4.0804	1.9221	0.7921	3.6481	0.4900	0.1024	12.9627
	II	Exp. value	10.00	15.71	23.75	33.57	46.07	53.21	56.79	60.36	65.36	68.93	
		Calc. value	9.35	16.41	24.21	34.21	45.83	52.94	57.93	61.69	64.67	67.11	
	$K = 0.0051996$	Diff. Δ	+0.65	−0.70	−0.46	−0.64	+0.24	+0.27	−1.14	−1.33	+0.69	+1.82	+3.67−4.27=−0.60
	$K' = 0.70248$	Δ^2	0.4225	0.4900	0.2116	0.4096	0.0576	0.0729	1.2996	1.7689	0.4761	3.3124	8.5212
Denat. glycinin	I	Exp. value	12.13	18.52	27.66	38.80	52.04	58.35	65.36	71.05	74.75	78.45	
		Calc. value	11.82	19.31	27.49	38.22	51.40	59.91	66.08	70.81	74.58	77.65	
	$K = 0.20916$	Diff. Δ	+0.31	−0.79	+0.17	+0.58	+0.64	−1.56	−0.72	+0.24	+0.17	+0.80	+2.91−3.07=−0.16
	$K' = 0.58324$	Δ^2	0.0961	0.6241	0.0289	0.3364	0.4096	2.4336	0.5184	0.0576	0.0289	0.6400	5.1736
	II	Exp. value	12.13	18.52	27.66	38.80	52.04	58.35	65.36	71.05	74.75	78.45	
		Calc. value	10.53	19.21	28.81	40.79	53.97	61.54	66.61	70.31	73.14	75.40	
	$K = 0.068877$	Difi. Δ	+1.60	−0.69	−1.15	−1.99	−1.93	−3.19	−1.25	+0.74	+1.61	+3.05	+7.60−10.20=−3.20
	$K' = 0.76723$	Δ^2	2.5600	0.4761	1.3225	3.9601	3.7249	10.1761	1.5625	0.5476	2.5921	9.3025	36.2244

TABLE CVII

The results of application of equations I and II to the data obtained in decomposition by papain, 1/100 solution

		E	0.1	0.25	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	Summation
Glycinin	I	Exp. value	10.71	21.43	32.86	47.86	61.07	66.96	73.04	77.14	80.89	84.50	
		Calc. value	12.34	20.95	30.53	45.08	58.23	67.61	74.12	78.90	82.55	85.40	
	$K = 0.24479$	Diff. Δ	−1.63	+0.48	+2.33	+4.78	+2.84	−0.65	−1.08	−1.76	−1.66	−0.90	+10.43−7.68=+2.75
	$K' = 0.63096$	Δ^2	2.6569	0.2304	5.4289	22.8484	8.0656	0.4225	1.1664	3.0976	2.7556	0.8100	47.4823
	II	Exp. value	10.71	21.43	32.86	47.86	61.07	66.96	73.04	77.14	80.89	84.50	
		Calc. value	10.77	21.02	32.60	46.79	61.52	69.40	74.40	77.89	80.48	82.49	
	$K = 0.0087946$	Diff. Δ	−0.06	+0.41	+0.26	+1.07	−0.45	−2.44	−1.36	−0.75	+0.41	+2.01	+4.16−5.06=−0.90
	$K' = 0.86232$	Δ^2	0.0036	0.1681	0.0676	1.1449	0.2025	5.9536	1.8496	0.5625	0.1681	4.0401	14.1606
Denat. glycinin	I	Exp. value	13.47	23.23	37.71	—	69.36	76.77	83.50	87.20	91.75	94.28	
		Calc. value	13.57	23.98	35.73	—	68.32	78.13	84.33	88.49	91.36	93.45	
	$K = 0.30957$	Diff. Δ	−0.10	−0.75	+1.98	—	+1.04	−1.36	−0.83	−1.29	+0.39	+0.83	+4.24−4.33=−0.09
	$K' = 0.68937$	Δ^2	0.0100	0.5625	3.9204	—	1.0816	1.8496	0.6889	1.6641	0.1521	0.6889	10.6181
	II	Exp. value	13.47	23.23	37.71	—	69.36	76.77	83.50	87.20	91.75	94.28	
		Calc. value	10.89	24.24	39.86	—	73.98	81.31	85.48	88.15	90.01	91.38	
	$K = 0.013726$	Diff. Δ	+2.58	−1.01	−2.15	—	−4.62	−4.54	−1.98	−0.95	+1.74	+2.90	+7.22−15.25=−8.03
	$K' = 1.05031$	Δ^2	6.6564	1.0201	4.6225	—	21.3444	20.6116	3.9204	0.9025	3.0276	8.4100	70.5551

TABLE CVIII

The values of K, K' and probable errors in application of equations I and II to the data obtained in decomposition by papain

	Papain, 1/200 solution			
	Range of decomposition	K	K'	P.E.
Glycinin I	10.00—68.93	0.17043	0.56528	0.81
Glycinin II	,,	0.0051996	0.70248	0.66
Denat. glycinin I	12.13—78.45	0.20916	0.58324	0.54
Denat. glycinin II	,,	0.006877	0.76723	1.35

	Papain, 1/100 solution			
	Range of decomposition	K	K'	P.E.
Glycinin I	10.71—84.50	0.24479	0.63096	1.55
Glycinin II	,,	0.0087946	0.86232	0.85
Denat. glycinin I	13.47—94.28	0.30957	0.68937	0.78
Denat. glycinin II	,,	0.013726	1.05031	2.01

To the data of decomposition of glycinin, equation II was better applicable than I in all cases, while to the data of the denatured glycinin, it was the opposite. The author saw, for the first time, that the best applicable equations were not the same for the two proteins.

Some discussion has been made on the applicability of the equations, $\frac{1}{E^{K'}} \log \frac{a}{a-x} = K$ and $\frac{1}{E^{K'}} \frac{x}{a(a-x)} = K$, to the experimental data of both normal and denatured glycinins in the decomposition by pepsin, pancreatin and papain, taking the relative quantity of enzyme as a variable. To the data of each result either equation I or II is satisfactorily applicable. To the data of peptic and pancreatic decomposition, the best applicable equations are the same for both proteins, while to the data of papain they are not the same. The difference of the results in the application of equations between the two proteins will be summarized later.

(iii) Studies on enzymic decomposition taking the quantity of substrate as a variable

The author decomposed both the normal and denatured glycinins with pepsin, pancreatin and papain, taking as a variable either time or the relative quantity of enzyme. The equations, $\frac{1}{t^{K'}}\log\frac{a}{a-x}=K$ and $\frac{1}{t^{K'}}\frac{x}{a(a-x)}=K$, or their modifications were applied and the equation best applicable to the data of each different experiment was determined. The author next proposes to find the equation applicable to the data of decomposition of both proteins taking the quantity of substrate as a variable.

A. Experiment

The experimental condition was almost the same with that in the previous experiments. In each experiment the quantity of protein dissolved in NaOH was taken as a variable. Different quantities of 0.1 g. to 1.5 g. of protein were dissolved in 100 cc. of 1/100 N NaOH. 10 cc. of each solution was treated as follows :

10 cc. of protein solution + 12.5 cc. of buffer solution + 6.5 cc. of water + 1 cc. of enzyme solution ; digested for one hour at 40°C.; + 3 cc. of 1/5 N NaOH or 1/10 N HCl + 7 cc. of 20% solution of CCl_3–COOH ; N in 5 cc. of the filtrate was determined.

The buffer solutions of Clark and Lubs were used. The P_H values were 1.2 and 1.8 for pepsin, 7.4 for pancreatin and 6.2 for papain. At the end of decomposition, to prevent any further digestion the following solutions were added, 3 cc. of 1/5 N NaOH in the case of pepsin, 3 cc. of 1/10 N HCl in both cases in pancreatin and papain. The enzyme solutions used were the same with that in case of the previous experiments. Each quantity of substrate decomposed is given in percentage of each quantity of substrate taken.

The result of decomposition of the denatured glycinin at P_H 1.8 did not give about the same datum in several experiments when the quantity of substrate over 0.7 or 0.8 g. was taken. Therefore only the data obtained with quantities of substrate below 0.6 g. are tabulated in TABLE CIX. The data obtained with pancreatin are given in TABLE CX, and those obtained with papain in TABLE CXI. As given in these tables, the decomposition of glycinin by pepsin was more progressive

than that of the denatured, while the decomposition by pancreatin or papain was the contrary.

TABLE CIX

The result of decomposition of glycinin by pepsin

	S	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.5
P_H 1.2	Glycinin x (%)	—	77.32	66.37	56.67	47.64	44.17	40.00	36.67	33.99	32.02	23.63
	Denat. glycinin ,,	87.20	75.09	63.66	52.42	46.08	39.88	35.22	31.74	29.26	27.64	18.82
P_H 1.8	Glycinin x (%)	—	82.26	71.37	59.76	55.12	49.48	46.07	41.55	37.86	34.64	24.27
	Denat. glycinin ,,	—	76.05	63.04	52.17	43.42	39.27	—	—	—	—	—

TABLE CX

The result of decomposition of proteins by pancreatin

S	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.2	1.5
Glycinin x (%)	35.36	28.57	22.50	19.29	16.40	15.61	14.20	13.33	13.07	12.32	11.29
Denat. glycinin ,,	78.89	67.34	58.08	49.70	46.05	41.56	37.37	32.55	30.71	25.25	21.28
Glycinin ,,	45.18	37.14	29.46	25.71	23.00	21.11	19.29	17.62	16.50	15.18	13.57
Denat. glycinin ,,	—	86.75	78.00	71.51	66.92	61.79	57.46	53.65	49.96	43.82	37.66

TABLE CXI

The result of decomposition of proteins by papain

S	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.2	1.5
Glycinin x (%)	88.57	79.24	73.14	69.53	63.98	60.00	54.05	48.86	43.75	38.57
Denat. glycin ,,	90.28	86.87	84.85	80.00	76.48	70.71	67.12	—	—	—

B. Application of equations to experimental data

To find some equation applicable to the data above given the modifi-

cations of the equations, $\frac{1}{t^{K'}}\log\frac{a}{a-x}=K$ and $\frac{1}{t^{K'}}\frac{x}{a(a-x)}=K$, were considered. In case of the experiment when either time or the relative quantity of enzyme is taken as a variable, x increases with the increase of the value of t or E. But in the case of this experiment, x decreases according to the increase of the value of the variable, S. Therefore to apply the two equations, S and $a-x$ were used instead of t and x in the equations respectively thus,

$$\frac{1}{S^{K'}}\log\frac{a}{x}=K \quad \text{..... I,}$$

$$\frac{1}{S^{K'}}\frac{a-x}{a\cdot x}=K \quad \text{.....II.}$$

These modified equations were applied to the data to test their applicability.

1. Application to the data obtained in decomposition by pepsin

In the applied results which are given in the following tables, the value of $a-x$ instead of the value of x is given as the experimental value, however, there occurs no impropriety to estimate the applicability of the equations, for, although in this case the sign of each difference (Δ) is contrary to that in the case where x is noted as the experimental value, the probable errors in both cases are quite equal. Therefore, for convenience of calculation the value of $a-x$ will be noted as the experimental value.

Glycinin, P_H 1.2

The applied result to the whole data is given in TABLE CXII. The equation often shows high applicability if it is applied to the data omitting their one or two special data, even when the applicability is very low to the whole data. Therefore the applicability of the equations to the limitted data was examined. The calculated results which were gained in applying the equation to the data for the range of S(0.3−1.5) and are also given in Table CXIII. As seen in the table the applicability of quation II is higher than that of I, but not satisfactory because the occurrence of a positive or negative difference is not irregular. Probable errors were calculated from the table and are tabulated with the values of K and K' in TABLE CXIII.

TABLE CXII

The results of application of equations I and II to the data obtained in decomposition of glycinin by pepsin at 1.2

		S	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.5	Summation
For the whole data	I	Exp. value	22.68	33.63	43.33	52.36	55.83	60.00	63.33	66.01	67.98	76.37	
		Calc. value	25.71	34.31	42.31	47.83	53.26	58.01	62.19	65.90	69.19	81.09	
	$K = 0.51126$	Diff. Δ	−3.03	−0.68	+1.02	+4.53	+2.57	+1.99	+1.14	+0.11	−1.21	−4.72	+11.36−9.64=+1.72
	$K' = 0.85540$	Δ^2	9.1809	0.4624	1.0404	20.5209	6.6049	3.9601	1.2996	0.0121	1.4641	22.2784	66.8238
	II	Exp. value	22.68	33.63	43.33	52.36	55.83	60.00	63.33	66.01	67.98	76.37	
		Calc. value	24.50	34.46	42.55	49.13	54.55	59.04	62 83	66.04	68.79	78.13	
	$K = 0.022043$	Diff. Δ	−1.82	−0.83	+0.78	+3.23	+1.28	+0.96	+0.50	−0.03	−0.81	−1.76	+6.75−5.25=+1.50
	$K' = 1.15073$	Δ^2	3.3124	0.6889	0.6084	10.4329	1.6384	0.9216	0.2500	0.0009	0.6561	3.0976	21.6072
For the data of S: 0.3—1.5	I	Exp. value	—	33.63	43.33	52.36	55.83	60.00	63.33	66.01	67.98	76.37	
		Calc. value	—	36.82	43.58	49.28	54.18	58.45	62.19	65.51	68.46	79.28	
	$K = 0.50725$	Diff. Δ	—	−3.19	−0.25	+3.08	+1.65	+1.55	+1.14	+0.50	−0.48	−2.91	+7.92−6.83=+1.09
	$K' = 0.76549$	Δ^2	—	10.1761	0.0625	9.4864	2.7225	2.4025	1.2996	0.2500	0.2304	8.4681	35.0981
	II	Exp. value	—	33.63	43.33	52.36	55.83	60.00	63.33	66.01	67.98	76.73	
		Calc. value	—	35.84	43.60	49.76	54.98	59.24	62.83	65.87	68.49	77.45	
	$K = 0.0217375$	Diff. Δ	—	−2.21	−0.27	+2.60	+0.85	+0.76	+0.50	+0.14	−0.51	−1.08	+4.85−4.07=+0.78
	$K' = 1.12818$	Δ^2	—	4.8841	0.0729	6.7600	0.7225	0.5776	0.2500	0.0196	0.2601	1.1664	14.7132

TABLE CXIII

The values of K, K' and probable errors obtained from the data given in TABLE CXII

	S: 0.2–1.5			S: 0.3–1.5		
	K	K'	P.E.	K	K'	P.E.
I	0.51126	0.85540	±1.83	0.500725	0.76549	±1.41
II	0.0220425	1.19073	±1.05	0.0217375	1.12818	±0.91

The value of K or K' of equation II is almost constant for different ranges of S, and each probable error is not so large. On the other hand, the value of K' of equation I is not constant and the probable error is always large. Therefore equation II can be recognized as better applicable. If one notices the fact that the occurrence of a positive or negative difference is not irregular, the applicability of the equation, $\frac{1}{S^{K'}} \frac{a-x}{a \cdot x} = K$, seems to be not quite satisfactory. However, as the probable error is not very large the equation is applicable without great inaccuracy.

From the above examination, it is inferred that the velocity of enzymic decomposition taking the quantity of substrate as a variable has intimate relation to the equation, $\frac{1}{S^{K'}} \log \frac{a}{x} = K$ or $\frac{1}{S^{K'}} \frac{a-x}{a \cdot x} = K$, which are originated from $\frac{1}{t^{K'}} \log \frac{a}{a-x} = K$ and $\frac{1}{t^{K'}} \frac{x}{a(a-x)} = K$ respectively.

Denatured glycinin, P_H 1.2

The results of application of the equations are given in TABLE CXIV. Probable errors were calculated and are tabulated with the values of K and K' in TABLE CX.

The decomposition of the denatured glycinin at P_H 1.2 taking the quantity of substrate as a variable can be recognized to follow the equation, $\frac{1}{S^{K'}} \frac{a-x}{a \cdot x} = K$, as in the case of glycinin.

TABLE CXIV

The results of application of equations I and II to the data obtained in decomposition of denatured glycinin by pepsin at P_H 1.2

		S	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.5	Summation
S: 0.1—1.5	I	Exp. value	12.80	24.91	36.34	47.58	52.92	60.12	64.78	68.26	70.74	72.36	81.18	
		Calc. value	14.29	25.72	32.24	43.65	50.76	56.92	62.27	66.92	70.97	74.51	86.56	
	$K = 0.59356$	Diff. Δ	−1.49	−0.81	+4.10	+3.93	+2.16	+3.20	+2.51	+1.34	−0.23	−2.15	−5.38	+17.24−10.06=+7.18
	$K' = 0.94774$	Δ^2	2.2201	0.6561	16.8100	15.4449	4.6656	10.2400	6.3001	1.7956	0.0529	4.6225	28 9444	91.7522
	II	Exp. value	12.80	24.91	36.34	47 58	52.92	60.12	64.78	68.26	70.74	72.36	81.18	
		Calc. value	14.12	27.61	38.42	46.93	53.69	59.13	63.56	67 22	70.29	72.89	81.48	
	$K = 0.02689$	Diff Δ	−1.32	−2.70	−2.08	+0.65	−0.77	+0.99	+1.22	+1 04	+0.45	−0.53	−0.30	+4.35−7.70=−3.35
	$K' = 1.21355$	Δ^2	1.7424	7.2900	4.3264	0.4225	0.5929	0.9801	1.4884	1.0816	0.2025	0.2809	0.0900	18.4977
S: 0.2—1.5	I	Exp. value	—	24.91	36.34	47.58	52.92	60.12	64.78	68.26	70.74	72.36	81.18	
		Calc. value	—	27.69	36.98	44.86	51.56	57.33	62.31	66.64	70.43	73.74	85.23	
	$K = 0.58070$	Diff. Δ	—	−2.78	−0.64	+2.72	+1.36	+2.79	+2.47	+1.62	+0.31	−1.38	−4.05	+11.27−8.85=+2.42
	$K' = 0.88305$	Δ^2	—	7.7284	0.4096	7.3984	1.8496	7.7841	6.1009	2.6244	0.0961	1.9044	16.4025	52.2984
	II	Exp. value	—	24.91	36.34	47.58	52.92	60.12	64.78	68.26	70.74	72.36	81.18	
		Calc. value	—	26.02	37.12	46.02	53.13	58.86	63.53	67.39	70.61	73.32	82.49	
	$K = 0.027478$	Diff. Δ	—	−1.11	−0.78	+1.56	−0.21	+1.26	+1.25	+0.87	+0.13	−0.96	−1.31	+5.07−4.37=+0.70
	$K' = 1.27739$	Δ^2	—	1.2321	0.6084	2.4336	0.0441	1.5876	1.5625	0.7569	0.0169	0.9216	1.7261	10.8798

TABLE CXV

The values of K, K' and probable errors obtained from the data given in TABLE CXIV

	S: 0.1–1.5			S: 0.2–1.5		
	K	K'	P.E.	K	K'	P.E.
I	0.59356	0.94774	±2.04	0.58070	0.88305	±1.63
II	0.02689	1.21355	±0.92	0.027478	1.27739	±0.74

Glycinin, P_H 1.8

The applied results are given in TABLE CXVI. Probable errors were calculated which will be tabulated with the values of K and K' in TABLE CXVII.

Equation II is always applicable better than I. The occurrence of a positive or negative difference (Δ) is satisfactory in the result calculated for the data of S (0.3 − 1.5).

Denatured glycinin, P_H 1.8

The results of application of equations I and II will be given in TABLE CXVIII.

Probable errors were calculated with values as follows:

I	II
±1.03	±0.61

From the above calculated results the higher applicability of equation II may be recognized also to the data of decomposition of the denatured glycinin as to that of the normal glycinin.

We next examined the applicability of equations I and II to the data obtained in peptic decomposition of the normal and denatured glycinins when the quantity of substrate was taken as a variable. The values of K and K' and probable error are tabulated in TABLE CXIX. The value of either K or K' of the better applicable equation, II, for the denatured glycinin is larger than the respective value of the normal glycinin.

2. Application to the data obtained in decomposition by pancreatin

The applicability of equations I and II to the data of S (0.02–0.15) was considered from the results given in TABLES CXX and CXXI.

TABLE CXVI

The results of application of equations I and II to data obtained in decomposition of glycinin by pepsin at P_H 1.8

		S	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.5	Summation
S: 0.2—1.5	I	Exp. value	17.74	28.63	40.24	44.88	50.52	53.93	58.45	62.14	65.36	75.73	
		Calc. value	20.52	28.75	36.04	42.52	48.30	53.46	58.09	62.23	65.95	79.62	
	K = 0.46792	Diff. Δ	−2.78	−0.12	+4.20	+2.36	+2.22	+0.47	+0.36	−0.09	−0.59	−3.89	+9.61−7.47=+2.14
	K' = 0.96043	Δ^2	7.7284	0.0144	17.6400	5.5696	4.9284	0.2209	0.1296	0.0081	0.3481	15.1321	51.7196
	II	Exp. value	17.74	28.63	40.24	44.88	50.52	53.93	58.45	62.14	65.36	75.73	
		Calc. value	19.29	28.75	36.91	43.83	49.69	54.65	58.88	62.51	65.64	76.33	
	K =0.019102	Diff. Δ	−1.55	−0.12	+3.33	+1.05	+0.83	−0.72	−0.43	−0.37	−0.28	−0.60	+5.21−4.07=+1.14
	K'= 1.29139	Δ^2	2.4025	0.0144	11.0889	1.1025	0.6889	0.5184	0.1849	0.1369	0.0784	0.3600	16.5758
S: 0.3—1.5	I	Exp. value	—	28.63	40.24	44.88	50.52	53.93	58.45	62.14	65.36	75.73	
		Calc. value	—	31.23	38.08	44.02	49.30	53.95	58.09	61.80	64.28	77.52	
	K = 0.45753	Diff. Δ	—	−2.60	+2.16	+0.86	+1.22	−0.02	+0.36	+0.34	+1.08	−1.79	+6.02−4.41=+1.61
	K'= 0.85934	Δ^2	—	6.7600	4.6656	0.7396	1.4884	0.0004	0.1296	0.1156	1.1664	3.2041	18.2697
	II	Exp. palue	—	28.63	40.24	44.88	50.52	53.93	58.45	62.14	65.36	75.73	
		Calc. value	—	30.05	37.95	44.58	50.15	54.87	58.88	62.33	55.32	75.59	
	K =0.018832	Diff. Δ	—	−1.42	+2.29	+0.30	+0.37	−0.94	−0.43	−0.19	+0.04	+0.14	+3.14−2.98=+0.16
	K'= 1.22731	Δ^2	—	2.0164	5.2441	0.0900	0.1369	0.8836	0.1849	0.0361	0.0016	0.0196	8.6132

TABLE CXVII

The values of K, K' and probable errors obtained from the data given in the previous table

	S: 0.2–1.5			S: 0.3–1.5		
	K	K'	P.E.	K	K'	P.E.
I	0.46792	0.96043	±1.62	0.45753	0.85934	±1.02
II	0.019102	1.29139	±0.91	0.018832	1.22731	±0.70

TABLE CXVIII

The results of application of equations I and II to the data in decomposition of denatured glycinin by pepsin at P_H 1.8

	S	0.2	0.3	0.4	0.5	0.6	Summation
I	Exp. value	23.95	36.96	47.83	56.58	60.73	
	Calc. value	24.70	36.27	46.50	55.38	62.98	
K = 0.77313	Diff. Δ	−0.75	−0.69	+1.33	+1.20	−2.25	+3.22−3.00=+0.22
K' = 1.14138	Δ^2	0.5625	0.4761	1.7685	1.4400	5.0625	9.3100
II	Exp. value	23.95	36.96	47.83	56.58	60.75	
	Calc. value	24.29	36.50	47.23	55.47	62.00	
K = 0.34751	Diff. Δ	−0.34	+0.06	+0.60	+1.11	−1.27	+1.77−1.61=+0.16
K' = 1.48031	Δ^2	0.1156	0.0036	0.3600	1.2321	1.6129	3.3242

The occurrence of a positive or negative difference is not irregular, that is, the equations are not satisfactorily applicable to the data. From the data of these two tables, probable errors were calculated and are tabulated with the values of K and K' in TABLE CXXII.

To the data obtained in pancreatic decomposition of both normal and denatured glycinins taking the quantity of substrate as a variable, equation II is better applicable than I.

3. Application to the data obtained with papain

Equations I and II, were applied to the data obtained with papain. The applied results for the normal glycinin are given in TABLE CXXIII.

Probable errors were calculated and are tabulated with the values of K and K' in TABLE CXXIV. As given in these tables, both equations are not satisfactorily applicable to the data obtained with papain. The probable error obtained in application of equation II is smaller than that obtained with I for S (0.3–0.15), but it is larger for both cases of S (0.04–0.12) and S (0.04–0.10). Therefore, the better applicable equation can not be determined from these calculated results. But the values of either K or K' of equation II decreases always according to the decrease of range of S, while that of equation I varies in an irregular way as seen in TABLE CXXIV. From this point of view equation I is recognized to be better applicable than II.

TABLE CXIX

The values of constants and probable errors in application of equations I and II to data obtained in decomposition of normal and denatured glycinins by pepsin

P_H 1.2		Glycinin		Denat. glycinin	
	S	0.2–1.5	0.3–1.5	0.1–1.5	0.2–1.5
I	K	0.51126	0.500725	0.59356	0.58070
	K'	0.85540	0.76549	0.94774	0.88305
	P.E.	±1.83	±1.41	±2.04	±1.63
II	K	0.022043	0.0217375	0.02689	0.027478
	K'	1.19073	1.12818	1.21355	1.27739
	P.E.	±1.05	±0.91	±0.92	±0.74

P_H 1.8		Glycinin		Denat. glcinin
	S	0.2–1.5	0.3–1.5	0.2–0.6
I	K	0.46792	0.45753	0.77313
	K'	0.96043	0.85934	1.14138
	P.E.	±1.62	±1.02	±1.03
II	K	0.019102	0.018832	0.34751
	K'	1.29139	1.22731	1.48031
	P.E.	±0.91	±0.70	±0.61

The applied result of the equations to the data obtained with the denatured glycinin are given in TABLE CXXV. From the data in this table, probable errors were calculated which are tabulated with the values of K and K' in TABLE CXXVI.

TABLE CXX

The results of application of equations I and II to the data obtained in decomposition of normal and denatured glycinins by pancreatin, 1/1000 water solution

		S	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.10	0.12	0.15	Summation
Glycinin	I	Exp. value	64.64	71.43	77.50	80.71	83.60	84.39	85.80	86.67	86.93	87.68	88.71	
		Calc. value	67.27	72.76	76.51	79.31	81.49	83.26	84.73	85.97	87.04	88.79	90.74	
	$K = 0.32344$	Diff. Δ	−2.63	−1.33	+0.99	+1.40	+2.11	+1.13	+1 07	+0.70	−0.11	−1.11	−2.03	+7.40−7.21=+0.19
	$K' = 0.37531$	Δ^2	6.9169	1.7689	0.9801	1.96cc	4.4521	1.2769	1.1449	0.4900	0.0121	1.2321	4.1209	24.3549
	II	Exp. value	64.64	71.43	77.50	8c.71	83.60	84.39	85.80	86.67	86.93	87.68	88.71	
		Calc. value	66.18	72.72	76.86	79.73	81.89	83.57	84.92	86.03	86.97	88.46	90.09	
	$K = 0.28607$	Diff. Δ	−1.54	−1.29	+0.64	+c.98	+1.71	+0.82	+0.88	+0.64	−0.04	−0.78	−1.38	+5.67−5.03=+0.64
	$K' = 0.76232$	Δ^2	2.3716	1.6641	0.4096	0.9604	2.92r1	0.6724	0.7744	0.4c96	0.c016	0.6084	1.5044	12.7006
Denat. glycinin	I	Exp. value	21.11	32.66	41.92	50.30	53.95	58.44	62.63	67.45	69.29	74.75	78.72	
		Calc. value	23.75	32.54	40.13	46.74	52.53	57.62	62.12	66.10	69.64	75.66	82.29	
	$K = 0.63435$	Diff. Δ	−2.64	+0.12	+1.79	+3.56	+1.42	+0.82	+0.51	+1.35	−0.35	−0.91	−3.57	+9.57−7.47=+2.10
	$K' = 0.92027$	Δ^2	6.9696	0.0144	3.2041	12.6736	2.0164	0.6724	0.2601	1.8225	0.1225	0.8281	12.7449	41.3286
	II	Exp. value	21.11	32.66	41.92	50.30	53.95	58.44	62.63	67.45	69.29	74.75	78.72	
		Calc. value	22.13	32.47	41.12	48.26	54.16	59.06	63.17	66.65	69.62	74.37	79.49	
	$K = 0.45367$	Diff. Δ	−1.02	+0.19	+0.80	+2.04	−0.21	−0.62	−0.54	+0.80	−0.33	+0.38	−0.77	+4.21−3.49=+0.72
	$K' = 1.29669$	Δ^2	1.0404	0.0361	0.6400	4.1616	0.0441	0.3844	0.2916	0.6400	0.1c89	0.1444	0.5929	8.0844

TABLE CXXI

The results of application of equations I and II to the data obtained in decomposition of normal and denatured glycinins by pancreatin, $^1/_{500}$ water solution

		S	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.10	0 12	0.15	Summation
Glycinin	I	Exp. value	54.82	62.86	70.54	74.29	77.00	78.89	80.71	82.38	83.50	84.82	86.43	
		Calc. value	57.20	64.08	68.95	72.65	75.61	78.02	80.05	81.77	83.26	85.69	88.42	
	$K = 0.35270$	Diff. Δ	− 2.38	− 1.22	+1.59	+1.64	+1.39	+0.87	+0.66	+0.61	+0.24	− 0.87	− 1.99	+7.00−6.46=+0.54
	$K' = 0.46276$	Δ^2	5.6644	1.4884	2.5281	2.6896	1.9321	0.7569	0.4356	0.3721	0.0576	0.7569	3.9601	20.6418
	II	Exp. value	54.82	62.86	70.54	74.29	77.00	78.89	80.71	82.38	83.50	84.82	86.43	
		Calc. value	56.05	64.22	69.57	73.40	76.29	78.55	80.39	81.90	83.18	85.22	87.42	
	$K = 0.34375$	Diff. Δ	− 1.23	− 1.36	+0.97	+0.89	+0.71	+0.34	+0.32	+0.48	+0.32	− 0.40	− 0.99	+4.03−3.98=+0.05
	$K' = 0.84202$	Δ^2	1.5129	1.8496	0.9409	0.7921	0.5041	0.1156	0.1024	0.2304	0.1024	0.1600	0.9801	7.2905
Denat. glycinin	I	Exp. value	—	13.25	22.00	28.49	33.08	38.21	42.54	46.35	50.04	56.18	62.34	
		Calc. value	—	15.70	21.22	26.58	31.73	36.64	41.30	45.70	49.84	57.36	66.84	
	$K = 0.63565$	Diff. Δ	—	− 2.45	+0.78	+1.91	+1.35	+1.57	+1.24	+0.65	+0.20	− 1.18	− 4.50	+7.70−8.13=−0.43
	$K' = 1.15907$	Δ^2	—	6.0025	0.6084	3.6481	1.8225	2.4649	1.5376	0.4225	0.0400	1.3924	20.2500	38.1889
	II	Exp. value	—	13.25	22.00	28.49	33.08	38.21	42.54	46.35	50.04	56.18	62.34	
		Calc. value	—	15.63	21.71	27.50	32.87	37.81	42.30	46.37	50.06	56.42	63.91	
	$K = 0.25326$	Diff. Δ	—	− 2.38	+0.29	+0.99	+0.21	+0.40	+0.24	− 0.02	− 0.02	− 0.24	− 1.57	+2.13−4.23=−2.10
	$K' = 1.40249$	Δ^2	—	5.6644	0.0841	0.9801	0.0441	0.1600	0.0576	0.0004	0.0004	0.0576	2.4649	9.5136

TABLE CXXII

The values of K and K' and probable errors in application of equations I and II to the data obtained in decomposition of normal and denatured glycinins by pancreatin

		Pancreatin 1/1000			Paacreatin 1/500		
		K	K'	P.E.	K	K'	P.E.
Glycinin	I	0.32344	0.37531	±1.05	0.35270	0.46276	±0.97
	II	0.28607	0.76232	±0.76	0.34375	0.84202	±0.58
Denat. glycinin	I	0.63435	0.92027	±1.37	0.63565	1.15907	±1.39
	II	0.45367	1.29669	±0.61	0.25326	1.40249	±0.69

The decomposition of the denatured glycinin by papain taking the quantity of substrate as a variable seems to follow equation I rather than II.

From the above examination, it is recognized that when the quantity of substrate is taken as a variable in the decomposition of normal and denatured glycinins by papain, the applicability of equation I to the data is higher than that of II.

(iv) Comparison of the normal glycinin with the denatured in the results of application of equations

The author decomposed the normal and denatured glycinins with pepsin, pancreatin and papain taking (1) time, (2) the relative quantity of enzyme and (3) the quantity of substrate as a variable. To the data of each experiment, the equations $\frac{1}{t^{K'}}\log\frac{a}{a-x}=K$ (I) and $\frac{1}{t^{K'}}\frac{x}{a(a-x)}=K$ (II) or their modified forms, $\frac{1}{E^{K'}}\log\frac{a}{a-x}=K$ (I) and $\frac{1}{E^{K'}}\frac{x}{a(a-x)}=K$ (II) or $\frac{1}{S^{K'}}\log\frac{a}{x}=K$ (I) and $\frac{1}{S^{K'}}\frac{a-x}{a\cdot x}=K$ (II), were applied respectively and their applicability was examined, from the results of which it is recognized that one of the equations is always well applicable to the data obtained in the experiment of one kind. The two proteins will be compared with each other from the results of application of equations on pages 294 and 295.

TABLE CXXIII

The results of application of equations I and II to the data obtained in decomposition of glycinin by papain

		S	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.10	0.12	0.15	Summation
S: 0.03—0.15	I	Exp. value	11.43	20.27	26.86	30.47	36.02	40.00	45.95	51.14	56.25	61.43	
		Calc. value	13.86	19.23	24 59	29.83	34 90	37.15	44.41	48.81	56.85	67.05	
	$K = 0.71073$	Diff. Δ	−2.43	+ 1.04	+2.27	+0.64	+ 1.12	+ 2.85	+ 1.54	+ 2.33	− 0.60	− 5.62	+11.79−8.65=−3.14
	$K' = 1.24711$	Δ^2	5.9049	1.0816	5.1529	0.4096	1.2544	8.1225	2.3716	5.4289	0.3600	31.5844	61.6708
	II	Exp. value	11.43	20.27	26.86	30.47	36.02	40.00	45.95	51.14	56.25	61.43	
		Calc. value	13.23	19.16	25.02	30.62	35.86	40.69	45.11	49.13	56.09	64.26	
	$K = 0.32949$	Diff. Δ	− 1.80	+ 1.11	+ 1.84	− 0.15	+ 0.16	− 0.69	+ 0.84	+ 2.01	+ 0.16	− 2.83	+6.12−5.47=−0.65
	$K' = 1.53297$	Δ^2	3.2400	1.2321	3.3856	0.0225	0.0256	0.4761	0.7056	4.0401	0.0256	8.0089	21.1621
S: 0.04—0.12	I	Exp. value	—	20.27	26.86	30.47	36.02	40.00	45.95	51.14	56.25	—	
		Calc. value	—	20.81	26.15	31.30	36.23	40.92	46.11	49.53	57.13	—	
	$K = 0.64613$	Diff. Δ	—	− 0.54	+ 0.71	− 0.83	− 0.21	− 0.92	− 0.16	+ 1.61	− 0.88	—	+2.32−3.54=−1.22
	$K' = 1.17348$	Δ^2	—	0.2916	0.5041	0.6889	0.0441	0.8464	0.0256	2.5921	0.7744	—	5.7672
	II	Exp. value	—	20.27	26.86	30.47	36.02	40.00	45.05	51.14	56.25	—	
		Calc. value	—	20.30	26.14	31.64	36.20	41.40	45.66	49.18	56.20	—	
	$K = 0.29033$	Diff. Δ	—	−0.03	+0.72	−1.17	−0.18	−1.40	+0.29	+1.96	+ 0.05	—	+3.02−2.78=+0.24
	$K' = 1.47117$	Δ^2	—	0.0009	0.5184	1.3689	0.0324	1.9600	0.0841	3.8416	0.0025	—	7.8088

S: 0.04—0.10		S	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.10	0.12	0.15	Summation
	I	Exp. value	—	20.27	26.86	30.47	36.02	40.00	45.95	51.14	—	—	
		Calc. value	—	20.64	26.08	31.33	36.35	41.14	45.66	49.95	—	—	
	K =0.67321	Diff. Δ	—	−0.37	+0.78	−0.86	−0.33	−1.14	+0.29	+1.19	—	—	+2.26−2.70=−0.44
	K'=1.19527	Δ^2	—	0.1369	0.6084	0.7396	0.1089	1.2996	0.0841	1.4161	—	—	4.3936
	II	Exp. value	—	20.27	26.86	30.47	36.02	40.00	45.95	51.14	—	—	
		Calc. value	—	20.32	26.15	31.63	36.72	41.39	45.64	49.49	—	—	
	K =0.28855	Diff. Δ	—	−0.05	+0.71	−1.16	−0.70	−1.39	+0.31	+1.65	—	—	+2.67−3.30=−0.63
	K'=1.46899	Δ^2	—	0.0025	0.5041	1.3456	0.4900	1.9321	0.0961	2.7225	—	—	7.0929

TABLE CXXIV

The values of K, K' and probable errors in application of equations I and II to the data obtained in decomposition of glycinin by papain

S	0.03–0.15			0.04–0.12			0.04–0.10		
	K	K'	P.E.	K	K'	P.E.	K	K'	P.E.
I	0.71073	1.24711	±1.77	0.64613	1.17348	±0.61	0.67321	1.19527	±0.58
II	0.32949	1.53297	±1.03	0.29033	1.47117	±0.72	0.28855	1.46899	±0.73

TABLE CXXV

The results of application of equations I and II to the data obtained in decomposition of denatured glycinin by papain

		S	0.03	0.04	0.05	0.06	0.07	0.08	0.09	Summation
S: 0.03–0.09	I	Exp. value	9.72	13.13	15.15	20.00	23.52	29.29	32.88	
		Calc. value	9.20	12.91	16.71	20.52	24.31	28.04	31.70	
	$K = 0.52700$	Diff. Δ	+0.52	+0.22	−1.56	−0.52	−0.79	+1.25	+1.18	+3.17−2.87=+0.30
	$K' = 1.25077$	Δ^2	0.2705	0.0484	2.4336	0.2704	0.6241	1.5625	1.3924	6.6018
	II	Exp. value	9.72	13.13	15.15	20.00	23.52	29.29	32.88	
		Calc. value	9.06	12.94	16.85	20.71	24.45	28.03	31.45	
	$K = 0.13037$	Diff. Δ	+0.66	+0.19	−1.70	−0.71	−0.93	+1.26	+1.43	+3.54−3.34=+0.20
	$K' = 1.38996$	Δ^2	0.4356	0.0361	2.8900	0.5041	0.8649	1.5876	2.0449	8.3632
S: 0.03–0.08	I	Exp. value	9.72	13.13	15.15	20.00	23.52	29.29	—	
		Calc. value	9.30	12.94	16.61	20.29	23.91	27.49	—	
	$K = 0.47674$	Diff. Δ	+0.42	+0.19	−1.46	−0.29	−0.39	+1.80	—	+2.41−2.14=+0.27
	$K' = 1.21412$	Δ^2	0.1764	0.0361	2.1316	0.0841	0.1521	3.2400	—	5.8203
	II	Exp. value	9.72	13.13	15.15	20.00	23.52	29.29	—	
		Calc. value	9.22	12.98	16.73	20.40	23.95	27.36	—	
	$K = 0.110085$	Diff. Δ	+0.50	+0.15	−1.58	−0.40	−0.43	+1.93	—	+2.58−2.41=+0.17
	$K' = 1.33636$	Δ^2	0.2500	0.0225	2.4964	0.1600	0.1849	3.7249	—	6.8387

TABLE CXXVI

The values of K, K' and probable errors in application of equations I and II to the data obtained in decomposition of denatured glycinin by papain

	S: 0.03–0.09			S: 0.03–0.08		
	K	K'	P.E.	K	K'	P.E.
I	0.52700	1.25077	±0.71	0.47674	1.21412	±0.73
II	0.13037	1.38996	±0.80	0.110085	1.33636	±0.79

Comparison of the results of application to the data obtained in decomposition, when time was taken as a variable

In this decomposition, the velocity is determined by the sum of both positive and negative factors, one of which is the velocity of decomposing the substrate and the other is the retarding influence caused by the decomposition product. The intensity of the former influence varies with the specificity of chemical structure of substrate, while that of the latter may be proportional to the quantity of decomposed product. To the author's supposition the retarding influence of the decomposed product may be generally not so intensive as some investigators considered and the difference of decomposition velocity between normal and denatured glycinins may due chiefly to the difference of the chemical structure of the two proteins.

The decomposition of both proteins by either pepsin or papain followed equation II. In the case of pepsin the value of K was greater in the normal glycinin but the value of K' was greater in the denatured while in the case of papain these values were the opposite respectively (TABLE LXXIII). The decomposition by pancreatin followed equation I in which the value of K was greater in the normal glycinin than in the denatured, while the value of K' was the opposite (TABLE LXXXVI).

Comparison of the results of application to the data obtained in decomposition taking the relative quantity of enzyme as a variable

The peptic decomposition of both proteins followed equation I in which the value of K was greater in the normal glycinin than in the

denatured, but K' was the opposite. (TABLE CII). The results of pancreatic decomposition were also satisfied by equation I, where the value of either K or K' for the denatured glycinin was greater than that for the normal (TABLE CV). For the data of decomposition by papain, the satisfactory equation was not the same for the normal and denatured glycinins. The former followed equation II and the latter equation I.

Comparison of the results of application to the data obtained in decomposition taking the quantity of substrate as a variable

The peptic decomposition of both proteins by pepsin followed equation II. The values of both K and K' were greater in the denatnred glycinin than in the normal (TABLE CXIX). The data of decomposition by pancreatin were satisfactorily expressed by equation II. The value of K' was greater in the denatured glycinin but the value of K was not constantly determined. In the decomposition by papain the best applicable equation was recognized to be I where the value of K was greater in the normal glycinin.

The values of K and K' of the equation which was best applicable to the data obtained with the normal glycinin are compared with those of the same equation determined for the denatured glycinin in the following table where G and D denote the value of either K or K' for the normal and denatured proteins respectively.

TABLE CXXVII

Comparison of the values of K and K' in application of equations I and II to the data of decomposition of normal and denatured glycinins in various kinds of experiment

Variable	Pepsin		Pancreatin		Papain	
	K	K'	K	K'	K	K'
Time	II $G>D$	$G<D$	I $G>D$	$G<D$	II $G>D$	$G<D$
Quantity of enzyme	I $G>D$	$G<D$	I $G<D$	$G<D$	—	—
Quanttiy of substrate	II $G<D$	$G<D$	II indefinite	$G<D$	I $G>D$	$G<D$

Part II. Studies on Soy Bean Oil

Studies on soy bean oil are just as necessary as studies on soy bean proteins in order to increase the utilization of two principal components of soy beans. The author undertook some experiment with soy bean oil upon two subjects (1) the decomposition of the oil with some enzymes, (1) the viscosity of the binary mixture of the oil and its solvents.

I. Studies on enzymic decomposition of soy bean oil

There are four principal processes to decompose oil or fat, the autoclave process, sulphuric acid process, Twitschel's process and the enzyme process. The author employing the last process made some experiment on soy bean oil using papain and castor bean powder.

Samples

Refined soy bean oil manfactured at the Shimizu Mill of the Hōnen Oil Co., Ltd. was taken. By the standard experimental methods of oil and fat[83], 1 cc. of the oil was determined to correspond to 5.6813 cc. of 1/2 N NaOH in a mean value. This datum was used through the experiment to calculate the degree of decomposition.

The same papain as had been used in the proteolytic decomposition was utilized. Castor bean powder was prepared from castor beans produced in Chiba profecture. At first, to get lipase powder from castor beans Tanaka's method of preparation of lipase powder from the residue obtained by the complete extraction of oil with ether was used. But the product had no activity of lipase. The powder made from the residue of castor beans after extracting oil thoroughly with alcohol had also no lipase activity. But the powder which was prepared from the residue obtained by incomplete extraction of oil with a hand press had powerful activity of lipase. That lipase is extracted out by such solvent as alcohol or ether is clear. Therefore, for the preparation of lipase, another of Tanaka's methods was used in the following way. Castor beans were tritulated well in a mortar, wrapped up with clean linen cloth which had been washed in boiling water, and were pressed well with a hand press. The residue thus obtained was 170 grams which was taken in a mortar and mixed well with several times its quantity 1/10 N acetic acid drop by drop. Then the mixture was taken in a beaker and was kept in water at 32°C. for 30 mins. Then it was

taken with water into a beaker of 5 litres and washed three times with water by decantation and then filtered, pressed between sheets of filter paper and dried at below 40°C. About 40 grams of brownish grey powder like rice bran were obtained. After it was sieved through 0.2 m.m. mesh, the powder was used as the sample of castor bean lipase. The water content was 2.78%.

(i) Decomposition taking time as a variable

1. Papain

Sandberg and Brand[71] proved that papain lipase was activated by $CaCl_2$. The optimum quantity of $CaCl_2$ for soy bean oil was determined in the following way:

1 cc. of soy bean oil + 3 cc. of buffer solution of P_H 6.2 + 0.1 g. of papain + various quantities of $CaCl_2 \cdot 6H_2O$; digested at 40°C. for 1½ hours.

Various quantities below 0.07 g. of crystalline $CaCl_2 \cdot 6H_2O$ were used. After a certain time of decomposition, each was titrated with ½ N NaOH with a result given in TABLE CXXVIII.

TABLE CXXVIII

The result of decomposition of soy bean oil by papain in the presence of different quantities of calcium chloride

$CaCl_2 \cdot 6H_2O$, g.	0	0.02	0.03	0.05	0.07
cc. of 1/2 N NaOH	0.41	0.68	0.69	0.69	0.53
% of decomposition	7.22	11.97	12.15	12.15	9.33

As given above,* 0.03 g. was the optimum quantity, accordingly was used in each following experiment.

In the decomposition of oil by lipase, it is very necessary to keep the mixture in a well homogenized state of emulsion. If this point is neglected or if the mixture does not keep such condition, the decomposition is very slow even though the lipase itself is powerful. Usually, as oil is easy to separate from the mixture after a certain time, the treatment of mixing or shaking is indispensable to promote the enzymic

* The datum does not show the absolute quantity of decomposition because the number of cc. titrated in blank test was neglected.

decomposition of oil. To keep the mixture in emulsified state, the buffer solution, $CaCl_2$ solution and lipase powder were stirred well with a platinum wire and then soy bean oil was added. After shaking well by hand for 1 min., the tube containing the mixture was sunk in a water bath at 40°C. At thirty minute intervals it was shaken for 1 min. After each time of decomposition, the mixture was taken in another beaker with 8 cc. of 85% alcohol, shaken well and then titrated using phenolphthalein as indicator. The condition of experiment was as follows:

3 cc. of Clark and Lubs's buffer solutisn, P_H 6.2 + 1 cc. of 3% $CaCl_2 \cdot 6H_2O$ + 0.15 g. of papain + 1 cc. of soy bean oil; decomposed at 40°C.

A blank test in the absence of soy bean oil was made for each time. The result is given below.

TABLE CXXIX

The result of decomposition of soy bean oil by papain

Time (mins.)	30	60	90	120	180	240	390	1800	2880
cc. of 1/2 N NaOH	0.33	0.47	0.62	0.75	0.87	0.92	1.11	1.52	1.77
% of decomp.	5.83	8.30	10.94	13.24	15.36	16.24	19.59	26.83	31.24

2. Castor bean powder

Decomposition was examined as follows with the castor bean powder prepared in the way previously described.

1 cc. of buffer solution, P_H 6.2 + 2 cc. of soy bean oil + 0.25 g. or 0.5 g. of castor bean powder; decomposed at 40°C.

The miscibility of soy bean oil with lipase powder was much better than that in case of papain. The mixture was shaken well by hand once every 30 mins. The results are given in TABLE CXXX.

3. Application of equations to experimental data

The author has already proved the satisfactory application of the equations, $\frac{1}{t^{K'}} \log \frac{a}{a-x} = K$ and $\frac{1}{t^{K'}} \frac{x}{a(a-x)} = K$, to the data of proteolytic decomposition. Here, to the data of oil decomposition, he also tried the application of these equations with results as given in

TABLE CXXX

The results of decomposition of soy bean oil by the lipase of castor bean powder

0.2 g. powder	Time (mins.)	30	60	120	160	200	240	
	%	17.60	22.36	26.58	30.80	33.62	37.49	
0.3 g. powder	Time (mins.)	30	60	90	120	180	240	300	350
	%	24.82	33.26	40.80	46.48	55.00	61.02	64.68	66.62
0.5 g. powder	Time (mins.)	30	60	90	120	180	240	300	330
	%	42.24	54.21	53.80	70.93	77.00	80.52	85.45	86.94

TABLE CXXXI. Each probable error was calculated by the formula $\pm 0.6745 \times \sqrt{\frac{\Sigma \Delta^2}{n-1}}$ with results as ± 1.45 for equation I and ± 1.08 for II. The applicability of equation II is thus shown to be higher than that of I. The author proved further that equation II was better applicable than I to the data of olive oil decomposition obtained by Sandberg.[71] In both calculated results for the data of the present author and of Sandberg, a positive or negative difference does not occur irregularly, that is, the equation can not be said to be perfectly applicable. Although the reason why the equation is not perfect for these data can not be made clear here, equation II is surely better than I.

Equations I and II were applied to the data of three results obtained with castor bean powder with calculated results as will be given in TABLE CXXXII. The probable errors were calculated and are compared in TABLE CXXXIII. As shown in this table, it is clear that the decomposition of soy bean oil by castor bean powder follows the equation, $\frac{1}{t^{K'}} \log \frac{a}{a-x} = K$. The author proved that equation I was also applicable better than II to Willstätter's data[81] and to Nicloux's data[57] obtained with castor bean lipase. It is interesting that the equation best applicable to the data of decomposition by castor bean lipase is not the same with that in the case of papain lipase decomposition.

From the calculated results, the values of K and K' of equation I are cited in TABLE CXXXIV. As given in this table, in the decomposition by castor bean lipase taking time as a variable, there can be found no regular change of the value of either K or K' accord-

TABLE CXXXI

The results of application of equations I and II to the data obtained with papain

	Time (mins.)	30	60	90	120	180	240	390	1800	2880	Summation
I	Exp. value	5.83	8.30	10 94	13.24	15.36	16.24	19.59	26.83	31.24	
	Calc. value	7.15	9.14	10.58	11.72	13.52	14.94	23.35	29.27	33.88	
$K = 0.c08878$	Diff. Δ	− 1.32	− 0.84	+ 0.36	+ 1.52	+ 1.84	+ 1.30	− 3.76	− 2.44	− 2.64	+5.02−11.00=−5.98
$K' = 0.37754$	Δ^2	1.7424	0.7056	0.1296	2.3104	3.3856	1.69c0	14.1376	5.9536	6.9696	37.0244
II	Exp. value	5.83	8.30	10.94	13.24	15.36	16.24	19.59	26.83	31.24	
	Calc. value	7.01	9.49	10.62	11.81	13.65	15.12	17.87	29.03	33.19	
$K=0.0c018534$	Diff. Δ	− 1.18	− 1.19	+ 0.32	+ 1.43	+ 1.71	+ 1.12	+ 1.72	− 2.:0	− 1.95	+6.30−6.52=−0.22
$K'=0.41283$	Δ^2	1.3724	1.4161	0.1024	2.0449	2.9241	1.2544	2.9584	4.84c0	3.8025	20.7352

TABLE CXXXII

The results of application of equations I and II to the data obtained with the lipase of caster bean powder

0.2 g. of powder		Time (mins.)	30	60	120	170	200	240	Summation
	I	Exp. value	17.60	22.36	26.58	30.80	33.62	37.49	
		Calc. value	17.23	22.15	28.32	31.23	33.64	35.72	
	$K = 0.020496$	Diff. Δ	+ 0.37	+ 0.21	− 1.74	− 0.43	− 0.02	+ 1.77	+2.35−2.19=+0.16
	$K' = 0.40828$	Δ^2	0.1369	0.0441	3.0276	0.1849	0.0004	3.1329	6.5268
	II	Exp. value	17.60	22.36	26.58	30.80	33.62	37.49	
		Calc. value	17.05	22.19	28.35	31.19	33.49	35.44	
	$K = 0.00052$	Diff. Δ	+ 0.55	+ 0.17	− 1.77	− 0.39	+ 0.13	+ 2.05	+2.90−2.16=+0.74
	$K' = 0.47219$	Δ^2	0.3025	0 0289	3.1329	0.1521	0.0169	4.2025	7.8358

		Time (mins.)	30	60	90	120	180	240	300	350	Summation
0.3 g. of powder	I	Exp. value	24.82	33.26	40.80	46.48	55.00	61.02	64.68	66.62	
		Calc. value	24.56	34.15	40.89	46.16	54.13	60.06	64.71	67.92	
	$K = 0.01775$	Diff. Δ	+0.26	−0.89	−0.09	+0.32	+0.87	+0.96	−0.03	−1.30	+2.41−2.31=+0.10
	$K' = 0.56772$	Δ^2	0.0676	0.7921	0.0081	0.1024	0.7569	0.9216	0.0009	1.6900	4.3396
	II	Exp. value	24.82	33.26	40.80	46.48	55.00	61.02	64.68	66.62	
		Calc. value	23.54	34.35	41.63	47.05	54.79	60.15	64.16	66.85	
	$K = 2.2848 \cdot 10^{-4}$	Diff. Δ	+1.28	−1.09	−0.83	−0.57	+0.21	+0.87	+0.52	−0.23	+2.88−2.72=+0.16
	$K' = 0.76463$	Δ^2	1.6384	1.1881	0.6889	0.3249	0.0441	0.7569	0.2704	0.0529	4.9646
0.5 g. of powder	I	Exp. value	42 24	54.21	63.80	70.93	77.00	80.52	85.45	86.94	
		Calc. value	43.39	55.40	63.36	69.02	76.72	81.76	85.32	86.73	
	$K = 0.038774$	Diff. Δ	−1.15	−1.19	+0.44	+1.91	+0.28	−1.24	+0.13	+0.21	+2.97−3.58=−0.61
	$K' = 0.53788$	Δ^2	1.3225	1.4161	0.1936	3.6481	0.0784	1.5376	0.0169	0.0441	8.2573
	II	Exp. value	42.24	54.21	63.80	70.93	77.00	80.52	85.45	86.94	
		Calc. value	40 18	55.94	64.82	71.52	77.68	80.33	84.48	85.85	
	$K = 2.9663 \cdot 10^{-4}$	Diff. Δ	+2.06	−1.73	−1.02	−0.59	−0.68	+0.19	+0.97	+1.09	+4.31−4.02=+0.29
	$K' = 0.91754$	Δ^2	4.2436	2.9929	1.0404	0.3481	0.4624	0.0361	0.9409	1.1881	11.2525

ing to the quantity of enzyme. However, the value of K is largest in 0.5 g. enzyme, smallest in 0.3 g. and the value of K' is largest in 0.3 g. and smallest in 0.2 g. enzyme.

TABLE CXXXIII

The values of probable errors calculated from the data in TABLE CXXXII

Lipase powder, g.	0.2	0.3	0.5
I	±0.77	±0.53	±0.73
II	±0.84	±0.57	±0.86

TABLE CXXXIV

The values of K and K' in application of equation I for the data obtained with castor bean lipase

Lipase powder, g.	0.2	0.3	0.5
K	0.020496	0.01775	0.038774
K'	0.40828	0.56772	0.53788

(ii) Decomposition taking the relative quantity of enzyme as a variable

Soy bean oil was decomposed with papain and castor bean powder taking the relative quantity of enzyme as a variable.

Samples and experimental methods were the same with those in the case of the previous experiment except the kind of variable.

TABLE CXXXV

The result of decomposition of oil obtained with papain

E	0.05	0.10	0.15	0.20	0 30	0.40	0.50
x (%)	6.51	10.56	12.85	15.84	19.71	24.47	28.34

1. Papain

Different quantities of 0.05 to 0.50 g. were used. The condition of the experiment is given below.

3 cc. of buffer solution, P_H 6.2 + 1 cc. of 3% $CaCl_2 \cdot 6H_2O$ + 1 cc. of oil; decomposed for 6 hours at 40°C.

The result is given in TABEE CXXXV.

2. Castor bean powder

The condition of the experiment is given below.

1 cc. of buffer solution, P_H 6.2 + 2 cc. of oil + 0.05 − 0.5 g. of enzyme powder; decomposed at 40°C. for 4 hours.

TABLE CXXXVI

The result of decomposition of oil obtained in the lipase digestion with castor bean powder

E	0.05	0.10	0.15	0.20	0.25	0.30	0.40	0.50
x (%)	7.00	15.40	40.04	52.36	62.48	68.64	79.20	83.60

The data are plotted in Fig II. The latter part of the curve is convex, but the first part is concave. In the cases with a small quantity of enzyme, all the mixture of buffer solution, enzyme and oil did not mixed well even after shaking and enzyme powder separated from the buffer solution. If a smaller quantity of buffer solution, 0.5 cc., was used, all the mixture remains for a comparatively long time as in the cases for the latter part of the curve. The result obtained with 0.5 cc. of buffer solution is given in TABLE CXXXVII and plotted in Fig. II.

TABLE CXXXVII

The result of decomposition of oil obtained with different quantities of buffer solution

E	0.05	0.10	0.15
x (%) for 1 cc. of buffer sol.	7.00	15.40	40.04
x (%) for 0.5 cc. of buffer sol	16.19	30.68	42.20

When much lipase powder was used with so small a quantity of buffer solution as 0.5 cc., the mixture was too viscous to become homogeneous even if it was shaken. Therefore, in such an experiment as the above, it is desirable to keep each mixture equally viscous by regulating the quantity of buffer solution. So the data given in the next table which are extracted from the data of TABLES CXXXVI and CXXXVII are more reasonable than the data of each table as the data of decomposition by E (0.05–0.50).

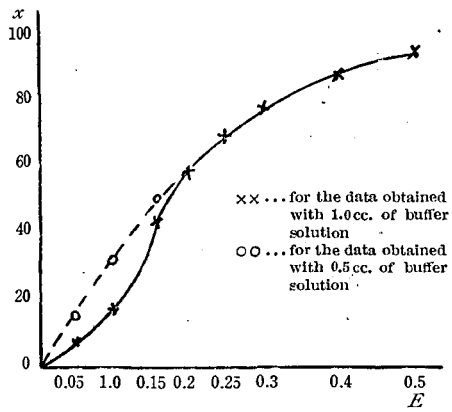


Fig. II

TABLE CXXXVIII

The reasonable result of decomposition of oil for different quantities of castor bean powder

E	0.05	0.10	0.15	0.20	0.25	0.30	0.40	0.50
x (%)	16.19	30.68	42.20	52.36	62.48	68.64	79.20	83.60

The result obtained with the oil in a quantity of one half that in the previous case is given below.

TABLE CXXXIX

The result of decomposition of oil in the lipase digestion with castor bean powder

E	0.05	0.10	0.15	0.20	0.3	0.4	0.5	0.6
x (%)	11.97	26.40	43.65	61.60	77.09	86.24	95.14	96.02

As in the previous case, the decomposition by a small quantity of enzyme was comparatively very small. In each case of E below 0.15 g., 0.5 cc. of buffer solution was used and in the other cases 1.0 cc. was used; then the result obtained is as follows:

TABLE CXL

The result of decomposition of oil in the lipase digestion with castor bean powder

E	0.05	0.10	0.15	0.20	0.30	0.40	0.50	0.60
x (%)	22.88	38.72	51.04	62.13	76.38	86.59	94.16	97.15

The curve of this result is very smooth and has no minimum.

3. Application of equations to experimental data

The author applied the equations, $\frac{1}{E^{K'}}\log\frac{a}{a-x}=K$ (I) and $\frac{1}{E^{K'}}\frac{x}{a(a-x)}=K$ (II), to the previously given data of decomposition of soy bean oil as to the data of proteolytic decomposition.

Papain

The calculated results for the data of E (0.05—0.5) and E (0.05—0.4) will be given in TABLES CXLI and CXLII. From these two tables, probable errors were calculated and are compared in TABLE CXLIII. Equation I was always much better applicable than II and the occurrence of a positive or negative difference can be seen irregular in the applied result for the range of E (0.05–0.4). He also proved that the equation was satisfactorily applicable to Sandberg's data.[71]

Castor bean powder

The author applied equations I and II to the data given in TABLE CXL as shown in TABLE CXLIV. The probable errrors calculated from the results are ±1.14 for equation I and ±4.06 for II. Equation I is surely better applicable than II.

TABLE CXLI

The results of application of equations I and II to the data obtained with papain given in TABLE CXXXV

	E	0.05	0.10	0.15	0.20	0.30	0.40	0.50	Summation
I	Exp. value	6.51	10.56	12.85	15.84	19.71	24.47	28.34	
	Calc. value	6.49	11.02	13.17	15.90	20.41	24.26	27.65	
$K = 0.284107$	Diff. Δ	+0.02	−0.46	−0.32	−0.06	−0.70	+0.21	+0.69	+0.92−1.54=−0.62
$K' = 0.68302$	Δ^2	0.0004	0.2116	0.1004	0.0036	0.4900	0.0441	0.4761	1.3262
II	Exp. value	6.51	10.56	12.85	15.84	19.71	24.47	28.34	
	Calc. value	6.40	10.26	13.38	16.05	20.52	24.21	27.37	
$K = 0.0063001$	Diff. Δ	+0.11	+0.30	−0.53	−0.21	−0.81	+0.26	+0.97	+1.64−1.55=+0.09
$K' = 0.74112$	Δ^2	0.0121	0.0900	0.2809	0.0441	0.6561	0.0676	0.9409	2.0917

TABLE CXLII

The results of application nf equations I and II to the data obtained with papain omitting the highest value of E

	E	0.05	0.10	0.15	0.20	0.30	0.40	Summation
I	Exp. value	6.51	10.56	12.85	15.84	19.71	24.47	
	Calc. value	6.55	10.23	13.21	15.79	20.20	23.94	
$K = 0.21990$	Diff. Δ	−0.04	+0.33	−0.36	+0.05	−0.49	+0.53	+0.91−0.89=+0.02
$K' = 0.67150$	Δ^2	0.0016	0.1089	0.1296	0.0025	0.2401	0.2809	0.7636
II	Exp. value	6.51	10.56	12.85	15.84	19.71	24.47	
	Calc. value	6.49	10.27	13.30	15.89	20.20	23.75	
$K = 0.006038$	Diff. Δ	+0.02	+0.29	−0.45	−0.05	−0.49	+0.72	+1.03−0.99=+0.04
$K' = 0.72219$	Δ^2	0.0004	0.0841	0.2025	0.0025	0.2401	0.5184	1.0480

TABLE CXLIII

The value of probable error for different range of application

	I	II
E : 0.05–0.5	±0.35	±0.44
E : 0.05–0.4	±0.29	±0.35

As demonstrated above, the equation, $\frac{1}{E^{K'}} \log \frac{a}{a-x} = K$, is regarded to be applicable well to the data which are obtained in the decomposition by papain or castor bean lipase taking the relative quantity of enzyme as a variable.

(iii) Decomposition taking the quantity of substrate as a variable

Soy bean oil was decomposed taking the quantity of substrate as a variable.

1. Papain

The experimental condition is briefly given below.

1 cc. of buffer solution, P_H 6.2 + 1 cc. of 3% $CaCl_2 \cdot 6H_2O$ + (0.5–3.5) cc. of oil; decomposed at 40°C. for 5 hours.

The result is given in TABLE CXLV.

2. Castor bean powder

The experimental condition is briefly given below.

1 cc. of buffer solution, P_H 6.2 + (0.5–3.0) cc. of oil + 0.3 or 0.4g. of enzyme powder; decomposed at 40°C. for 4 hours.

The result is shown in TABLE CXLVI.

3. Application of equations to experimental data

As in the case of decomposition by proteolytic enzymes, the equations, $\frac{1}{S^{K'}} \log \frac{a}{x} = K$ (I) and $\frac{1}{S^{K'}} \frac{a-x}{a \cdot x} = K$ (II), were applied to the data of lipase decomposition and their applicabilities were compared.

Papain

The applied results for the data of papain decomposition are given in TABLE CXLVII and the calculated probable errors in TABLE

TABLE CXLIV

The results of application of equations I and II to the data given in TABLE CXL

	E	0.05	0.10	0.15	0.20	0.30	0.40	0.50	0.60	Summation
I	Exp. value	22.88	38.72	51.04	62.13	76.38	86.59	94.16	97.15	
	Calc. value	21.67	38.96	53.01	63.98	79.03	87.90	93.07	96.05	
$K = 0.41709$	Diff. Δ	+1.21	−0.24	−1.97	−1.85	−2.65	−1.31	+1.09	+1.10	+3.40−8.02=−4.62
$K' = 1.04857$	Δ^2	1.4641	0.0576	3.8809	3.4225	7.0225	1.7161	1.1881	1.2100	19.9618
II	Exp. value	22.88	38.72	51.04	62.13	76.38	86.59	94.16	97.15	
	Calc. value	15.12	39.56	58.02	70.15	83.23	89.40	92.72	94.69	
$K = 0.45711$	Diff. Δ	+7.76	−0.84	−6.98	−8.02	−6.85	−2.81	+1.44	+2.46	+11.66−25.50=−13.84
$K' = 1.84418$	Δ^2	60.2176	0.7056	48.7204	64.3204	46.9225	7.8961	2.0736	6.0516	236.9078

TABLE CXLV

The result of decomposition of oil by papain

S	0.5	1.0	1.5	2.0	2.5	3.0	3.5
$X(\%)$	19.06	17.83	15.30	12.97	11.12	9.53	8.27

TABLE CXLVI

The result of decomposition of oil by castor bean powder

Powder	S	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.3 g.	x (%)	85.08	62.84	49.78	41.66	35.70	30.89	27.13	23.96
0.4 g.	x (%)	95.56	88.88	82.13	76.12	71.10	66.59	61.82	58.53

TABLE CXLVII

The result of application of the equations I and II to the data given obtained with papain

		S	0.5	1.0	1.5	2.0	2.5	3.0	3.5	Summation
S: 0.5–3.5	I	Exp. value	19.06	17.83	15.30	12.97	11.12	9.53	8.27	
		Calc. value	20.71	16.36	13.86	12.48	11.01	10.08	9.32	
	$K = 0.65767$	Diff. Δ	−1.65	+1.47	+1.44	+0.49	+0.11	−0.55	−1.05	+3.51−3.25=−0.25
	$K' = 0.28481$	Δ^2	2.7225	2.1609	2.0736	0.2401	0.0121	0.3025	1.1025	8.6142
	II	Exp. value	19.06	17.83	15.30	12.97	11.12	9.53	8.27	
		Calc. value	21.54	16.24	13.66	12.05	10.91	10.05	9.38	
	$K = 0.051571$	Diff. Δ	−2.48	+1.59	+1.64	+0.92	+0.21	−0.52	−1.11	+4.36−4.11=+0.25
	$K' = 0.50137$	Δ^2	6.1504	2.5281	2.6896	0.8464	0.0441	0 2704	1.2321	13.7611
S: 1.0–3.5	I	Exp. value	—	17.83	15.30	12.97	11.12	9.53	8.27	
		Calc. value	—	18.46	14.87	12.55	10.89	9.62	8.62	
	$K = 0.73373$	Diff. Δ	—	−0.63	+0.43	+0.42	+0.23	−0.09	−0.35	+1.08−1.07=+0.01
	$K' = 0.29686$	Δ^2	—	0.3969	0.1849	0.1764	0.0529	0.0081	0.1225	0.9417
	II	Exp. value	—	17.83	15.30	12.97	11.12	9.53	8.27	
		Calc. value	—	18.72	14.76	12.40	10.79	9.62	8.72	
	$K = 0.043431$	Diff. Δ	—	−0.89	+0.54	+0.57	+0.33	−0.09	−0.45	+1.44−1.43=+0.01
	$K' = 0.70236$	Δ^2	—	0.7921	0.2736	0.3249	0.1089	0.0081	0.2025	1.7101

CXLVIII. The values of K and K' of equation I are tabulated in TABLE CXLIX.

TABLE CXLVIII

The values of probable errors calculated from the data in TABLE CXLVII

S	I	II
S : 0.5–3.5	±0.82	±1.02
S : 1.0–3.5	±0.29	±0.39

TABLE CXLIX

The values of K and K' of equation I extracted from TABLE CXLVII

S : 0.5–3.5		S : 1.0–3.5	
K	K'	K	K'
0.65767	0.28481	0.73373	0.29686

The values of K or K' differ from each other between two cases of S (0.5–3.5) and S (1.0–3.5) and the probable error is remarkably small in the latter case. From the calculated results of above two tables, it is certain that equation I is better applicable that II.

Castor bean powder

The results of application of equations I and II to the data of TABLE CXLVI will be given in TABLE CL. Probable errors were calculated with results given in TABLE CLI. As clearly seen in this table, the equation, $\frac{1}{S^{K'}} \frac{a-x}{a \cdot x} = K$, was surely applicable well to the data obtained in the decomposition of soy bean oil by castor bean powder taking the quantity of substrate as a variable. The author reached the same conclusion for Jalander's data[37] of the decomposition of olive oil by castor bean lipase.

Soy bean oil was decomposed by papain and castor bean powder taking (1) time, (2) the relative quantity of enzyme and (3) the quantity of substrate as a variable. Castor bean lipase is more active than papain. As to the data of proteolytic decomposition, the applicability of the

TABLE CL

The results of application of equations I and II to the data obtained with castor bean powder

		S	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	Summation
0.3 g. of powder	I	Exp. value	14.92	37.16	50.22	58.34	64.30	69.11	72.87	76.04	
		Calc. value	17.95	32.84	45.06	55.09	63.39	69.98	75.53	80.02	
	$K = 0.17285$	Diff. Δ	− 3.03	+ 4.32	+ 5.16	+ 3.25	+ 0.99	− 0.87	− 2.66	− 3.98	+13.72−10.54=+3.18
	$K' = 1.00831$	Δ^2	9.1809	18.6624	26.6256	10.5625	0.9801	0.7569	7.0756	15.8404	89.6844
	II	Exp. value	14.92	37.16	50.22	58.34	64.30	69.11	72.87	76.04	
		Calc. value	16.93	34.22	47.38	57.02	64.25	69.69	73.90	77.23	
	$K = 5.20275$	Diff. Δ	− 2.01	+ 2.94	+ 2.84	+ 1.32	+ 0.05	− 0.58	− 1.03	− 1.19	+7.15−4.81=+2.34
	$K' = 1.35253$	Δ^2	4.0401	8.6436	8.0656	1.7424	0.0025	0.3364	1.0609	1.4161	25.3076
0.4 g. of powder	I	Exp. value	4.44	11.12	17.87	23.88	28.90	33.41	38.18	41.47	
		Calc. value	4.85	10.66	16.64	22.56	28.31	33.82	39.05	44.00	
	$K = 0.0489556$	Diff. Δ	− 0.41	+ 0.46	+ 1.23	+ 1.32	+ 0.59	− 0.41	− 0.87	− 2.53	+3.60−4.22=−0.62
	$K' = 1.18131$	Δ^2	0.1681	0.2116	1.5129	1.7424	0.3481	0.1681	0.7569	6.4009	11.3090
	II	Exp. value	4.44	11.12	17.87	23.88	28.90	33.41	38.18	41.47	
		Calc. value	4.69	10.86	17.14	23.11	28.72	33.83	38.47	42.68	
	$K = 0.0012161$	Diff. Δ	− 0.25	+ 0.26	+ 0.73	+ 0.77	+ 0.18	− 0.42	− 0.29	− 1.21	+1.94−2.17=−0.23
	$K' = 1.30596$	Δ^2	0.0625	0.0676	0.5329	0.5929	0.0324	0.1764	0.0841	1.4641	3.0129

TABLE CLI

The values of probable errors calculated from the data of TABLE CL

0.3 g. powder		0.4 g. powder	
I	II	I	II
±2.41	±1.28	±0.86	±0.44

TABLE CLII

The equation best applicable to the data of decomposition of oil determined for different kinds of experiment

Variable\Enzyme	Papain	Castor bean powder
Time	II	I
Rel. quantity of enzyme	I	I
Quantity of substrate	I	II

equations, $\frac{1}{t^{K'}}\log\frac{a}{a-x}=K$ (I) and $\frac{1}{t^{K'}}\frac{x}{a(a-x)}=K$ (II), and their modified forms to the data of each experiment was examined and the sufficiently applicable equation for each result was determined as given in TABLE CLII. Between papain and castor bean powder, the satisfactory equation was the same in the second kind of experiment but was not the same in the first and third kinds of experiment. It is clear that only an equation is constantly best applicable to the data of any result obtained with an enzyme in a certain kind of experiment.

II. Studies on the viscosity of binary mixtures of soy bean oil and its solvents

As the viscosity is a specific property of each kind of oil, it must be an important consideration in oil examination. Therefore a good many reports on the viscosity of soy bean oil have already been made by various investigators, but most of them are the results obtained by the direct determination of viscosity with oil itself. The present author attempted the study of the viscosity from different angle. The soy bean oil was diluted with various solvents at different concentrations.

The viscosity of each solution was measured, and some new equations formulated by the author were applied to the data thus obtained. As a result a new calculation method was found in applying the equations. There are many studies on the relation between the chemical structure of a pure chemical and its viscosity. At the same time there are many reports regarding the viscosity of binary mixtures, but most of them are not the results of the consideration on the chemical nature of each component except the recent study of Errera.[22] Principal investigations hitherto made on the viscosity of pure chemical and binary mixtures may be reviewed.

Graham[31] reported that in each homologous series of alcohol, ether and fatty acid, the higher the homologous was, the more its viscosity increased. On the same problem Rellstab[67] carried an elaborate experiment and found that the increase of viscosity was proportional to the increase of CH_2 group in a homologous series. According to Guerout,[33] higher alcohols showed higher viscosity, but the increase of viscosity was not regular, and the difference of viscosity between methyl and ethyl alcohols was remarkably large. He made it clear that there was found no relation between density and flow time. Pribram and Handl[65] determined the viscosity of many organic substances and confirmed Rellstab's result. They found that the substitution of a halogen or NO_2 to H increased the viscosity of the substance. They compared the viscosities obtained with iso and normal compounds of various substances and that obtained with alcohol and its corresponding ketonic acid. Gartenmeister[25] made many experiments on various kinds of chemicals. According to him, the following relations were recognized between the molecular weight M and the coefficient of viscosity ξ.

(1) $\xi/M=\text{const.}$

(2) $\xi/M^2=\text{const.}$

The first was applicable, within the limit of experimental error, to those chemicals containing the same number of carbon atom. With few exceptions, this was also true with temperature. Between two chemicals differing each other by a CH_2 group, the second was applicable to all series at all temperatures. Gartenmeister said that ξ/M^2 was a physicochemical constant and not a constant in mathematical meaning. He observed the increase of viscosity with the increase of a hydroxyl group. According to Thorp and Rodger,[80] in homologous series the viscosity increased with the increase of CH_2 group, but this increase was comparatively small between two substances of greater molecular weight. Between a normal

and its corresponding iso compound, the same result was also observed. The temperature coefficient of viscosity was much larger in a non-associated solution than in an associated. With homologous series the lower compounds were sometimes exceptional to the above rules as in cases of other physical properties. For instance the viscosity of formic or acetic acid was higher than that of propionic acid at all temperatures below 10°C. Dunstan and Thole[21] recognized the following relation,

$$\log \xi = aM + b$$

where ξ was the coefficient of viscosity, M molecular weight, a a general constant, b a constant for a homologous series. According to them, this equation was not applicable to the lower series but it could very satisfactorily be applied to the higher series. They determined the logarithmic increments of various groups and atoms at 20°C.

The principal studies on the relation between the viscosity and chemical structure have been cited. There have been several propositions for the expression of the viscosity of binary mixtures with various factors which will be given below.

(1) Linear form. The equation,

$$A = A_1 V_1 + A_2 V_2$$

where A is the viscosity of a mixture and where A_1, A_2, are the viscosities of two respective components and V_1, V_2, the volumes taken, is known as linear form.

(2) Bingham's equation. Bingham[8] came to the conclusion that the fluidity, not the viscosity, is a linear function of concentration. He proposed the following equation,

$$\frac{1}{A} = \frac{V_1}{A_1} + \frac{V_2}{A_2}$$

which is called Bingham's equation.

(3) Arrhenius's equation. Taking H for the viscosity of a mixture, and taking A and B for the viscosities of respective components, x and y the volumes or the numbers of gram equivalent in 1 litre calculated from the number of grams in each volume, Arrhenius[5] formulated the following equation,

$$H(x \cdot y) = A^x B^y$$

or

$$\log H = x \log A + y \log B.$$

(4) Kendall and Lees[9] established the equation,

$$\log N = M_1 \log N_1 + M_2 \log N_2$$

where N is the viscosity of a mixture, and where N_1, N_2 are the viscosities of components and M_1, M_2 the molecular concentrations of components in 1 cc. of the mixture.

(5) Kandall and Monroe's equation. Kendall and Monroe[40] proposed the equation,

$$n^{\frac{1}{3}} = an_1^{\frac{1}{3}} + bn_2^{\frac{1}{3}}$$

where n is the viscosity of the mixture and where n_1, n_2 are the viscosities and a, b the molecular concentrations of respective components.

Among these equations, the linear form seems to be most unsatisfactory. Guy[34] determined the viscosities of binary mixtures of glycerin and water, glycerin and methyl alcohol, and glycerin and ethyl alcohol, and reported that the viscosity of the mixture was not the sum of the viscosities of both components, agreeing Jones and Schmidt's result. Arrhenius, Yanik etc. regarded the limited applicability of Arrhenius's equation. According to Arrhenius, his own equation was applicable within 1% error only to a mixture of which one component consisted less than 10% by volume. Thus it is clear that the applicablility of his equation is imperfect. Yanik[85] discussed the Kendall and Monroe equation. According to him it was applicable to the data obtained with the mixtures of ethyl iodide and carbon tetrachloride at 50°C., of methyl alcohol and ethyl iodide at 20°C., and of toluene and bromobenzene or of toluene and chlorobezne. The applicability was generally higher at higher temperatures than lower, but was not always satisfactory.

In short, all the equations given above are not suitable for general application but are pretty well applicable to some special results. All of these equations are based on the quantities or volumes and viscosities of both components, i.e., the viscosity of a mixture is expressed by the combination of viscosities (physical property) and some of other factors of both components. Recently Errera[22] applied the theory of polarity to the viscosity of a binary mixture. By the polarity of each component, he classified the viscosity curves found by many preceding investigators into two kinds, i.e., concave and convex curves. If one component is non-polar and the other is either non-polar or dipolar, the viscosity curve shows concave, while if both components are dipolal the curve shows convex. The characteristic of his study is in the examination of only the viscosity of a binary mixture, leaving out the viscosities of the components.

TABEE CLIII

Specific viscosities of binary mixtures of soy bean oil

Carbon tetrachloride		Acetone		Petroleum ether	
Conc. of oil	Spec. visc.	Conc. of oil	Spec. visc.	Conc. of oil	Spec. visc.
80	41.25	79.58	13.09	90.14	31.500
70	25.62	70.01	7.13	80.01	15.829
60	16.74	59.68	4.13	69.15	8.037
50	10.05	49.91	2.62	60.00	4.624
40	6.50	40.79	1.70	49.63	2.786
30	3.72	30.17	1.13	41.65	1.987
20	2.16	19.97	0.76	39.71	1.769
10	1.16	10.01	0.54	35.03	1.457
0	0.583	0	0.421	30.16	1.221
				20.43	0.867
				0	0.469

Ether		Benzene		Chloroform	
Conc. of oil	Spec. visc.	Conc. of oil	Spec. visc.	Conc. of oil	Spec. visc.
90.09	26.137	89.37	34.588	90.10	49.137
79.66	11.737	79.93	18.985	79.78	31.025
70.06	6.266	69.47	10.749	72.45	21.139
59.36	3.243	59.75	6.390	60.09	10.907
54.91	2.589	49.88	4.148	49.82	6.393
49.94	2.081	39.86	2.718	39.90	4.116
44.63	1.607	30.05	1.868	29.87	2.295
41.50	1.380	19.93	1.308	19.94	1.321
39.84	1.287	10.00	0.933	9.96	0.699
0	0.338	0	0.680	0	0.373

From the results of applying certain equations to the author's experimental data, it may be inferred that there is an intimate relation between the viscosity of a binary mixture and the chemical nature of the components. This opens a new field in the investigation of binary mixtures.

(i) Experiment

Soy bean oil identical with the sample in the previous experiment was again taken. The following six chemicals were used as solvents.

(a) Carbon tetrachloride. Prepared by Kojima.
(b) Acetone. " " "

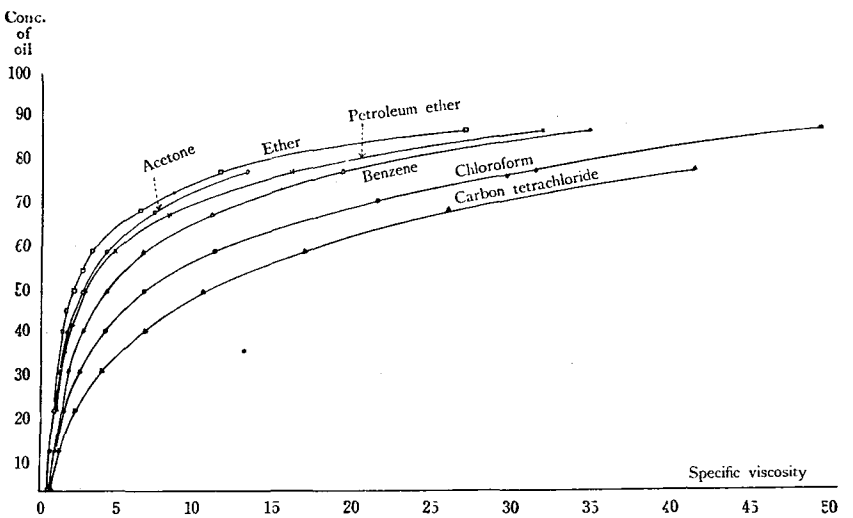


Fig. III

(c) Petroleum ether. B.P. 40°—60°C., prepared by Nakarai.
(d) Ethyl ether. Prepared by Kojima.
(e) Benzene. ,, ,, ,,
(f) Chloroform. ,, ,, ,,

Soy bean oil was diluted with a solvent at various concentrations (by weight). The viscosity was determined with 5 cc. of each liquid at 15°C. by means of an Ostwald's viscosimeter. The results were calculated in syecific viscosity and are shown in TABLE CLIII. The data are traced in Fig. III. All the curves are convex. At the same concentration of soy bean oil in various solvents, the viscosity is highest in the mixture of ether and oil, and lowest in that of carbon tetrachloride and oil. The solvents are shown below in the order of the degree of viscosity at the same concentration of the oil.

Ether, acetone, petroleum ether, benzene, chloroform, carbon tetrachloride.

The specific viscosities of these chemicals were as follows:

TABLE CLIV

Specific viscosities of solvents

Ether	0.338	Petroleum ether...	0.469
Chloroform	0.373	Carbon tetrachloride... ...	0.583
Acetone...	0.421	Benzene	0.680

There can be found no specific relation between the viscosity of a solvent and the viscosity of the mixture of the oil and the solvent. Then it may be considered that the viscosity of a binary mixture is the result of the relative properties of the components.

(ii) Application of equations to experimental data

As it is already noted that all the equations hitherto given for the calculation of the specific viscosity of binary mixture are inadequate, the author endeavoured to find some other equations which may be more adequately applicable. By the application of the equation, $\frac{1}{t^{K'}}\log\frac{a}{a-x}=K$ or $\frac{1}{t^{K'}}\frac{x}{a(a-x)}=K$, which have already been discussed before, to the author's data of results of decomposition by proteolytic enzymes and lipase, as well as to the data obtained by many other investigators in decomposition by various enzymes, it has been verified that these equations or their modifications were quite sufficient for the application to the results obtained by taking any of the variables, such as *time*, *the relative quantity of enzyme* or *the quantity of substrate*. From the fact that they are generally applicable and also from their nature, these equations may be recognized as general formulae of the equations of the first order and second order respectively. Modified forms of these equations were applied to the results of viscosity test of the binary mixture of soy bean oil given in TABLE CLIII. Let V represent the specific viscosity, d the concentration of the oil. In applying the above equations, V and d were used instead of t and x respectively sush as,

$$\frac{1}{V^{K'}}\log\frac{a}{a-d}=K \quad\ldots\ldots(\mathrm{I}), \qquad \frac{1}{V^{K'}}\frac{d}{a(a-d)}=K\ldots\ldots(\mathrm{II}).$$

The following examination of applicability of the equations was made under a hypothesis that the curves of experimental results may fall on the curves of any of these equations. However, in the above data in the case where the concentration of the oil is zero the viscosity of the mixture means that of the solvent itself and consequently gives some definite value. Therefore the curve of each result does not pass through the zero point in the figure, while the curve of any of the above equations does pass through zero point. But as the value of the viscosity of any solvent was very small, it is uncertain that whether it may be neglected or not in applying the equations. Here the applicability was examined by the following three methods.

Method A

A method which is based on the treatment that the curve of experimental data and that of the equation are considered for the same axis of abscissa

As above stated, $V=0$ in case $d=0$ in the equation, while the experimental curve does not pass through the original point. In applying the equations, the question is how to treat the value of specific viscosity of the solvent. Let V_{exp} represent the experimental value of viscosity, V_{apl} the value to be used in applying the equations and V_s the viscosity of a solvent. In finding the values of K and K' by the method of least square, the values of V_{apl} are taken under various considerations as follows:

The value of V_s was neglected. The experimental value of each specific viscosity was used directly in the application, viz.,

$$V_{apl}=V_{exp} \quad \text{.....(1).}$$

The value gained by the substraction of V_s from each experimental value was used, viz.,

$$V_{apl}=V_{exp}-V_s \quad \text{.....(2).}$$

As the viscosity of the oil itself has no relation to that of a solvent and as the viscosity of the liquid should be the viscosity of the solvent, if the concentration of the oil were zero, the values of V_{apl} were calculated in the following manner, where V_s is equal to zero in case of 100% oil liquid and V_{exp} is V_s in case of zero precent oil liquid.

$$V_{apl}=V_{exp}-V_s(a-d)\times 1/100 \quad \text{.....(3)}$$

Besides these formulae, the following two were used for trials.

$$V_{apl}=V_{exp}-V_s\left(1-\log\frac{a}{a-d}\right) \quad \text{.....(4)}$$

$$V_{apl}=V_{exp}-V_s\left(1-\log\frac{a}{a-d}\right)^n \quad \text{.....(5)}$$

The equations were also applied using the values of V_{apl} which had been calculated by formulae other than those mentioned above. But all the results thus obtained were more unsatisfactory. No attention is, therefore, given here as to their description. The applied results using the values of V_{apl} calculated by the formulae (1)—(5) are given in the following tables where (1), (2), etc. denotes the formulae applied.

TABLE CLV

The result of application of equation $\frac{1}{V^{K'}} \log \frac{a}{a-d} = K$ to the data obtained with carbon tetrachloride and soy bean oil mixture

		80	70	60	50	40	30	20	10	Summations
(1) $K = 0.051435$ $K' = 0.73782$	V_{apl}	41.52	25.62	16.74	10.50	6.50	3.72	2.16	1.16	
	V_{cal}	36.05	24.20	16.63	11.33	7.45	4.55	2.39	0.85	
	Diff.	+5.47	+1.42	+0.11	−0.83	−0.95	−0.83	−0.23	+0.31	+7.31−2.84=+4.47
(2) $K = 0.069189$ $K' = 0.63689$	V_{apl}	40.94	25.04	16.16	9.91	5.92	3.14	1.57	0.58	
	V_{cal}	37.76	23.94	15.59	10.06	6.23	3.54	1.70	0.52	
	Diff.	+3.18	+1.10	+0.57	−0.15	−0.31	−0.40	−0.13	+0.06	+4.91−0.99=+3.92
(3) $K = 0.064147$ $K' = 0.65101$	V_{apl}	41.40	25.40	16.50	10.21	6.15	3.31	1.69	0.64	
	V_{cal}	38.82	34.71	16.15	10.44	6.50	3.72	1.79	0.56	
	Diff.	+2.58	+0.69	+0.35	−0.23	−0.35	−0.41	−0.10	+0.08	+3.70−1.09=+2.61
(4) $K = 0.069403$ $K' = 0.63131$	V_{apl}	41.14	25.19	16.27	10.00	5.98	3.18	1.61	0.59	
	V_{cal}	38.80	24.50	15.90	10.22	6.30	3.57	1.70	0.52	
	Diff.	+2.34	+0.69	+0.37	−0.22	−0.32	−0.39	−0.09	+0.07	+3.47−1.02=+2.45
(5) $K = 0.068323$ $K' = 0.63646$	V_{apl}	41.21	25.21	16.32	10.04	6.01	3.20	1.62	0.62	
	V_{cal}	38.61	24.47	15.93	8.97	6.36	4.56	1.73	0.53	
	Diff.	+2.60	+0.74	+0.39	+1.07	−0.35	−1.36	−0.11	+0.09	+4.89−1.82=+3.07

TABLE CLVI

The result of application of equation $\frac{1}{V^{K'}} \frac{d}{a(a-d)} = K$ to the data obtained with carbon tetrachloride and soy bean oil mixture

		80	70	60	50	40	30	20	10	Summation
(3) $K = 0.001574$ $K' = 0.81567$	V_{apl}	41.40	25.40	16.50	10.21	6.15	3.31	1.69	0.64	
	V_{cal}	53.44	20.58	15.88	9.66	5.88	3.42	1.77	0.65	
	Diff.	−12.04	+4.82	+0.62	+0.55	+0.27	−0.11	−0.08	−0.01	+6.26−12.24=−5.98
(4) $K = 0.0013686$ $K' = 0.82207$	V_{apl}	41.14	25.19	16 27	10.00	5.98	3.18	1.61	0.59	
	V_{cal}	48.74	25.30	14.78	9.03	5.51	3.22	1.67	0.63	
	Diff.	−7.60	−0.11	+1.49	+0.97	+0.47	−0.04	−0.06	−0.04	+2.93−7.58=−4.92

TABLE CLVII

The result of application of equations, $\frac{1}{V^{K'}} \log \frac{a}{a-d} = K$ (I) and $\frac{1}{V^{K'}} \frac{d}{a(a-d)} = K$ (II), for the solutions of oil concentration up to 70% in carbon tetrachloride and soy bean oil mixture

Conc. of oil		70	60	50	40	30	20	10	Summation
(I) (3) $K = 0.06615$ $K' = 0.65057$	V_{apl}	25.40	16.50	10.21	6.15	3.31	1.69	0.64	
	V_{cal}	23.70	15.57	10.14	6.34	3.65	1.78	0.56	
	Diff.	+1.70	+0.93	+0.07	−0.19	−0.34	−0.09	+0.08	+2.78−0.62=+2.16
(II) (3) $K = 0.0016365$ $K' = 0.77325$	V_{apl}	25.40	16.50	10.21	6.15	3.31	1.69	0.64	
	V_{cal}	23.09	15.58	10.39	6.15	3.47	1.73	0.62	
	Diff.	+2.31	+0.92	−0.18	0.00	−0.16	−0.04	−0.02	+3.25−0.38=+2.87

Conc. of oil		70	60	50	40	30	20	10	Summation
(I) (4) $K=0.048477$ $K'=0.63868$	V_{apl}	25.19	16.27	10.00	5.98	3.18	1.61	0.59	
	V_{cal}	24.82	15.56	10.05	6.23	3.55	1.70	0.53	
	Diff.	+0.37	+0.71	−0.05	−0.25	−0.37	−0.09	+0.06	+1.14−0.76=+0.38
(II) (4) $K=0.0016807$ $K'=0.79381$	V_{apl}	25.19	16.27	10.00	5.98	3.18	1.61	0.59	
	V_{cal}	27.50	15.76	9.46	5.67	3.25	1.65	0.59	
	Diff.	−2.31	+0.51	+0.54	+0.31	−0.07	−0.04	0.00	+1.36−2.42=−1.06

TABLE CLVIII

The result of application of equation $\frac{1}{V^{K'}}\log\frac{a}{a-d}=K$ to the data obtained with acetone and soy bean oil mixture

Conc. of oil		79.58	70.01	59.68	49.91	40.79	30.17	19.97	10.01	Summation
(2) $K=0.17864$ $K'=0.57878$	V_{apl}	12.67	6.70	3.71	2.20	1.28	0.71	0.34	0.12	
	V_{cal}	10.33	6.40	3.94	2.45	1.52	0.79	0.35	0.11	
	Diff.	+2.34	+0.30	−0.23	−0.25	−0.24	−0.08	−0.01	+0.01	+2.65−0.81=+1.84
(3) $K=0.16215$ $K'=0.61895$	V_{apl}	13.01	7.00	3.96	2.41	1.44	0.84	0.42	0.17	
	V_{cal}	10.38	6.64	4.21	2.71	1.73	0.94	0.63	0.13	
	Diff.	+2.63	+0.36	−0.25	−0.30	−0.29	−0.10	−0.21	+0.04	+3.03−1.15=+1.88
(4) $K=0.17636$ $K'=0.55946$	V_{apl}	12.81	6.81	3.79	2.26	1.33	0.74	0.36	0.13	
	V_{cal}	11.45	6.98	4.22	2.59	1.58	0.80	0.34	0.09	
	Diff.	+1.36	−0.17	−0.43	−0.33	−0.25	−0.06	+0.02	+0.04	+1.42−1.24=+0.18

TABLE CLIX

The result of application of equation $\frac{1}{V^{K'}} \frac{d}{a(a-d)} = K$ to the data obtained with acetone and soy bean oil mixture

Conc. of oil		79.58	70.01	59.68	49.91	40.79	30.17	19.97	10.01	Summation
(3) $K = 0.0049309$ $K' = 0.80693$	V_{apl}	13.01	7.00	3.96	2.41	1.44	0.84	0.42	0.17	
	V_{cal}	12.96	6.87	3.91	2.39	1.51	0.85	0.43	0.16	
	Diff.	+0.05	+0.13	+0.05	+0 02	−0.07	−0.01	−0.01	+0.01	+0.26−0.09=+0.17
(5) $K = 0.0054039$ $K' = 0.77022$	V_{apl}	12.81	6.81	3.79	2.26	1.33	0.74	0.36	0.13	
	V_{cal}	13.00	6.69	3.70	2.21	1.37	0.75	0.37	0.13	
	Diff.	−0.19	+0.12	+0.09	+0.05	−0.04	−0.01	−0.01	0.00	+0.26−0.25=+0.01

Naturally the values of V_{apl} in different calculations are not equal. When the values of K and K' are found, the value of V_{cal} can be found, by substituting the values of d_{apl}, a (100) and the values of K and K' in the original equation.

Carbon tetrachloride and soy bean oil mixture

As shown in TABLE CLV the difference between V_{apl} and V_{cal} is positive in the higher concentrations of oil, bearing negative at the lower with an exception of the lowest, 10% concentration of oil, which again becomes positive. The occurrence of the positive or negative difference must be irregular if the applied equation is perfectly sufficient for an experimental result. From this point of view, the results of calculation are not satisfactory. This impropriety was surely caused by the fact that the equations were applied to the experimental data whose curve does not actually pass through the zero point. But these calculated results drew the author's attention to the fact that in any of them, even though it does not show the perfect application of the equation, each value of $V_{apl}-V_{cal}$ or the sum of all differences is not so large. Among the calculated results in TABLE CLV, it is clear at a glance that the result of (4) shows the best application and that of (3) comes second. Next, the equation, $\frac{1}{V^{K'}}\frac{d}{a(a-d)}=K$, was applied using the values of V_{apl} obtained from formulae (3) and (4) on page 319 with results which were also better than other calculated results as shown in TABLE CLVI.

The higher applicability is seen in the result of (4) as in the previous case. If the two calculated results of two equations, $\frac{1}{V^{K'}}\log\frac{a}{a-d}=K$ and $\frac{1}{V^{K'}}\frac{d}{a(a-d)}=K$, using the values of V_{apl} of (4) are compared with each other, it is evident that the former equation is better applicable than the latter. As the difference at the highest concentration is especially large, the equations were applied by omitting the corresponding datum with results given in TABLE CLVII. As shown in this table, if the equation applied to the data of result for oil concentration from 10 to 70 %, the differences between V_{apl} and V_{cal} become very small. In this case again the equation, $\frac{1}{V^{K'}}\log\frac{a}{a-d}=K$, is better applicable than $\frac{1}{V^{K'}}\frac{d}{a(a-d)}=K$.

From the above facts, it may be said that the equation,

$\frac{1}{V^{K'}} \log \frac{a}{a-d} = K$, can be applied with reasonable accuracy to the data obtained in the viscosity test of the binary mixture of soy bean oil and carbon tetrachloride between certain limits of concentration of oil. In this case with the values of V_{apl} calculated by formula (4) the equation shows the highest applicability.

Acetone and soy bean oil mixture

The equation, $\frac{1}{V^{K'}} \log \frac{a}{a-d} = K$, was first applied to the data obtained with the mixture with results given in TABLE CLVIII. As in the case of carbon tetrachloride and soy bean oil mixture, the result of calculation in the above table with the value of V_{apl} calculated by formula (4) also shows the highest applicability of the equation. Next, the results of the application of $\frac{1}{V^{K'}} \frac{d}{a(a-d)} = K$ are shown in TABLE CLIX.

From the above two tables, it can be seen that the equation, $\frac{1}{V^{K'}} \frac{d}{a(a-d)} = K$, is far better applicable than $\frac{1}{V^{K'}} \log \frac{a}{a-d} = K$. This is contrary to the results in the case of carbon tetrachloride and soy bean oil mixture.

To the other experimental data the application of the equations was also tried using the values of V_{apl} calculated by formulae (3) and (4) on page 319. The values of V_{apl} calculated by formula (4) gave more satisfactory results than those calculated by formulae (1), (2) and (3) just as seen in the preceding two cases. The results of application of the equations to other experimental data are given below omitting the figures. The equations, $\frac{1}{V^{K'}} \log \frac{a}{a-d} = K$ and $\frac{1}{V^{K'}} \frac{d}{a(a-d)} = K$, will be denoted by equations I and II respectively.

Petroleum ether and soy bean oil mixture

Equation I was recognized as far better than II. Although the differences for the higher concentrations were not so great as in the former case, the application of the two equations was also tried by omitting the first one or two data of the highest concentration. In the calculated results for *d*: 20.43—90.14 and for *d*: 20.43—80.01, equation I was better applicable than II while in the result for *d*: 20.43—69.15 the latter was better. The sum of the absolute differences in the result for *d*: 20.43—90.14 was larger than that for *d*: 20.43—80.01 and the

most of it was due to the negative quantities. In the calculated result of equation II, the applicability increased according to the decrease of the range of concentration of oil.

Ether and soy bean oil mixture

Equation I was better than II in the result for *d*: 39.84—90.09 while equation II was better for the narrower range of *d*.

Benzene and soy bean oil mixture

In the result obtained by the values of V_{apl} calculated by formula (3), equation II was better. If the values of V_{apl} calculated by formula (4) were used, no perceptible difference between the two equations could be seen when applied to the data of *d*: 10.00—89.37. But equation II was distinctly better for the data of *d*: 10.00—79.93.

Chloroform and soy bean oil mixture

In the applied results obtained by using the values of V_{apl} calculated by formulae (3) and (4), equation I was better in the application to *d*: 9.96—90.10, while the equation II was better for the range of *d*: 9.96—79.78.

The results of application of two equations, $\frac{1}{V^{K'}}\log\frac{a}{a-d}=K$ (I) and $\frac{1}{V^{K'}}\frac{d}{a(a-d)}=K$ (II), to the author's data of viscosity test of the binary mixture of soy bean oil and its solvents using the values of V_{apl} calculated by various formulae on page 319 are summarized as follows:

1. The values of V_{apl} calculated by the following two equations

$$V_{apl}=V_{exp}-V_s(a-d)$$

$$V_{apl}=V_{exp}-V_s\left(1-\log\frac{a}{a-d}\right)$$

showed the highest applicability of the equations where V_s represents the specific viscosity of a solvent; V_{exp}, that of experimental result; *d*, concentration of oil in percentage; and $a=100$. No perceptible difference can be seen between these two equations.

2. From the results of application of equations I and II, it may be said that the two equations are not quite sufficient to express the viscosity change of the binary mixture of soy bean oil if the positive or negative difference, the sum of differences, and the equality of positive and negative quantilies in the total of differences are observed in detail in each calculated result. But the applied results can be classified into two as follows:

(a) A certain equation is always better applicable than the other in spite of the range of the concentration of oil in the binary mixture with any of the following solvents. These solvents with each applicable equation are given below.

Carbon tetrachloride	Acetone	Benzene
I	II	II

(b) The applicability of the equations is variable according to the applied range of the concentration of oil in the binary mixture with following solvents.

Petroleum ether, Ether, Chloroform.

3. The difference, $V_{apl}-V_{cal}$, is very small in each case when the equations are applied to the data of an experimental result for the concentration less than 70 or 80% oil mixture.

Method B

A method of application based on the treatment that the curve of an experimental result and that of one of the equations are considered for the same axis of ordinate and each different axis of abscissa

From the results of the application of equations, $\frac{1}{V^{K'}}\log\frac{a}{a-d}=K$ (I) and $\frac{1}{V^{K'}}\frac{d}{a(a-d)}=K$ (II), as discussed under "method A," the author has derived an inferrence that the equations may have a close relation to the experimental curves. The applicability however, was not necessarily perfect and the best applicable equation was not always same in the applications to the different groups of data with each different range of concentration of oil in the binary mixture with petroleum ether or chloroform. In other words it may be said that the equations give only an apparent satisfaction for the application. There shall be only one of such equations and not any more to be applied sufficiently for a binary mixture when different limits of concentration are taken for calculation. From this point of view, the author endeavoured to find some other method in applying the equations, which is more suitable for calculation than method A. Now it is necessary to study the nature of the two equations. Equation I will be taken first for consideration. First of all it must be noticed that the curve of this equation passes exactly through the original point as previously stated. The equation can be written in the following way.

$$\log a - \log (a-d) = KV^{K'}$$

$$\frac{a}{a-d} = e^{mK,\,K'} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (6)$$

As can be easily known from this equation the nearer d approaches a the greater becomes the value of the left side. If $\lim_{d\to a}$, then $V\to\infty$, thus the curve of equation(6) must have a straight line which passes through the point for $d=a$ and is parallel to the axis of abscissa for its asymptote. Such a characteristic nature must be considered in applying the equation to experimental data, for the viscosity of soy bean oil ($d=a$) gives a certain finite value not an infinite in measurement. Now in applying the equation, after the values of K and K' are found, the values of V_{cal} are calculated by the following equation,

$$\log V = \log\left\{\log\left(\log\frac{a}{a-d}\right) - \log K\right\} \div K'$$

which is a logarithmic form of the original equation. The value of $\log\left(\log\frac{a}{a-d}\right)$ is zero when $d=90$. Consequently, the value of V may be calculated from the values of two constants, K and K', only in this case. Even with a little increase of the value of d over 90 the value of $\log\left(\log\frac{a}{a-d}\right)$, accordingly the value of V_{cal} becomes very large, then $V_{cal}\to\infty$ if $\lim_{a\to 100}$. Therefore, the difference, $V_{exp}-V_{cal}$, becomes very large when the value of d increases over 90. Here the author gave a consideration for the modification of the value of d in the equation so that the curve of equation may coincide with the experimental curve, and considered value 90 for 100 per cent concentration. To see if it works satisfactorily, the author studied the curves by plotting them with above idea. In Fig. IV, DE represents an experimental curve;

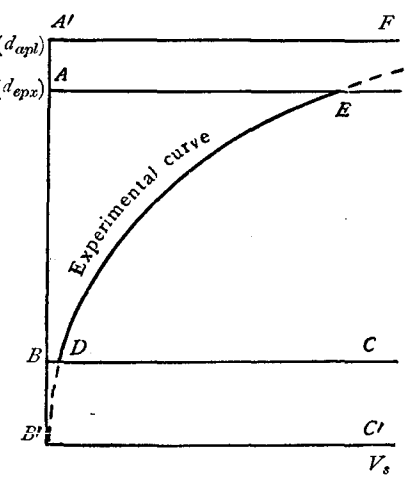


Fig. IV

and AB and BC, the axes of coordinates for plotting the curve. The experimental curve is extended at the both ends maintaining the curvature of original curve as much as possible. Then the curve ED intersects with AB at a point B'. A straight line $A'F$ which is parallel to BC is supposed to be the asymptote for the curve $B'E$.

The author considered new coordinat-axes ($A'B'$, $B'C'$) for the curve of the equation. In other words, he tried to apply the equation under a supposition that the curve $B'E$ had such curvature as it coincided with that of the equation. If the supposition is true, the applicability of the equation based on the supposition must be perfect. Let the value from B to B' be represented by p.

To apply the equation to the experimental data using the values of d for new axes, it is necessary to find first the value of p. If the value of p is not given, the value of d for new axes can not be found. Therefore the author obtained several values for p upon plotting the curve from the experimental result. With each value of p the values of d_{apl} are calculated by the following formula,

$$d_{apl}=\frac{(d_{exp}+p)100}{(100+p)\frac{100}{90}}=\frac{(d_{exp}+p)90}{(100+p)} \quad \ldots\ldots\ldots\ldots\ldots\ldots (7).$$

The values of V_{exp} on the other hand are used as the values of V_{apl}. The values of V_{apl} and those of d_{apl} are thus used in applying the equation. The values of K and K' and the values of V_{cal} are calculated in the manner described under "method A." The author is now going to study whether or not the best application of the equation given by the most satisfactory value of p is perfect. The appliability of the equation will be examined later in each calculated result.

Next, a consideration will be given with the equation $\frac{1}{V^{K'}}\frac{d}{a(a-d)}=K$. The curve of this equation has a straight line which passes through the point for a and is parallel to the axis of abscissa for its asymptote. This is easily understood if the equation is given in such a form as $V^{K'}=\frac{d}{Ka(a-d)}$. As in the case of equation I, it is also necessary to secure a formula for calculating the values of d_{apl} in applying equation II. To find the values of K and K' in the the application

of the equation, whatever values of d_{apl} be applied, the logarithmic form of the equation.

$$\log K+K' \log V=\log d-\log a-\log (a-d)$$

is used. If the value of the right side is represented by c, the equation becomes

$$\log V=(c-\log K)\div K'.$$

Similar to the case in consideration of equation I, certain new coordinate-axes are also considered for the curve of equation II. It is necessary to give a value for the new coordinate-axes to each plotted point of expepimental result in applying equation II. As in case of equation I, the value 100 for new coordinates should not be given to the point for $d_{exp}(100)+p$ (the point for 100% oil). To find the most satisfactory value for $d_{exp}(100)+p$ for applying the equation, the values of c were calculated for various values of d with following result.

TABLE CLX

The values of $\log d-\log a-\log (a-d)$ for various values of d in the application of equation II

d	c	d	c
100	$+\infty$	90.909	−1.0000
99.999	2.999996	90.00	−1.04576
99.99	1.99996	80	−1.38794
99.9	0.99996	50	−2.0000
99.0099	$\fallingdotseq 0$	1	−2.95424
99	−0.00436	0	−4.0000
95	−0.72125		

The value of c is -4 at the original point and increases to $+\infty$ if $\lim_{d\to 100}$. To get a finite value of V_{cal} for 100% oil in applying the equation, the author gave to the point for $d_{exp}(100)+p$ two different values as will be shown in (i) and (ii) to determine the values of d_{exp} for the new coordinate-axes.

(i) In applying equation I, a value 90 of d_{apl} was given to the

point for $d_{exp}(100)+p$ by which the application was almost satisfactory. It was supposed that in the case of equation II, the application might be satisfactory if the values of d_{apl} for the new axes were calculated by giving a value near 90 to the point for $d_{exp}(100)+p$. For a trial a value 90.909 was given to the point where the value of c is equal to -1, an integer.

(ii) To the point for $d_{exp}(100)+p$, a value 99.0099 was given where the value of c is equal to zero as it is at the point for $d_{avl}(90)$ in applying equation I.

Therefore the values of d_{exp} for the new coordinate-axes, the values of d_{apl}, are calculated respectively for (i) and (ii) by the following formulae.

$$d_{apl}=\frac{(d_{exp}+p)\,100}{(100+p)\frac{100}{90.909}}=\frac{(d_{exp}+p)\,90.909}{(100+p)} \quad \ldots\ldots\ldots\ldots (8)$$

$$d_{apl}=\frac{(d_{exp}+p)\,100}{(100+p)\frac{100}{99.0099}}=\frac{(d_{exp}+p)\,99.0099}{(100+p)} \quad \ldots\ldots\ldots\ldots (9)$$

Using the values of d_{apl} calculated by these formulae, equation II was applied to experimental data. It was recognized that the values of d_{apl} calculated by formula (9) showed higher applicability than those calculated by formula (8). The applied results by using formula (8) will be omitted here.

Comparing the results obtained in the application of equation I by using formula (7) with those obtained in the application of equation II by using formula (9), the former equation was more applicable than the latter to the experimental data obtained with carbon tetrachloride or chloroform while the latter was more satisfactory for the data with other solvents. However, the applicability of the equation bacame lower when the term of the highest concentration of oil in the experiment was taken.

Carbon tetrachloride and soy bean oil mixture

As the value of p, 20, 25, 35, 45, 55, 57.5, 59, 59.5, 60 and 64 were taken in applying equation I. Some of the calculated results are shown in TABLE CLXI.

If the differences of one group are compared with those of another, it is clearly be seen that the result is most satisfactory when p equals 55. In this case the sum of positive differences is about equal to that

TABLE CLXI

The result of application of equation I, using formula (9) to the data obtained with carbon tetrachloride and soy bean oil mixture

Conc. of oil (d_{exp})		80	70	60	50	40	30	20	10	Summation of diffs.
$p = 20$	d_{apl}	75.0	67.5	60.0	52.5	45.0	37.5	30.0	15.0	
$K = 0.0088054$	V_{exp}	41.25	25.62	16.74	10.50	6.50	3.72	2.16	1.16	
$K' = 0.53643$	V_{cal}	36.01	24.35	16.64	11.30	7.51	4.79	2.87	0.66	$+7.11-3.59=+3.52$
	Diff. Δ	+5.24	+1.27	+0.10	−0.80	−1.01	−1.07	−0.71	+0.50	$\Sigma\lvert\Delta\rvert=10.70$
$p = 35$	d_{apl}	76.67	70.00	63.33	56.67	50.00	43.33	36.67	23.33	
$K = 0.129137$	V_{exp}	41.25	25.62	16.74	10.50	6.50	3.72	2.16	1.16	
$K' = 0.43402$	V_{cal}	38.85	25.08	16.48	10.83	7.05	4.44	2.69	0.77	$+3.61-2.11=+1.50$
	Diff. Δ	+2.42	+0.54	+0.26	−0.33	−0.53	−0.72	−0.53	+0.39	$\Sigma\lvert\Delta\rvert=5.72$
$p = 45$	d_{apl}	77.59	71.38	65.17	58.97	52.76	46.55	40.34	27.93	
$K = 0.155246$	V_{exp}	41.25	25.62	16.74	10.50	6.50	3.72	2.16	1.16	
$K' = 0.38788$	V_{cal}	39.81	25.27	16.27	10.53	6.76	4.66	2.58	0.80	$+2.62-1.65=+0.97$
	Diff. Δ	+1.44	+0.35	+0.47	−0.03	−0.26	−0.94	−0.42	+0.36	$\Sigma\lvert\Delta\rvert=4.27$
$p = 55$	d_{apl}	78.39	72.58	66.97	60.97	55.16	49.36	43.55	31.94	
$K = 0.17680$	V_{exp}	41.25	25.62	16.74	10.50	6.50	3.72	2.16	1.16	
$K' = 0.35662$	V_{cal}	41.08	25.60	16.31	10.48	6.70	3.96	2.72	0.85	$+0.95-1.00=-0.05$
	Diff. Δ	+0.17	+0.02	+0.43	+0.02	−0.20	−0.24	−0.56	+0.31	$\Sigma\lvert\Delta\rvert=1.95$

Conc. of oil (d_{exp})		80	70	60	50	40	30	20	10	Summation of diffs.
$p = 57$	d_{apl}	78.54	72.80	67.07	61.34	55.61	49.87	44.14	32.68	
$K = 0.181175$	V_{exp}	41.25	25.62	16.74	10.50	6.50	3.72	2.16	1.16	
$K' = 0.35091$	V_{cal}	41.30	25.62	16.29	10.45	6.68	4.20	2.58	0.86	$+0.80-1.13=-0.33$
	Diff. Δ	−0.05	0.00	+0.45	+0.05	−0.18	−0.48	−0.42	+0.30	$\Sigma\lvert\Delta\rvert=1.93$
$p = 59$	d_{apl}	78.67	73.01	67.35	61.69	56.03	50.37	47.71	33.39	
$K = 0.185474$	V_{exp}	41.25	25.62	16.74	10.50	6.50	3.72	2.16	1.18	
$K' = 0.34531$	V_{cal}	41.42	25.67	16.29	10.42	6.65	4.19	2.63	0.87	$+0.82-1.31=-0.49$
	Diff. Δ	−0.17	−0.05	+0.45	+0.08	−0.15	−0.47	−0.47	+0.29	$\Sigma\lvert\Delta\rvert=2.13$
$p = 60$	d_{apl}	78.75	73.13	67.50	61.88	56.25	50.63	45.00	33.75	
$K = 0.187674$	V_{exp}	41.25	25.62	16.74	10.50	6.50	3.72	2.16	1.16	
$K' = 0.34263$	V_{cal}	41.50	25.69	16.28	10.42	6.64	4.19	2 58	0.87	$+0.83-1.35=-0.52$
	Diff. Δ	−0.25	−0.07	+0.46	+0.08	−0.14	−0.47	−0.42	+0.29	$\Sigma\lvert\Delta\rvert=2.18$
$p = 64$	d_{apl}	79.02	73.54	68.05	62.56	57.07	51.59	46.10	35.12	
$K = 0.19618$	V_{exp}	41.25	25.62	16.74	10.50	6.50	3.72	2.16	1.16	
$K' = 0.33235$	V_{cal}	41.80	27.61	16.26	10.36	6.60	4.16	2.57	0.88	$+0.90-3.49=-2.59$
	Diff. Δ	−0.55	−1.99	+0.48	+0.14	−0.10	−0.44	−0.41	+0.28	$\Sigma\lvert\Delta\rvert=4.39$

TABLE CLXII

The result of application of equation I, using formula (7) to the data obtained with chloroform and soy bean oil muxture

Conc. of oil (d_{exp})		90.10	79.78	72.45	60.09	49.82	39.90	29.87	19.94	9.96	Summation of diffs.
$p = 18$	d_{apl}	82.45	74.57	68.99	59.56	51.73	44.16	36.51	28.93	21.38	
$K = 0.129906$	V_{exp}	49.139	51.025	21.139	10.907	6.393	4.116	2.295	1.321	0.699	
$K' = 0.46875$	V_{cal}	48.427	28.537	20.183	11.436	7.073	4.318	2.493	1.327	0.608	$+4.247-1.615=+2.632$
	Diff. Δ	+0.712	+2.488	+0.956	−0.529	−0.680	−0.202	−0.198	−0.006	+0.091	$\Sigma\|\Delta\|=5.862$
$p = 19$	d_{apl}	82.51	74.71	69.16	60.16	52.05	44.55	36.96	29.45	21.90	
$K = 0.13364$	V_{exp}	49.139	31.025	21.139	10.907	6.393	4.116	2.295	1.321	0.699	
$K' = 0.44742$	V_{cal}	48.261	28.375	20.081	11.857	7.001	4.279	2.474	1.323	0.613	$+4.672-1.902=+2.770$
	Diff. Δ	+0.878	+2.650	+1.058	−0.950	−0.608	−0.163	−0.179	−0.002	+0.086	$\Sigma\|\Delta\|=6.574$
$p = 20$	d_{apl}	82.58	74.84	69.34	60.07	52.37	44.93	37.40	29.96	22.47	
$K = 0.13463$	V_{exp}	49.139	31.025	21.139	10.907	6.393	4.116	2.295	1.321	0.699	
$K' = 0.44020$	V_{cal}	49.304	28.889	20.323	10.863	7.051	4.299	2.482	1.331	0.621	$+3.074-1.203=+1.871$
	Diff. Δ	−0.165	+2.136	+0.816	+0.044	−0.658	−0.183	−0.187	−0.010	+0.078	$\Sigma\|\Delta\|=4.277$
$p = 21$	d_{apl}	82.63	74.69	69.51	60.31	52.68	45.30	37.84	30.45	23.03	
$K = 0.13948$	V_{exp}	49.139	31.025	21.139	10.907	6.393	4.116	2.295	1.321	0.699	
$K' = 0.43617$	V_{cal}	48.797	28 009	21.145	11.280	6.953	4.244	2.458	1.325	0.622	$+3.435-1.234=+2.201$
	Diff. Δ	+0.342	+3.016	−0.006	−0.373	−0.560	−0.128	−0.163	−0.004	+0.077	$\Sigma\|\Delta\|=4.669$
$p = 22$	d_{apl}	82.70	75.08	69.68	60.56	52.98	45.66	38.26	30.94	23.58	
$K = 0.14241$	V_{exp}	49.139	31.025	21.139	10.907	6.393	4.116	2.295	1.321	0.695	
$K' = 0.42989$	V_{cal}	49.474	28.761	20.185	11.313	6.950	4.015	2.325	1.326	0.630	$+3.388-1.333=+2.055$
	Diff. Δ	−0.335	+2.264	+0.954	−0.406	−0.557	+0.101	−0.030	−0.005	+0.065	$\Sigma\|\Delta\|=4.721$
$p = 25$	d_{apl}	82.87	75.44	70.16	61.26	53.87	46.73	39 51	32.36	25.17	
$K = 0.15109$	V_{exp}	49.139	31.025	21.139	10.907	6.393	4.116	2.295	1.321	0.699	
$K' = 0.41469$	V_{cal}	50.159	28.917	20.173	11.224	6.872	4.181	2.429	1.325	0.644	$+3.129-2.019=+1.110$
	Diff. Δ	−1.020	+2.108	+0.966	−0.317	−0.479	−0.065	−0.134	−0.004	+0.055	$\Sigma\|\Delta\|=5.148$

TABLE CLXIII

Ths result of application of equation II using formula (9) to the obtained with acetone and soy bean oil mixure

Conc. of oil (d_{exp})		79.58	70.01	59.68	49.91	40.79	30.17	19.97	10.01	Summation
$p = 60$	d_{apl}	86.37	80.45	74.06	68.01	62.37	55.80	49.49	43.32	
$K = 0.042315$	V_{exp}	13.09	7.13	4.13	2.62	1.70	1.13	0.76	0.54	
$K' = 0.65263$	V_{cal}	13.57	6.76	4.00	2.55	1.74	1.15	0.78	0.53	$+0.58-0.56=+0.02$
	Diff. Δ	−0.48	+0.37	+0.13	+0.07	−0.04	−0.02	−0.02	+0.01	$\Sigma\lvert\Delta\rvert=1.14$

TABLE CLXIV

The result of application of equation II using formula (9) to the data obtained with petroleum ether and soy bean oil mixture

Conc. of oil (d_{exp})		80.01	69.15	60.00	49.63	41.65	39.71	35.03	30.16	20.43	Summation
$p = 36$	d_{apl}	84.46	76.55	69.89	62.34	56.53	55.12	51.71	48.16	41.08	
$K = 0.0080945$	V_{exp}	15.829	8.034	4.624	2.786	1.987	1.769	1.457	1.221	0.867	
$K' = 0.68487$	V_{cal}	16.127	7.661	4.656	2.842	1.998	1.838	1.505	1.223	0.804	$+0.439-0.516=-0.077$
	Diff. Δ	−0.298	+0.376	−0.032	−0.056	−0.011	−0.069	−0.048	−0.002	+0.063	$\Sigma\lvert\Delta\rvert=0.955$

TABLE CLXV

The result of application of equation IIusing formula (9) to the data obtained with ether and soy bean oil mixture

Conc. of oil (d_{exp})		79.66	70.06	59.36	54.91	49.94	44.63	41.50	39.84	Summation
$p = 40$	d_{apl}	84.62	77.84	70.27	67.12	63.61	59.85	57.64	56.46	
$K = 0.0109405$	V_{exp}	11.737	6.266	3.243	2.589	2.081	1.607	1.380	1.287	
$K' = 0.64892$	V_{cal}	12.049	6.035	3.277	2.615	2.009	1.611	1.399	1.295	$+0.303-0.407=-0.104$
	Diff. Δ	−0.312	+0.231	−0.034	−0.026	+0.072	−0.004	−0.019	−0.012	$\Sigma\lvert\Delta\rvert=0.710$

of negative (0.95 : 1.00), and the sum of absolute differences (1.95) is less than that of any other result. If the value of p is either greater or less than 55 the balance of the positive and negative differences in the summation becomes worse in the calculated result.

Chloroform and soy bean oil mixture

As the value of p in applying equation I, 18, 19, 20, 21 and 25 were taken. The results are given in TABLE CLXII.

The result is most satisfactory when $p=20$. In the case where the value of p is either greater of less than 20 the sum of differences is strongly due to either the positive or negative quantity.

Acetone and soy bean oil mixture

A satisfactory result in the application of equation II was obtained by taking 60 as the value of p as shown in TABLE CLXIII.

Petroleum ether and soy bean oil mixture

The applicability of equation II became somewhat lower when the equation was applied to the experimental data with the highest concentration of oil. Equation II was always better applicable than I. The applied result of II for the range of d_{exp}: 20.43—80.01, taking 36 as the value of p is given in TABLE CLXIV.

Ether and soy bean oil mixture

A value 40 was given to p by which equation II gave satisfactory result as shown in TABLE CLXV.

Benezene and soy bean oil mixture

The applicability of equation II is high as shown in TABLE CLXVI when it is applied to the range of d_{exp}: 10.00—79.93 taking 40 as the value of p.

Method C

A method of application which is based on the treatment that the curve of an experimental result and that of the equation are considered for the same axis of abscissa and different axis of ordinate

The results calculated by method B gave small difference in all cases as shown in TABLES CLXI—CLXVI. However, the applicability was not quite sufficient. Therefore the author endeavoured to find some other method which might be better than method B for the application of the equations. He finally succeeded in finding a satisfactory method which was above described. For the value of

TABLE CLXVI

The result of application of equation II using formula (9) to the data obtained with benzene and soy bean oil mixture

Conc. of oil (d_{exp})		79.93	69.74	59.75	49.88	39.86	30.05	19.93	10.00	Summation of diffs.
$p = 40$	d_{apl}	84.82	77.61	70.54	63.56	56.48	49.54	42.38	35.36	
$K = 0.0059607$	V_{exp}	18.958	10.749	6.390	4.148	2.718	1.868	1.308	0.933	
$K' = 0.75473$	V_{cal}	19.399	10.305	6.312	4.148	2.804	1.937	1.321	0.892	$+0.563-0.609=-0.046$
	Diff. Δ	-0.441	-0.444	$+0.078$	0.000	-0.086	-0.069	-0.013	$+0.041$	$\Sigma\lvert\Delta\rvert=1.172$

TABLE CLXVII

The result of application of equations I and II by method C to the data obtained with carbon tetrachloride and soy bean oil mixture

Conc. of oil (d_{exp})		80	70	60	50	40	30	20	10	Summation of diffs.
(I)	d_{apl}	72.0	63.0	54.0	45.0	36.0	27 0	18.0	9.0	
$K = 0.0631643$	V_{apl}	40.67	25.04	16.16	9.92	5.92	3.14	1.58	0.58	
$K' = 0.60323$	V_{cal}	36.46	24.18	16.07	10.42	6.42	3.60	1.67	0.49	$+5.25-1.55=+3.70$
	Diff.	$+4.21$	$+0.86$	$+0.09$	-0.50	-0.50	-0.46	-0.09	$+0.09$	$\Sigma\lvert\Delta\rvert=6.80$
(II)	d_{apl}	79.21	69.31	59.40	49.50	39.60	29.70	19.80	9.90	
$K = 0.0016471$	V_{apl}	40.67	25.04	16.16	9.92	5.92	3.14	1.58	0.58	
$K' = 0.81218$	V_{cal}	47.83	25.12	14.72	8.99	5.48	3.19	1.65	0.61	$+2.81-7.39=-4.58$
	Diff. Δ	-7.16	-0.08	$+1.44$	-0.93	$+0.44$	-0.05	-0.07	-0.03	$\Sigma\lvert\Delta\rvert=10.20$

TABLE CLXVIII

The result of application of equations I and II by method C with chloroform and soy bean oil mixture

Conc. of oil (d_{exp})		90.10	79.78	72.45	60.09	49.82	39.90	29.87	19.94	9.96	Summation
(I)	d_{apl}	81.09	71.81	65.21	54.81	44.84	35.91	26.88	17.86	8.87	
$K = 0.08676$	V_{apl}	48.766	30.652	20.766	10.534	6.020	3.743	1.922	0.948	0.326	
$K' = 0.56061$	V_{cal}	43.941	26.947	19.489	11.730	7.005	4.171	2.228	0.973	0.255	
	Diff. Δ	+4.825	+3.705	+1.277	−1.196	−0.985	−0.428	−0.306	−0.025	+0.071	+9.878−2.940=+6.938 $\Sigma\lvert\Delta\rvert = 12.818$
(II)	d_{apl}	89.21	78.99	71.73	59.50	49.33	39.50	29.57	19.74	9.86	
$K = 0.0024457$	V_{apl}	48.766	30.652	20.766	10.534	6.020	3.743	1.922	0.948	0.326	
$K' = 0.81680$	V_{cal}	74.462	28.374	17.532	8.981	5.427	3.327	1.938	1.007	0.373	
	Diff.	−25.696	+2.278	+3.234	+1.553	+0.593	+0.416	−0.016	−0.059	−0.047	+8.074−25.818=−17.744 $\Sigma\lvert\Delta\rvert = 33.892$

TABLE CLXIX

The result of application of equations I and II by method C to the data obtained with acetone and soy bean oil mixture

Conc. of oil (d_{exp})		79.58	70.01	59.68	49.91	40.79	30.17	19.97	10.01	Summation of diffs.
(I)	d_{apl}	71.62	63.01	53.71	44 92	36.71	27.15	17.97	9.01	
$K = 0.15474$	V_{apl}	12.669	6.709	3.709	2.199	1.279	0.709	0.339	0.119	
$K' = 0.55442$	V_{cal}	9.752	6.369	4.017	2.532	1.569	1.236	0.347	0.091	$+3.285-1.466=+1.819$
	Diff. Δ	+2.917	+0.340	−0.308	−0.333	−0.290	−0.527	−0.008	+0.028	$\Sigma\lvert\Delta\rvert=4.751$
(II)	d_{apl}	78.79	69.32	59.09	49.09	40.39	29.87	19.77	9.91	
$K = 0.0054879$	V_{apl}	12.669	6.709	3.709	2.199	1.279	0.709	0.347	0.115	
$K' = 0.74900$	V_{cal}	12.849	6.615	3.642	2.160	1.325	0.713	0.343	0.117	$+0.206-0\ 230=-0.024$
	Diff. Δ	−0.180	+0.094	+0.067	+0.039	−0.046	−0.004	+0.004	+0.002	$\Sigma\lvert\Delta\rvert=0.436$

TABLE CLXX

The result of application of equations I and II by method C to the data obtained with petroleum ether and soy bean oil mixture

Conc. of oil (d_{exp})		80.01	69.15	60.00	49.63	41.65	39.71	25.03	30.16	20.43	Summation
(I)	d_{apl}	72.01	62.24	54.00	44.67	37.49	35.74	31.53	27.14	18.39	
$K = 0.15930$	V_{apl}	15.360	7.568	4.155	2.317	1.518	1.300	0.988	0.752	0.398	
$K' = 0.49040$	V_{cal}	10.514	7.324	4.615	2.653	1.657	1.464	1.068	0.741	0.299	$+5.200-1.179=+4.021$
	Diff. Δ	+4.846	+0.244	−0.460	−0.336	−0.139	−0.164	−0.080	+0.011	+0.099	$\Sigma\lvert\Delta\rvert=6.379$
(II)	d_{apl}	79.22	68.47	59.41	49.14	41.24	39.32	34.68	29.86	20.23	
$K = 0.0051033$	V_{apl}	15.360	7.568	4.155	2.317	1.518	1.300	0.988	0.752	0.398	
$K' = 0.72666$	V_{cal}	15.538	7.163	4.162	2.350	1.514	1.356	1.031	0.761	0.373	$+0.434-0.326=+0.108$
	Diff. Δ	−0.178	+0.405	−0.007	−0.033	+0.004	−0.056	−0.043	−0.009	+0.025	$\Sigma\lvert\Delta\rvert=0.760$

TABLE CLXXI

The result of application of equations, I and II by method to the data obtained with ether and soy bean oil mixture

Conc. of oil (d_{exp})		79.66	70.06	59.36	54.91	49.94	44.63	41.50	39.84	Summation of diffs.
(I)	d_{apl}	71.69	63.05	53.42	49.42	44.95	40.17	37.35	35.77	
$K = 0.202864$	V_{apl}	11.399	5.878	2.905	2.251	1.743	1.267	1.042	0.949	
$K' = 0.42289$	V_{cal}	11.472	5.978	3.214	2.440	1.783	1 250	1.001	0.880	$+1.054-0.638=+0.416$
	Diff. Δ	+0.927	−0.100	−0.309	−0.189	−0.040	+0.017	+0.041	+0.069	$\Sigma\|\Delta\|=1.692$
(II)	d_{apl}	78.87	69.37	58.77	54.37	49.45	44.19	41.09	39.45	
$K = 0.006727$	V_{apl}	11.399	5.878	2.905	2.251	1.743	1.267	1.042	0.949	
$K' = 0.69800$	V_{cal}	11.646	5.693	2.933	2.268	1.710	1.263	1.053	0.955	$+0.222-0.309=-0.087$
	Diff. Δ	−0.247	+0.185	−0.028	−0.017	+0.033	+0.004	−0.011	−0.006	$\Sigma\|\Delta\|=0.531$

TABLE CLXXII

The result of application of equations I and II by method C to the data obtained with benzene and soy bean oil mixture

Conc. of oil (d_{exp})		79.93	69.74	59.75	49.88	39.86	30.05	19.93	10.00	Summation of diffs.
(I)	d_{apl}	72.03	62.77	53.78	44.89	35.87	27.05	17.94	9.00	
$K = 0.11930$	V_{apl}	18.305	10.069	5.710	3.468	2.038	1.188	0.628	0.253	
$K' = 0.53023$	V_{cal}	18.058	11.180	7.016	4.307	2.476	1.298	0.538	0.133	$+0.457-3.804=-3.347$
	Diff. Δ	+0.247	−1.111	−1.306	−0.839	−0.438	−0.110	+0.090	+0.120	$\Sigma\|\Delta\|=4.261$
(II)	d_{apl}	79.14	69.05	59.16	49.39	39.47	29.75	19.73	9.90	
$K = 0.0035336$	V_{apl}	18.305	10.069	5.710	3.468	2.038	1.188	0.628	0.253	
$K' = 0.81491$	V_{cal}	18.409	9.595	5.648	3.479	2.121	1.249	0.641	0.239	$+0.550-0.272=+0.278$
	Diff. Δ	−0.104	+0.474	+0.062	−0.011	−0.083	−0.061	−0.013	+0.014	$\Sigma\|\Delta\|=0.822$

d_{apl} similar consideration was made as that mentioned under "method *B*." In this method the following formulae are used. As for the values of V_{apl}, a formula $V_{apl}=V_{exp}-V_s$ is used for both equations. As for the values of d_{apl}, the following formulae, $d_{apl}=d_{exp}\times 0.9$ (in case of equation I), and $d_{apl}=d_{exp}\times 0.990099$ (in case of equation II) are used. The relation between the applied and experimental values are illustrated in Fig. V. In the application of the equations by using these formulae, the best applicable equation to the experimental data of each group is quite constant for different ranges of concentration of oil as it was in the application by using method *B*. The calculated results are given in TABLES CLXVII—CLXXII.

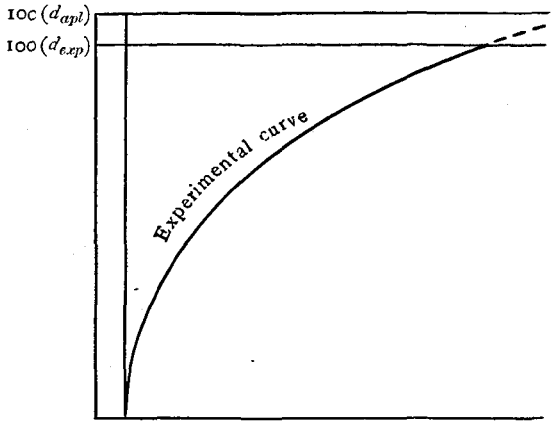


Fig. V

Carbon tetrachloride and soy bean oil mixture

The equations were applied to the data by method C and the applicabilities were compared with each other by the results given in TABLE CLXVII. As shown in this table equation I is better applicable than II.

Chloroform and soy bean oil mixture

The result of application is given in TABLE CLXVIII. In this case equation I is better than II, though the difference is not satisfactorily small.

Acetone and soy bean oil mixture

The equations were applied and compared as in the previous case. As shown in TABLE CLXIX equation II is much better applicable than I.

Petroleum ether and soy bean oil mixture

The applied result is given in TABLE CLXX. In this case equation II is also better than I.

Ether and soy bean oil mixture

As given in TABLE CLXXI, equation II shows not only slight differences, but also the satisfactory occurrence of the positive or negative difference.

Benzene and soy bean oil mixture

The result of application of the two equations is given in TABLE CLXXII. In this case equation II is also better than I.

As examined above in the applied results obtained by method C equation I is better applicable than II to the experimental data obtained with the binary mixture of soy bean oil and carbon tetrachloride, and also to the data obtained with the mixture with chloroform, while equation II is better applicable than equation I to the data obtained with other solvents. The best applicable equation for the data of each experimental result is the same in two cases of application by methods B and C. Method C is usually more satisfactory than method B with few exceptions. Moreover, to study the applicability of the equations even for one experimental result, extraordinary troublesome calculation is necessary in applying them by method B.

(iii) Discussion on the relation between the applicable equation and the kind of solvent in the binary mixture

The author applied the equations, $\frac{1}{V^{K'}}\log\frac{a}{a-d}=K$ and $\frac{1}{V^{K'}}\frac{d}{a(a-d)}=K$, to the experimental data of specific viscosity obtained with various binary mixtures of soy bean oil by various methods of application. The results of the application showed that method A was not satisfactory because even the best applicable equation for the data of an experimental result was sometimes uncertain for different ranges of concentration of oil. The results calculated by method B or C gave generally smaller differences, $V_{apl}-V_{cal}$. In general method C seems to be better than method B, although the better applicable equations determined by both methods for an experimental result are same. The calculated results did not always show the perfect applicability of the equations. However, the applicability of the equations is certainly and necessarily more satisfactory than that of any equation hitherto proposed. The best applicable equation for each binary mixture of soy bean oil which is determined by method B and C is given below with its solvent.

$\frac{1}{V^{K'}} \log \frac{a}{a-d} = K$: carbon tetrachloride, chloroform.

$\frac{1}{V^{K'}} \frac{d}{a(a-d)} = K$: acetone, petroleum ether, ether, benzene.

Among these solvents, petroleum ether is a mixture of various hydrocarbons. The structures of other solvents with each applicable equation are given below.

Equation I	Carbon tetrachloride	CCl_4
	Chloroform	$CHCl_3$
Equation II	Acetone	CH_3–CO–CH_3
	Ether	C_2H_5–O–C_2H_5
	Benzene	C_6H_6

The first two solvents belong to methane series. Equation II is applicable well to the binary mixture of soy bean oil and benzene or to that of soy bean oil and a chemical which has two $-CH_3$ or two $-C_2H_5$ groups such as acetone or ether respectively. The equations, $\frac{1}{t^{K'}} \log \frac{a}{a-x} = K$ and $\frac{1}{t^{K'}} \frac{x}{a(a-x)} = K$, and their modified forms were already proposed by the author as generally applicable equations in the kinetics of enzymes.* Now, the other modified equations, $\frac{1}{V^{K'}} \log \frac{a}{a-d} = K$ and $\frac{1}{V^{K'}} \frac{d}{a(a-d)} = K$, make one suppose that they have close relation to the viscosity of a binary mixture of soy bean oil and its solvents. When it is stated by the idea of Errera which was already reviewed on page 315, the change of the specific viscosity of the mixture of oil and a methane series, a non-polar substance, according to the concentration of oil, follows equation I, while that of the mixture of oil and the other solvent which is supposed to have symmetrical poles, follows equation II. Consequently, it is anticipated that the polarity of a solvent has much to do with the viscosity of the mixture of the oil and its solvent. If one considers that in the above equations when $K'=1$, the equations of the first and second order, $\frac{1}{V} \log \frac{a}{a-d} = K$ and $\frac{1}{V} \frac{d}{a(a-d)} = K$, result, an intimate relation between the equations and the kind of solvent in a binary mixture may

* The author has been reporting on this subject since April, 1931, on the Journ. Agr. Chem. Soc. (Japan).

be inferred. It can not be made clear in the binary mixture whether the oil, a mixture of glycerides of various fatty acids, is in a molecular particle or is in two separated groups, one is glycerin group which is not miscible with a solvent of oil and the other a group of fatty acids which are miscible with a solvent. At any rate it can be easily supposed from the result of the author's application of the equations that the oil exists in different forms in a binary mixture according to the kind of a solvent. It is quite natural to derive a thought that the viscosity of a binary mixture has an intimate relation to the chemical structure of both components, which however, has been almost neglected of a legitimate study. The author's application of the equations is quite independent of any study ever previously reported. Some new fundamental idea in the field of such investigation may be suggested from the author's investigation.

Summary and Conclusion

1. The distribution of nitrogen for different solvents, water; 10% saline solution; 0.2% NaOH solution, and nitrogen in the residue were determined with soy beans, soy bean meal and soy bean cakes (round and flat). Glycinin, the principal protein of soy beans, is denatured almost entirely into a glutelin-like protein which is soluble in NaOH solution through the industrial process of oil extraction. Thus about one half of total nitrogen of soy bean meal or of soy bean cake (round) is soluble in 0.2% NaOH.

2. On the physicochemical properties, natural glutelin of soy beans and the glutelin of soy bean meal were compared as follows; Let the former be denoted "N," the latter by "D," and glycinin by G. In elemental composition, G is highest, while "D" and "N" are lower in nitrogen and hydrogen contents. "D" is highest in carbon content and lowest in oxygen. From these facts, it is recognized that denatured glycinin originates from glycinin through two principal chemisms, deamination and hydration. It requires a greater quantity of HCl for "D" than for "N" to bring about the maximum turbidity of their alkali solutions. The free amino nitrogen content of "N" is higher than that of "D." The rotatory power of the alkali solution of "N" is higher than that of "D."

3. The influence of freezing upon the denatured glycinin of soy bean meal in alkali or various acid solutions was examined. The influence upon dilute sodium hydroxide solution was as follows:

(a) Freezing at $-7°$——$16°$C. for several days does not give the ultimate influence of freezing upon the protein solution.

(b) Some part of protein becomes insoluble, and is hard to dissolve again.

(c) The quantity of HCl needed to bring about the maximum turbidity of frozen sample of protein solution is smaller than that needed in case of not frozen sample.

(d) Sol state of protein solution changes into gel state through freezing.

(e) After the frozen sample melts, it slowly recovers its sol state even at below room temperature and the viscosity of the sample which was once increased by freezing decreases and approches that of the control.

(f) The melted sample which has been repeatedly frozen and melted is easily changeable into gel again at below the transition temperature. This transition point of a solution containing 0.05 g. nitrogen in 10 cc. is $-3°$C.

(g) The influence of freezing upon protein solution of hydrochloric, tartaric and citric acids was examined with the following results:

The acid solution of protein does not become viscous by freezing. This phenomenon is remarkably different from that of sodium hydroxide solution of protein. Especially much protein precipitates from the solution of HCl through freezing.

4. Differences of physicochemical properties between normal and the denatured glycinins were studied comparing necessarily with the differences between normal casein and its denatured form.

(a) The solubilities of both glycinins in various acid solutions in an ice-box were determined and compared. Solutions of different concentrations of hydrochloric, acetic, formic, oxalic malonic, tartaric and citric acids were used. In all cases the denatured glycinin is much less soluble than the normal with few exceptions in the case of tartaric acid. The difference of solubility between different proteins is remarkable in low concentrations but not so remarkable in high concentrations. The solubility of both glycinins in acetic, formic, malonic, tartaric and citric acids increases with the increase of concentration of acid while in the cases of hydrochloric and oxalic acids each certain concentration has the maximum solubility power, i.e., the lower or higher the concentration the lower the solubility. The filtration of a solution saturated with either normal or denatured glycinin is usually impossible over a limit of concentration of solvent. Denatured glycinin brings about the

impossibility of filtration with a smaller quantity than in case of the normal.

(b) The refractive index of acid or alkali solution of the denatured glycinin is higher than that of the normal.

(c) The rotatory power of the denatured glycinin or casein in alkali solution is higher than that of the corresponding normal protein.

(d) The denatured glycinin or casein is richer in free NH_2 and free COOH than the corresponding normal protein.

(e) The turbidity and surface tension of protein alkali solution which resulted by the addition of HCl was determined and comparison made between the normal and denatured glycinins or caseins. The quantity of HCl which is necessary to give the maximum turbidity or surface tension to the denatured glycinin or casein solution is less than that for the corresponding normal protein. So the apparent isoelectric points were measured by the electric method and compared with each other. The isoelectric point of the denatured protein is distinctly more acidic than that of the corresponding normal protein.

(f) The viscosity of protein alkali and acid solutions was determined. As a solvent of protein, sodium hydroxide, hydrochloric, oxalic, acetic, formic, malonic, tartaric and citric acids at different concentrations were taken. The viscosity of the denatured glycinin or casein is always higher than that of the normal. From this fact the ionization degree of the denatured protein is inferred to be higher than that of the normal. Such an inference agrees with the results of determination of free NH_2 and COOH groups.

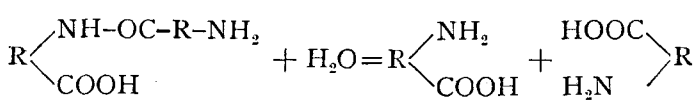
(g) In the decomposition of the glycinins with HCl, H_2SO_4 or NaOH of various concentrations, various types of nitrogen such as ammonia, amino nitrogen and digested form of the normal glycinin were compared with those of the denatured. The results show that in the decomposition by HCl the normal protein always evolves more ammonia than the denatured. In case of 5% HCl, the quantity of free amino nitrogen obtained with the denatured glycinin, both in absolute quantity and in percentage of total nitrogen, is more than that obtained with the normal. But in case of 10% HCl, the denatured glycinin releases more free amino nitrogen than the normal in decomposition of less than a half quantity of substrate, while in decomposition of over that quantity it is the contrary. In case of 20% HCl, the difference between the two kinds of protein is large at the beginning of decomposition, but the difference becomes gradually less, and in the

decomposition of over the half quantity there is almost no difference. The digested protein obtained with the normal glycinin, may be regarded as more than that with the denatured, however the percentage of total nitrogen is higher in the decomposition of the latter protein. The same tendency of decomposition of both proteins as in the case of HCl is observed in case of H_2SO_4. The result of decomposition by NaOH is observed as follows: The quantity of each form of nitrogen obtained in the decomposition by 5% NaOH is larger in the normal protein. It is the same in the decomposition by 10% NaOH, but at the end of decomposition for 30 hours, the percentage of each form of nitrogen in the denatured protein becomes about equal to that of the denatured.

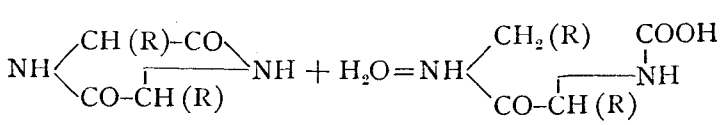
The quantity of each product obtained with a reagent of a certain concentration is compared with that obtained with another reagent of the same concentration as follows: Ammonia evolves remarkably much in case of NaOH, moderately and least in cases of H_2SO_4 and HCl respectively. It is a matter of course that ammonia evolves remarkably much in the decomposition by NaOH which is due to the evolution of a half arginine nitrogen. Free amino nitrogen evolves most in the decomposition by HCl, moderately in that by NaOH and least by H_2SO_4. The digested protein decreases in the order HCl, H_2SO_4 and NaOH.

5. From the results of various experiments and considering also some theories about the molecular structurse of protein which have been widely accepted, the author discussed and inferred the possible changes of molecular structures of glycinin in the industrial preparation of soy bean meal. These changes are classified into four kinds as follows:

(a) For the increase in free NH_2 and free COOH and decrease in keto group:



(b) For the increase in free COOH only and deerease in keto group:



(c) For the increase in free NH_2:

$$-CH_2-NH-C(NH)- + H_2O = -CH_2-NH(OH)\overset{|}{C}-NH_2$$

(d) For the hydration process at amide linkage:

$$-CONH_2 + H_2O = -COOH + NH_3$$

$$-CO\ NHOC + H_2O = -CONH_2 + -COOH$$

$$-CO\ NHOC + 2H_2O = 2-COOH + NH_3$$

These possible changes increase the terminal groups of NH_2 and COOH or enol group and decrease the keto group in denatured glycinin.

7. The degree of decomposition of normal glycinin by pepsin, pancreatin and papain was compared with that of the denatured. Peptic digestion of the normal glycinin is more rapid but digestion by the other two enzymes is slower. As a tryptic enzyme such as pancreatin is recognized as breaking up the anhydride structure of protein, the denatured glycinin may be supposed to be on the way to destruction.

8. To the data obtained in enzymic decomposition some equations were applied and the results of application were examined and compared. The results will be given below where G and D will denote the value of K or K' of any equation for the normal and denatured glycinins respectively.

(a) *For the decomposition taking time as a variable*

The decomposition by pepsin or papain follows satisfactorily Nakamura's equation, $\frac{1}{t^{K'}} \frac{x}{a(a-x)} = K$ (II), but that by pancreatin follows an equation, $\frac{1}{t^{K'}} \log \frac{a}{a-x} = K$ (I), which was formulated by the present author. For the present author's data the values of G and D of the best applicable equation are compared as follows:

Pepsin			Pancreatin			Papain		
	K	K'		K	K'		K	K'
II	G>D	G>D	I	G>D	G<D	II	G>D	G<D

(b) *For the decomposition taking the relative quantity of enzyme as a variable*

The decomposition follows either the equation, $\frac{1}{E^{K'}}\log\frac{a}{a-x}=K$ (I) or $\frac{1}{E^{K'}}\frac{x}{a(a-x)}=K$ (II), where E denotes the relative quantity of enzyme. These two equations are obtained by the modification of the preceding two equations. For the author's data obtained with papain the applicable equations were not the same for the normal and denatured glycinins, e. g., equation II was better for the former and equation I was better for the latter. The values of G and D of the best applicable equation I to the data obtained with pepsin and pancreatin are compared as follows:

Pepsin		Pancreatin	
K	K'	K	K'
G>D	G<D	G<D	G<D

(c) *For the decomposition taking the quantity of substrate as a variable*

The data of each experiment follows either $\frac{1}{S^{K'}}\log\frac{a}{x}=K$ (I) or $\frac{1}{S^{K'}}\frac{a-x}{a\cdot x}=K$ (II), in which S denotes the quantity of substrate and x the percentage of decomposed substrate. These two equations are also originated from $\frac{1}{t^{K'}}\log\frac{a}{a-x}=K$ and $\frac{1}{t^{K'}}\frac{x}{a(a-x)}=K$ respectively. In case of the decomposition by pancreatin the best applicable equation was II for the normal and denatured glycinins. The best applicable equation in the case of each enzyme for the two proteins and the values of G and D are compared as follows:

Pepsin			Pancreatin			Papain		
	K	K'		K	K'		K	K'
II	G<D	G<D	II	indefinite	G<D	I	G>D	G<D

9. For the data of decomposition obtained in experiment of any kind with one kind of substrate and one kind of enzyme, only one of the above two equations is always sufficiently applicable.

10. Studies on the decomposition of soy bean oil by papain and castor bean powder taking 1) time, 2) relative quantity of enzyme or 3) the quantity of substrate as a variable were made. Castor bean powder is more active than papain.

11. As to the data of proteolytic decomposition of the normal and denatured glycinins the same equations were also applied to the data of lipase digestion of the oil. The sufficiently applicable equation to the data of each experiment is given below where I and II denote the same equations which were shown under (a), (b) and (c) on pages 348 and 349.

Variable	Papain	Castor bean powder
Time	II	I
Relative quantity of enzyme	I	I
Quantity of substrate	I	II

12. The binary mixtures of soy bean oil at various concentrations were made with carbon tetrachloride, acetone, petroleum ether, benzene and chloroform. The specific viscosity of the mixture was measured by an Ostwald's viscosimeter. To the experimental data the equations, $\frac{1}{V^{K'}}\log\frac{a}{a-d}=K$ and $\frac{1}{V^{K'}}\frac{d}{a(a-d)}=K$, were applied using the values of V_{apl}, or of V_{apl} and d_{apl} calculated by three different methods, A, B and C as will be given below. The applicabilities of the equations were thus compared between different methods and between the two equations. In the formulae which will be given in each method, the experimental values will be represented by V_{exp}; the value of viscosity of a solvent, by V_s; the values of V and d which are used directly in application, by V_{apl} and d_{apl} respectively.

Method A: A method which is based on the treatment that the curve of experimental data and that of the equation are considered for the same axis of abscissa

Several formulae were proposed under the supposition above mentioned for calculating the values of V_{apl} as given below.

$$V_{apl}=V_{exp} \quad \cdots\cdots(1)$$

$$V_{apl}=V_{exp}-V_s \quad \cdots\cdots(2)$$

$$V_{apl}=V_{exp}-V_s(a-d) \quad \cdots\cdots(3)$$

$$V_{apl}=V_{exp}-V_s\left(1-\log\frac{a}{a-d}\right) \quad \cdots\cdots(4)$$

$$V_{apl}=V_{exp}-V_s\left(1-\log\frac{a}{a-d}\right)^n \quad \cdots\cdots(5)$$

The values of V_{apl} of (3) or (4) gave the most satisfactory result in the application of the equations. No perceptible difference could not be seen between (3) and (4). Though the applicability of the equations was not quite perfect, the following points are recognized in the application of this method.

(i) A certain equation was always better applicable than the other in spite of the range of concentration of oil in the binary mixture with some solvents. Such solvents with each applicable equation are given below.

Carbon tetrachloride	Acetone	Benzene
I	II	II

(ii) The best applicable equation was not same for different ranges of concentration of oil with any of following solvents:

Petroleum ether, ether, chloroform.

In both cases of (i) and (ii), the differences, $V_{apl}-V_{cal}$, were very small when the equations were applied to the data of concentration less than 70 or 80% oil mixture. If the concentration over this limit was taken, the difference for that term was especially large.

Method B: A method based on the treatment that the curve of an experimental result and that of the equation are considered for the same axis of ordinate and different axis of abscissa

The experimental curve and the ordinate axis were supposed to intersect at a point below the original. The point on the ordinate axis was supposed to be the original point of the coordinate-axes for the equation. On the other hand, in the application of the equations whatever values of d and V are used, the calculated value of V for 100% oil is always an infinite value, $+\infty$. Therefore to get a finite value of V_{cal} for 100% oil, the experimental curve was considered for certain new coordinate-axes. Let the value between two original points above

mentioned be represented by p. To the point for d_{exp} (100)$+p$ a value 90.00 was given in applying equation I, a value 90.909 or 99.0099, in applying equation II. In other words, the value of each d_{apl} is calculated by one of the following formulae :

$$d_{apl}=\frac{(d_{exp}+p)\,100}{(100+p)\,\frac{100}{90}}=\frac{(d_{exp}+p)\,90}{(100+p)} \quad \cdots\cdots (6)$$

for the application of equation I,

$$d_{apl}=\frac{(d_{exp}+p)\,100}{(100+p)\,\frac{100}{90.909}}=\frac{(d_{exp}+p)\,90.909}{(100+p)} \quad \cdots\cdots (7)$$

or

$$d_{apl}=\frac{(d_{exp}+p)\,100}{(100+p)\,\frac{100}{99.0099}}=\frac{(d_{exp}+p)\,99.0099}{(100+p)} \quad \cdots\cdots (8)$$

for the application of equation II. The values of V_{exp} are used directly in the application, that is, $V_{apl}=V_{exp}$. Formula (8) was generally more satisfactory than (7) with few exceptions.

Method C : A method which is based on the treatment that the curve of an experimental result and that of the equation are considered for the same axis of abscissa and different axis of ordinate

In this method, the formulae, $V_{apl}=V_{exp}-V_s$ (for both equations), $d_{apl}=d_{exp}\times 0.9$ (for equation I) and $d_{apl}=d_{exp}\times 0.990099$ (for equation II) are used.

13. The applicability of the equation resulted by method B is always higher than that resulted by method A. Method C gives generally more satisfactory result than method B. In the results obtained by methods B and C for the data of an experimental result, the satisfactory equations are always same. As for the kind of solvent of the binary mixture of soy bean oil, equation I is better applicable than II for carbon tetrachloride and chloroform which are methane series. Equation II is better applicable than I for other solvents, which, except petroleum ether, have two alkyl groups each. Some relation between the viscosity of a binary mixture and the polarities of components was pointed out by Errera as already reviewed. Here the present author proposes new equations and the method of their applica-

tion quite independently from any study ever reported. From the results of such application it is certainly known that the equations have nearly satisfactory applicability for the data of specific viscosity of binary mixture relating closely to the chemical structure of components. Consequently, some new fundamental idea in the field of such investigation may be suggested.

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