

A STUDY ON COMBINATION OF GLUTELIN WITH CADMIUM IN RICE GRAIN

Masumi MORITSUGU

This paper deals with the relation between rice glutelin and cadmium which is usually found in rice in a very small amount. The results of this investigation, in which a rice sample containing a considerable amount of cadmium is used, are as follows: First, cadmium in rice is easily soluble in each dilute solution of hydrochloric acid and sodium hydroxide. Secondly, it is hardly soluble in water, sodium chloride solution, ethyl alcohol and ethyl ether. Thirdly, cadmium is not found in the refined rice glutelin which is purified by the repetition of alkali dissolution followed by acid precipitation. And fourthly, about eighty percent of cadmium derived from the initial sample is found in the rice glutelin precipitated at pH 6.0 from the extracted solution of dilute sodium hydroxide, but only a small portion of cadmium is found in the glutelin precipitated from the solution of pH lower than 5.0.

From the above finding, it is considered that cadmium have no relation with albumin, globulin, prolamin, fat and carbohydrate. In contrast to these organic substances, it may be concluded that glutelin in rice grain combines with cadmium, unless the intracellular solution of rice is so acidic that cadmium separates from the glutelin.

As regards the biochemical properties of cadmium in animal tissues, some valuable works have been made, on the cadmium containing protein extracted from equin kidney (Margoshes and Vallee 1957, Wacker and Vallee 1959, Kägi and Vallee 1961), on the cadmium binding in human body, assumed from cadmium intoxication (Mačák et al. 1954), and on the experimental binding of cadmium with albumin (Perkins 1961). However, we have only scanty information for plant materials.

We studied on the cadmium content of rice, and indicated that the rice containing 1 ppm or more of cadmium was being produced in zinc poisoned districts (Moritsugu and Kobayashi 1964). On that occasion, it was questioned what compounds combined with such a considerable amount of cadmium in rice grain. Hence, an investigation on the above question was made. But, as described in the former report (Moritsugu and Kobayashi) concerning cadmium distribution in rice grains, a considerable portion of cadmium was found in the part where a large portion of protein was present, i. e., cadmium content was nearly in proportion to protein content in rice grains. Consequently, this investigation is mainly directed to the relation between rice protein and cadmium.

MATERIAL AND METHODS

For fear of obtaining indistinct results, we used a rice sample containing a considerable amount of cadmium. It was obtained in 1961 from the Jintsu river basin in Toyama Prefecture where the severe damage from zinc and lead pollution of Kamioka Mine had been observed during the Second World War. It was a nonglutinous rice, but the variety name was unknown. The rice sample (unpolished rice) had been kept in an air dried condition in our laboratories from the harvest time to the beginning of analysis. The cadmium content of the sample was 0.63 ppm for unpolished rice and 0.58 ppm for polished rice which was prepared from the unpolished rice by the following method.

Before analysis, the unpolished rice was polished by a test cleaning machine, until the weight of polished rice decreased to 90 % of the unpolished rice (Moritsugu and Kobayashi). The polished rice was ground to rough flour less than 1 mm in diameter by a pulverizer for laboratory use.

Organic substances were extracted from this rice flour by use of an electric mixer. In the extraction care must be taken not to take time for the operation, and to prevent the degeneration of the extracted substances. As the suspension of rice flour is liable to become syrupy during glutelin extraction, the ordinary procedure of extraction (Agr. Chem., Kyoto Univ. 1952, Fig. 1) was simplified to overcome this difficulty. In the other words, a rough and rapid separation was adopted in this investigation. Therefore, the organic substances were not separated accurately.

The rice flour was transferred into a mixer glass together with a suitable extractant for each of the organic substances. After homogenization, the emulsion was separated into two phases, i. e. a supernatant liquid and a precipitate, by a centrifugal separator. A suitable volume of the supernatant liquid, containing the extracted organic substances, was used for cadmium determination. This extraction procedure will be described subsequently in detail.

The cadmium determination was made on the ash of the extracts by the colorimetric method of Saltzman (1953), slightly modified (Moritsugu 1964), and also by the square-wave polarography (Saino and Kobayashi 1963). Additionally, in the case of polarography, copper, lead and zinc were simultaneously determined with cadmium.

It is a matter of course that all the reagents employed in this investigation are the analytical grade. Water was purified by a pure water producer of mono-bed type with a mixture of cation and anion exchanging resins in one cylinder. From the blank determination along with the determination on the sample, it was observed that any other reagents and water gave no effect upon the analytical results excepting chloroform. Sometimes, dithizone was partially decomposed by chloroform; however, the trouble was eliminated by a precautional test.

The main equipments employed in this investigation were as follows; a square-

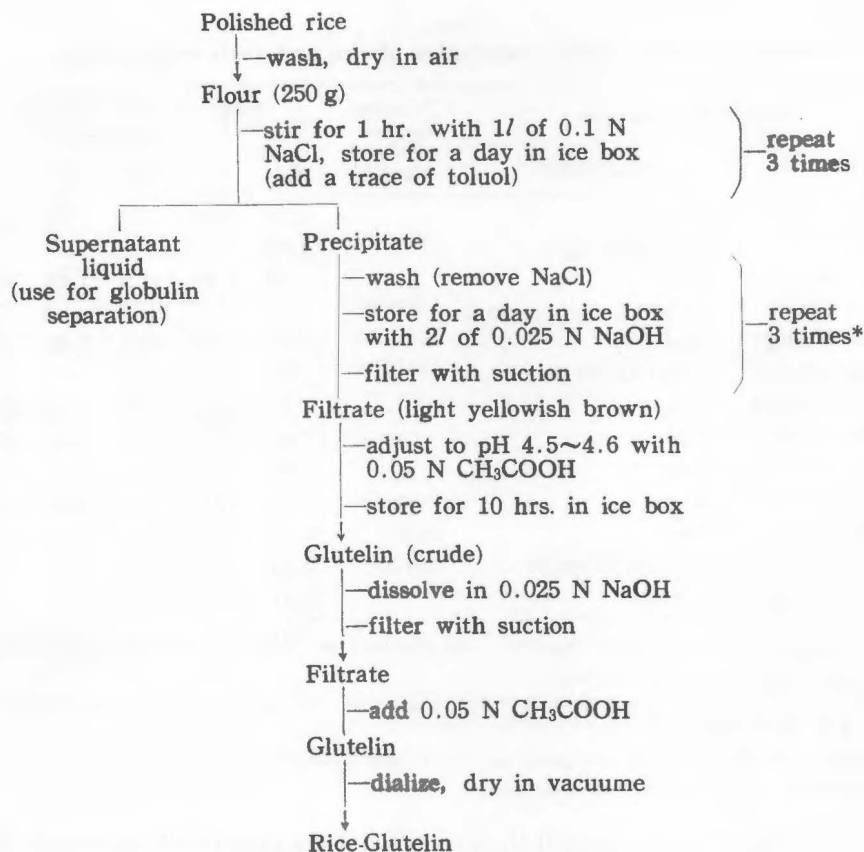


Fig. 1. Procedure of rice glutelin extraction by ordinary method (cited from Agr. Chem., Kyoto Univ. 1952)

*Starch becomes syrupy during this stage.

wave polarograph, a photoelectric spectrophotometer of Beckman type, a glass electrode pH meter, an electric muffle furnace with an automatic temperature controller, a centrifugal separator with maximum power of $17500 \times g$, an electric hot wind dryer, a test cleaning machine for rice polishing, a pulverizer for laboratory use, an electric mixer for domestic use, a shaker for separatory funnel and some other glasswares. The glasswares were washed with dilute hydrochloric acid and water.

RESULTS AND DISCUSSION

1. Cadmium Content in Several Extracts from Rice

The extraction was made on 50 g of the rice flour. The sample was transferred into a mixer glass together with 200 ml of the extractant shown in Table 1. After 5 minutes of vigorous stirring, the emulsion was separated into a

TABLE 1
Relation between organic substances of rice and their metal content

Method of preparation		Principal organic substance separated	Metal content ($\mu\text{g}/5\text{ g rice}$)				
Extractant	Precipitator		Colorimetric Cd	Polarographic			
			Cd	Cd	Cu	Pb	Zn
H ₂ O	None	Albumin	0.33	0.36	9.8	1.19	15.9
H ₂ O	CCl ₃ COOH (10%)		0.02	—	—	—	—
0.5 N NaCl	None	Globulin	0.33	0.30	18.3	1.89	30.9
0.5 N NaCl	(NH ₄) ₂ SO ₄ (half saturated)		0.24	—	—	—	—
0.025 N NaOH	None	Glutelin ¹⁾	2.02	1.89	44.1	3.65	56.6
0.025 N NaOH	dil. CH ₃ CCOH (to pH 4.5)		1.01	—	—	—	—
0.1 N NaOH	None	—	2.28	2.51	29.7	3.49	69.5
0.025 N HCl	None	—	2.41	2.44	9.9	0.41	87.4
0.1 N HCl	None	—	2.96	—	—	—	—
80% C ₂ H ₅ OH	None	Prolamin	0.00	0.00	3.9	0.03	10.6
C ₂ H ₅ -O-C ₂ H ₅	None	Fat ²⁾	0.00	—	—	—	—
Insoluble portion of 0.025 N NaOH		Starch ³⁾	0.05	—	—	—	—
5 g of initial rice flour		—	2.91	—	—	—	—

1) Other proteins are extracted together with glutelin, but glutelin content in polished rice is usually higher than other proteins.

2) Fat was extracted with a Soxhlet extraction apparatus, and analyzed on an amount equivalent to 5 g of rice flour.

3) White medium layer of precipitate in centrifugal collected was taken, and the amount equivalent to 5 g of rice was analyzed.

supernatant liquid and a precipitate by a centrifugal separator for 10 minutes at 2730 $\times g$. The cadmium determination was made on 20 ml of the supernatant liquid (or the precipitate from the equivalent volume of the supernatant liquid by addition of a suitable precipitator) which was equivalent to 5 g of the initial rice flour. The liquid was transferred into a ceramic crucible and gently evaporated to dryness on a sand bath. The residue was ignited in an electric muffle furnace set as 450°C until the carbon-free ash was obtained, by the aid of repetition of nitric acid moistening followed by muffle ignition. Cadmium in the ash was determined by the colorimetric or polarographic method which was described before.

As a result of this experiment, it has been clarified that cadmium in polished rice is easily soluble in dilute hydrochloric acid and dilute solution of sodium hydroxide, but it is hardly soluble in water, sodium chloride solution, 80 % solution of ethyl alcohol, and ethyl ether (Table 1).

Accordingly, it is considered that cadmium in rice has no relation to carbohydrate, fat, prolamin, globulin and albumin; however, there is a possibility that cadmium combines with rice glutelin. In other words, it is assumed that cadmium in rice is not present as a wandering impurity, and that the binding of cadmium with rice glutelin is rather chemical than physical.

Meanwhile, copper, lead and zinc, simultaneously determined by the square-

TABLE 2

Cadmium content of glutelin precipitated from the solutions of pH 6.0 and 4.5

pH ¹⁾		Cd content ($\mu\text{g}/5\text{g}$ rice)
6.0	Supernatant ²⁾	0.21
	Precipitate	2.01
4.5	Supernatant ²⁾	1.16
	Precipitate	1.01

1) pH was adjusted with dilute acetic acid.

2) By way of precaution, supernatant liquid was analyzed together with glutelin precipitate.

wave polarography, have some similarities to cadmium. However, the solubility of these metals in the several extractants have no peculiarity as in the case of cadmium. In other words, it is considered that these metals have no close relations with a specific organic substance as in the case of cadmium.

2. Cadmium Content of Glutelin Precipitated from the Solutions of Different pH

From the results of the above experiment, a possibility that cadmium in rice combined with rice glutelin was expected. For the confirmation of this possibility, cadmium in the rice glutelin purified from the extract of 0.025 N solution of sodium hydroxide by the repetition of alkali dissolution followed by acetic acid precipitation at pH 4.5 (Agr. Chem., Kyoto Univ.) was determined. But, no cadmium was found in this refined rice glutelin. Therefore, the accuracy of established purification method was unexpectedly demonstrated.

In this purification process, the first glutelin precipitate was separated from the extract of 0.025 N sodium hydroxide by adding dilute acetic acid to give a pH of 4.5. From the observation on that process, however, it was seen that the rice glutelin began to precipitate at pH about 6.5, and the major portion of the glutelin precipitated at the pH range 6.0 to 5.5.

As a preliminary experiment, accordingly, cadmium in the glutelin precipitated at pH 6.0 and at 4.5 was determined. By way of precaution, cadmium in each of the supernatant liquid was determined. These samples were prepared as follows. The mixer homogenization was made with 0.025 N solution of sodium hydroxide as described before, and the resultant emulsion was separated centrifugally. The pH of the supernatant liquid (20 ml) was adjusted separately to 6.0 and 4.5 with dilute acetic acid. The coagulated solution was centrifuged, and then the supernatant liquid and the precipitate was separately transferred into a ceramic crucible without washing. After gentle drying, ash was prepared by the dry method (Moritsugu). Cadmium was determined by the colorimetric method.

As shown in Table 2, it has been clarified that the glutelin precipitated at pH 6.0 contains the major portion of cadmium in the initial sample, whereas the other contains only about a half.

Thus, the experiment of the cadmium content of glutelin precipitated from

TABLE 3
Metal content of glutelin precipitated from the solutions of several pH

pH value	Dry weight of glutelin (mg/5g)	Metal content ¹⁾							
		Cd		Cu		Pb		Zn	
		(μ g/5g)	(ppm in glutelin)	(μ g/5g)	(ppm in glutelin)	(μ g/5g)	(ppm in glutelin)	(μ g/5g)	(ppm in glutelin)
6.5	152.5	1.14	7.5	5.5	36.1	1.60	10.5	25.9	169.8
6.0	243.3	2.45	10.1	6.2	25.5	2.18	9.0	40.4	166.0
5.5	244.8	1.22	5.0	7.5	30.6	2.91	11.9	33.9	138.4
5.0	241.3	1.19	4.9	6.2	25.7	2.76	11.4	41.7	172.8
4.5	244.0	0.94	3.9	8.6	35.2	2.85	11.7	19.1	78.3
4.0	221.7	0.23	1.0	5.2	23.5	1.88	8.7	9.3	42.9
6.0 ²⁾	trace	0.00	—	0.7	—	0.00	—	—	—
6.0 ³⁾	—	2.66	—	7.0	—	2.27	—	40.0	—
6.0 ⁴⁾	206.5	2.31	11.2	6.7	32.4	2.17	10.5	36.6	177.1
6.0 ⁵⁾	193.9	1.20	6.2	3.6	18.5	1.57	8.1	40.8	210.1

- 1) All the analysis were made by square-wave polarography. The figures of left side of each metal indicate the metal weight in glutelin derived from 5 g of rice (μ g/5g rice), while the right sides express the metal content in each of the dried glutelin (ppm in dried glutelin).
- 2) After the glutelin precipitate was removed at pH 4.0, the supernatant liquid was brought to pH 6.0 with dilute sodium hydroxide.
- 3) Precipitate was not washed.
- 4) Washing was made four times. All of the glutelins, excepting ³⁾ and ⁴⁾, were washed twice.
- 5) After the extraction with 0.1 N hydrochloric acid, the extract was neutralized to pH 6.0 with dilute sodium hydroxide. The precipitate was collected from this neutralized solution by centrifugation.

the solutions of several pH was made by use of the supernatant liquid of sodium hydroxide extract which was prepared from the above process. However, the time of the mixer homogenization was prolonged from 5 minutes to 10 minutes by way of precaution.

To 20 ml of the extract, dilute acetic acid was added in order to adjust the pH of the extracted solution to the following series, i. e. 6.5, 6.0, 5.5, 5.0, 4.5 and 4.0; the pH being measured with a glass electrode pH meter. The coagulated solution obtained from acidification was centrifuged as described before. The supernatant liquid was discarded, and the surface of each glutelin precipitate in the centrifugal tubes was carefully rinsed with water. The rinsings were discarded. After the addition of about ten ml each of pure water, gentle kneading by a glass rod was made. And the turbid solution was again centrifuged. This washing procedure was made twice; each of the washings being discarded. The final precipitate was transferred into a ceramic crucible. Transfer was completed by two washings. The solution in the ceramic crucible was gently heated in an electric hot wind dryer, set at 90°C. After weighing of dried glutelin, the cadmium determination was made on the ash prepared by dry method.

The result obtained is given in Table 3. It can be seen that the cadmium

content in glutelin is widely varied with the pH values at which glutelin is precipitated. Consequently, the glutelin precipitate obtained at pH 6.0 contains much cadmium, equivalent to about eighty percent of the initial sample, or equivalent to ninety percent or more of the alkaline extracted solution. However, the cadmium content in rice glutelin is rather low, and it is about ten ppm at maximum. In addition, it is observed that the cadmium content in glutelin precipitated from the solutions of lower pH decreases as the pH value is lowered. And compared with the result shown in Table 2, water washing process gives a small loss of cadmium to the glutelin precipitated at pH 6.0, whereas the remarkable loss is observed on the glutelin precipitated from the solutions of lower pH. In other words, it is to say that the combination capacity between cadmium and rice glutelin varies markedly with the pH of the solutions at which glutelin is separated.

In regard to copper, lead and zinc, simultaneously determined by the square-wave polarography, it is observed some similarities to cadmium in the relation between the metal content and the pH of the solution at which glutelin is separated. However, the effect of pH value on the metal content is a little smaller than in the case of cadmium, i. e. the specific pH range for the maximum metal content of the precipitate is uncertain as seen in Fig. 2. Among these metals, zinc shows similar character with cadmium. However, as shown in Table 1, zinc in rice is more soluble in water and sodium chloride solution than in the case of cadmium.

Therefore, it is considered that these metals have less close relation to glutelin, but a small portion of these metals may be combined with glutelin.

As can be seen in an additional remark ²⁾ in Table 3, it was examined whether or not a special protein was present in the supernatant liquid which separated the glutelin from the coagulated solution at pH 4.0. But no protein precipitate was obtained after the neutralization of the above supernatant liquid to pH 6.0 with dilute sodium hydroxide.

From the solubility test shown in additional remarks ³⁾ and ⁴⁾ in Table 3, it is observed that cadmium in glutelin precipitated at pH 6.0 is rather insoluble in water. In other words, it is considered that the loss of cadmium on water washing is mainly caused by the washing loss of glutelin and also by the decrease of impurities; because the cadmium content in dried glutelin washed four times ⁴⁾ is a little higher than that of twice washed glutelin, in spite of glutelin weight decreasing together with cadmium weight.

Furthermore, as shown in the other additional remark ⁵⁾ of Table 3, it was examined whether or not the glutelin containing cadmium is stable in the acid extract, i. e., the cadmium in the precipitate at pH 6.0 from the supernatant liquid prepared from the emulsion of the rice flour in 0.1 N solution of hydrochloric acid was determined. In this case, a considerable amount of cadmium is observed in the precipitate, equivalent to a little over forty percent of the initial sample, or of the extracted solution, as compared with the result in Table 1.

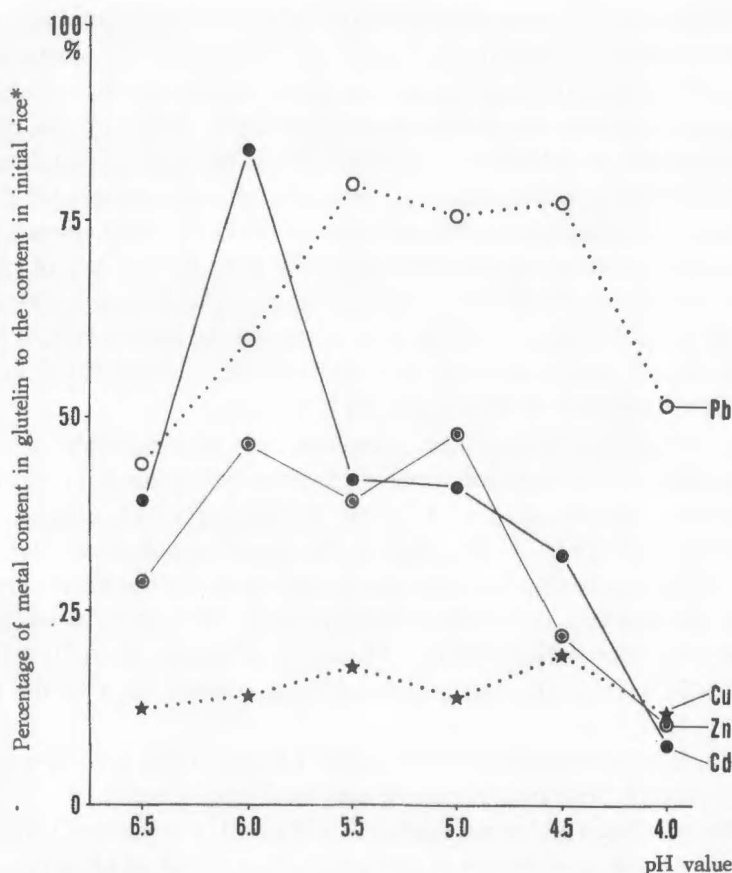


Fig. 2. Effect of pH on combination capacity of glutelin with metals.

*In case of cadmium, the content in initial rice ($2.91 \mu\text{g}/5 \text{ g}$ Table 1) was taken as 100 %. As for the other metals, the highest content of metals in Table 1 was supposed to be 100 %, i. e., $44.1 \mu\text{g}/5 \text{ g}$ for copper, $3.65 \mu\text{g}/5 \text{ g}$ for lead, $87.4 \mu\text{g}/5 \text{ g}$ for zinc were used as basis of calculation.

In consequence, it is to say that the glutelin containing cadmium resists acid considerably, and that the reaction of separation and combination between cadmium and glutelin seems rather reversible.

From the above investigation, the assumption may be given that cadmium in rice is combined with rice glutelin, because the greater part of cadmium in rice is easily soluble in the glutelin extractants, notwithstanding it is hardly soluble in the other extractants examined, and the pH value of the intracellular solution of rice falls probably within the range that cadmium can be combined with rice glutelin, i. e. nearly neutral. Even if we grant the above assumption, it is concluded that the rice glutelin contains the major portion of cadmium; because glutelin is the main protein in rice grain, and in regard to the cadmium distribution in rice grain, much cadmium is found in the part which contains a large portion of protein, as

described in the former report.

Additionally, it can hardly be said that such a cadmium protein extracted by Vallee et al. is found in this investigation, because the maximum cadmium content of rice glutelin precipitated from the sodium hydroxide extract at pH 6.0 is rather low, i. e. about ten ppm, and the stability of cadmium in the rice glutelin is insufficient, as compared with the results of Vallee et al.

SUMMARY

1) Using a sample which contains a considerable amount of cadmium (about 0.6 ppm for unpolished rice), we have investigated what organic substances combines with cadmium in rice grain.

2) The similar investigation is also carried out on copper, lead and zinc; a square-wave polarograph being used for the determination of these metals together with cadmium.

3) It seems that these metals, unlike cadmium, have no close relation to the specific organic substance. However, a small portion of these metals may have some relation with rice glutelin respectively.

4) From the solubility of cadmium in several extractants, it is considered that cadmium in rice has no relation with starch, fat, albumin, globulin and prolamin.

5) The bulk of cadmium is extracted in dilute sodium hydroxide (0.1 to 0.025 N) and dilute hydrochloric acid (0.1 N).

6) Although the refined rice glutelin contains no cadmium, the rice glutelin prepared from the extract of dilute sodium hydroxide by adjusting the pH to 6.0 contains a considerable amount of cadmium equivalent to ninety percent or more of the extract.

7) As regards the solubility of cadmium in the rice glutelin prepared from the dilute sodium hydroxide extract by adjusting the pH to 6.0, the metal is hardly soluble in water, but this is easily soluble in the dilute solution of weak acid such as acetic acid, needless to say in dilute hydrochloric acid.

8) While cadmium in glutelin precipitated at pH 6.0 is hardly soluble in water, cadmium in glutelin, prepared from dilute sodium hydroxide extract by adjusting the pH to lower values, is rather soluble in water.

9) The rice glutelin prepared from the extract of dilute hydrochloric acid by adjusting the pH to 6.0 contains about forty percent of cadmium in the initial sample or the extract.

10) As a result of the above experiments, it is considered that the relation between cadmium and rice glutelin is a sort of chemical combination rather than physical adsorption.

11) Furthermore, it may be given as a conclusion that the greater part of cadmium in rice combines with glutelin, unless the intracellular solution is

acidified so widely that cadmium separates from glutelin, i. e. below pH 5.0.

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LITERATURE CITED

- Kägi J. H. R. and Vallee B. L. 1961. Metallothionein: A cadmium and zinc-containing protein from equin renal cortex. *J. Biol. Chem.* 236: 2435—2442.
- Laboratory of Agricultural Chemistry, Kyoto University. 1952. 'Nogei-Kagaku Jikkensho' (in Japanese, Text Book for Experiment of Agricultural Chemistry). Vol. II, 3rd ed. p. 572, 'Sangyo-Tosho', Tokyo.
- Mačák V., Černoch M., Bartex J., Wiedermann D. and Šantavý F. 1954. Cadmium intoxication. *Lékařské Listy* 9: 27—30. cited from *Chemical Abstract* 48: 7783 a.
- Margoshes M. and Vallee B. L. 1957. A cadmium protein from equin kidney cortex. *J. Amer. Chem. Soc.* 79: 4813—4814.
- Moritsugu M. 1964. Interference on colorimetric microdetermination of cadmium with dithizone. (in Japanese) *Japan Analyst* 13: 64—66.
- Moritsugu M. 1964. An applied method for cadmium analysis of rice by colorimetric microdetermination with dithizone. (in Japanese) 'Nogaku-Kenkyu' (Report of Ohara Inst. Agr. Biol.) 50: 67—77.
- Moritsugu M. and Kobayashi J. 1964. Study on trace metals in bio materials, II. Cadmium content in polished rice. *Ber. Ohara Inst. landw. Biol.* 12: 145—158.
- Perkins D. J. 1961. Studies on the interaction of zinc, cadmium and mercuric ions with native and chemically modified human serum albumin. *Bioch. J.* 80: 668—672.
- Saino T. and Kobayashi J. 1963. Simultaneous quantitative determination of copper, lead, zinc and cadmium in polished rice by square-wave polarography. (in Japanese) 'Nogaku-Kenkyu' (Report of Ohara Inst, Agr. Biol.) 49: 189—216. (abstracted in *Ber. Ohara Inst. landw. Biol.* 12: A2, 1964)
- Wacker W. C. and Vallee B. L. 1959. Nucleic acids and metals, I. Chromium, manganese, nickel, iron, and other metals in ribonucleic acid from diverse biological sources. *J. Biol. Chem.* 234: 3257—3262.