STUDIES ON THE ARTIFICIAL DIGESTION ASSAY I. BASAL EXPERIMENT ON THE APPLICATION OF THE DIALYSIS METHOD

STUDIES ON THE ARTIFICIAL DIGESTION ASSAY I. BASAL EXPERIMENT ON THE APPLICATION OF THE DIALYSIS METHOD

By

Masao KAMETAKA and Kiyoo HATAKEYAMA

Department of Animal Husbandry, Faculty of Agriculture, Tohoku University, Sendai, Japan

(*Received May 31, 1952*)

In the later preliminary report,[1)2)] one of the writers has shown the results of the power of the digestive enzyme preparations of the domestic animal and of the power of the crude pepsin powder in the domestic animal examined by a one-dimensional diffusion method.

Then, the writers design a special dialysis apparatus, using three kinds of serous membrane of small intestine in horse, cattle and swine, and a parchment paper as dialysis membrane. In this paper we are investigating various conditions, under which glycine, chosen as the simplest form of amino acid, and three peptides are dialysed in our apparatus.

Experimental

Apparatus — Its construction details are shown in Fig. 1 and its operation in the photograph.

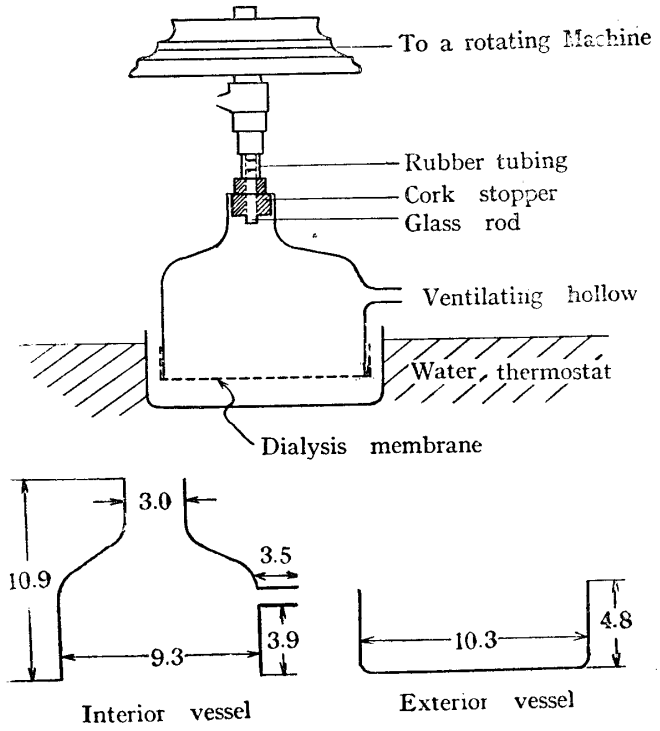
Dialysis membrane — The serous membrane of small intestine in horse is mainly used, comparing with the parchment paper and two serous membranes in cattle and swine. Serous membranes of small intestine are prepared according to the preparation method of casing[3)], used for sausage.

Method — First of all a dialysis membrane is stretched tight at the under side of the interior vessel. The residue of its membrane, wrapping the outside of this vessel, is closely tied with cotton thread. 200 ml. of distilled water is added into the exterior vessel and 100 ml. of the solution, containing certain weight of glycine (prepared by Merck Co. LTD.), into the above-mentioned interior vessel. Being connected to a rotating machine, the latter vessel is suspended in the former, as the surface of the glycine solution coincided with that of water in the apparatus.

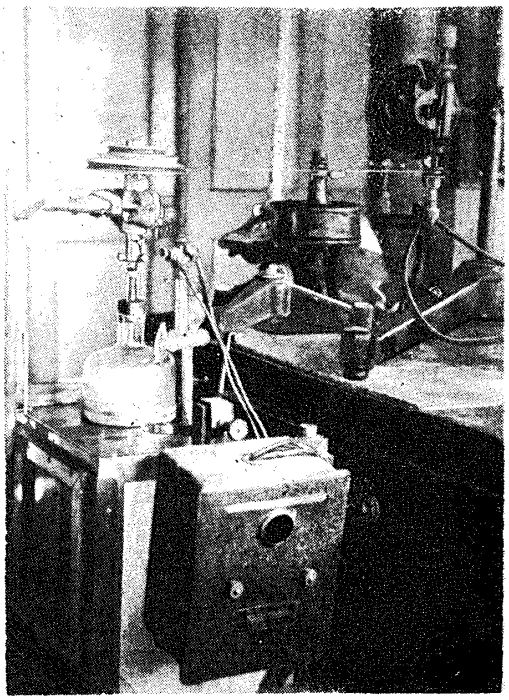
Determination — Glycine is determined by the formol titration method[4)]

Fig. 1 Construction details of dialysis apparatus.

(cm)



Photograph in operation



and nitrogen in peptides by the Kjeldahl method.

Calculation of dialysis coefficient — As the curve, obtained from the weight of dialysed solute every two hours resembles, in general, that of the Reaction of the First Order[5], we may calculate the velocity constant according to the Reaction, in order to compare easily the difference among their curves.

$$\frac{dx}{dt} = k\,(a-x) \qquad \ldots\ (\mathrm{I})$$

From which results k,

$$k = \frac{-\Sigma t \log y}{\Sigma t^2}$$

where

$$y = 1 - \frac{x}{a}$$

As it is seen that the area of dialysis membrane and the initial volume of dialysed solution are constant under our experimental conditions, k indicates the dialysis coefficient[6].

From Eq. (I)

$$a - x = 10^{-k't+c'}$$

$$x = 10^{c'}\,(1 - 10^{-k't}) \qquad \cdots\cdots\cdots \text{(II)}$$

When the coordinates of two points on the curve, for example, x_1 and t_1 as the 2nd hour, x_2 and t_2 as the 4th hour, are substituted to x and t in Eq. (II),

$$k' = \frac{-\log \dfrac{x_2 - x_1}{x_1}}{t_1}$$

indicates the approximative partial dialysis coefficient from the beginning to the 4th hour. k'' and k''' may be obtained, indicating these from the 4th hour to the 8th and from the 6th to the 12th respectively.

Then the standard value and its deviation may be calculated. Namely, from the following equation $x_c = 100\,(1 - 10^{-kt})$ the value of x_c is obtained. The σ-test is made according to the value of $\sigma = \sqrt{\dfrac{\Sigma\,(x - x_c)^2}{n - 1}}$. When $x_c + 2\sigma > x > x_c - 2\sigma$, the provability of experimental value as x is investigated closely.

Results

I) *Effect of Rotation on Dialysis Velocity*

a) When 0.3 g. of glycine in the interior solution is dialysed with rotation of none, 30 per min. and 60 per min. through the serous membrane of small intestine in horse and exterior water is exchanged for another 200 ml. of distilled water it every two hours, glycine dialysed in it is determined. Results obtained are shown in Table I and Fig. 2.

Table 1. Aggregate weight (%) of dialysed glycine, showing effect of rotation and dialysis coefficient when serous membrane in horse is used.

Hr. \ Rotation	None	30/min.	60/min.
2nd	21.67	29.44	31.79
4th	37.51	46.33	53.15
6th	50.15	58.67	66.85
8th	61.20	68.29	76.37
10th	68.81	75.78	83.05
12th	74.70	81.42	87.10
14th	79.61	85.72	90.29
Residue of solute	19.83	13.54	8.89
Total	99.44	99.26	99.04

Dialysis coefficient \ Rotation	None	30/min.	60/min.
k	0.05	0.06	0.08
k′	0.07	0.10	0.09
k″	0.05	0.08	0.09
k‴	0.05	0.07	0.08

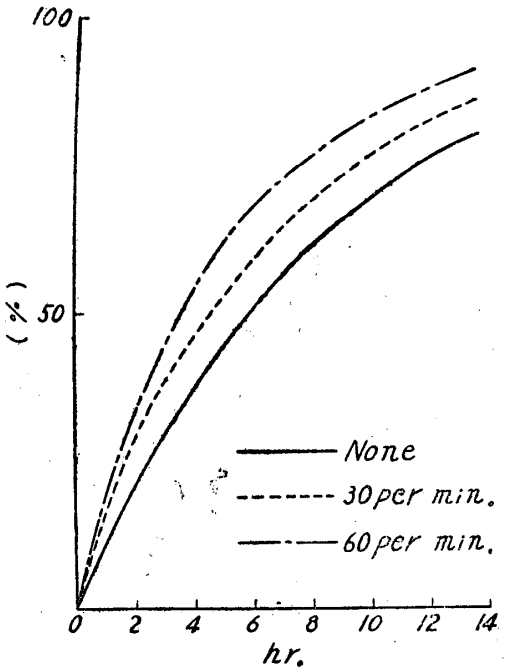


Fig. 2. Dialysis curve, showing effect of rotation when serous membrane in horse is used.

b) When the parchment paper is used as dialysis membrane in place of the serous membrane of small intestine in horse, glycine dialysed by the same method as a) is determined. Results obtained are shown Table 2 and Fig. 3.

Table 2. Aggregate weight (%) of dialysed glycine, showing effect of ratation and dialysis coefficient when parchment paper is used.

Hr. \ Rotation	None	30/min.	60/min.
2nd	24.34	32.71	36.29
4th	40.41	51.72	58.74
6th	54.90	65.46	73.79
8th	66.19	76.24	84.73
10th	74.32	83.87	92.29
12th	81.10	88.39	96.70
14th	85.43	91.08	99.28
Residue of solute	15.01	6.09	4.66
Total	100.44	97.16	103.94

Dialysis coefficient \ Rotation	None	30/min.	60/min.
k	0.06	0.08	0.11
k′	0.09	0.10	0.10
k″	0.05	0.08	0.09
k‴	0.05	0.08	0.08

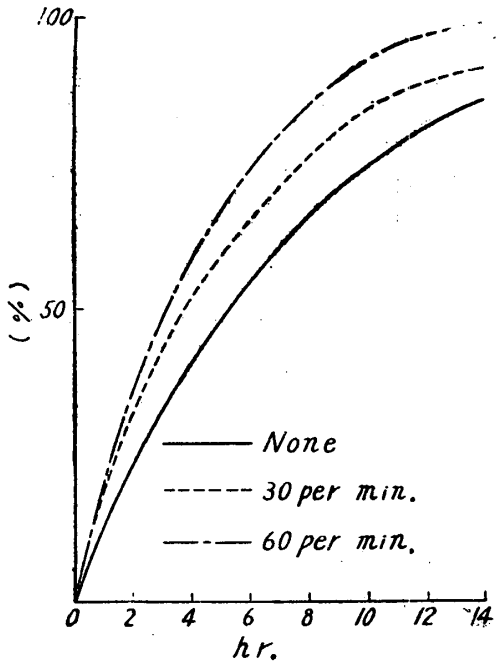


Fig. 3. Dialysis curve, showing effect of rotation when parchment paper is used

c) Effect of concentration of glycine in the interior solution on dialysis velocity is tested with rotation of 30 per min., using the serous membrane of

Table 3. Aggregate weight (%) of dialysed glycine, showing effect of concentration of glycine in interior solution on dialysis velocity and dialysis coefficient.

Hr. \ Concentration of glycine	0.2 g.	0.4 g.
2nd	25.64	28.46
4th	44.06	48.65
6th	57.47	62.74
8th	67.01	72.80
10th	73.68	79.93
12th	78.91	86.12
14th	83.15	92.01
Residue of solute	12.49	11.88
Total	95.64	103.89

Dialysis coefficient \ Concentration of glycine	0.2 g.	0.4 g.
k	0.06	0.07
k′	0.08	0.09
k″	0.07	0.08
k‴	0.07	0.07

small intestine in horse as dialysis membrane. Results obtained are shown in Table 3 and Fig. 4.

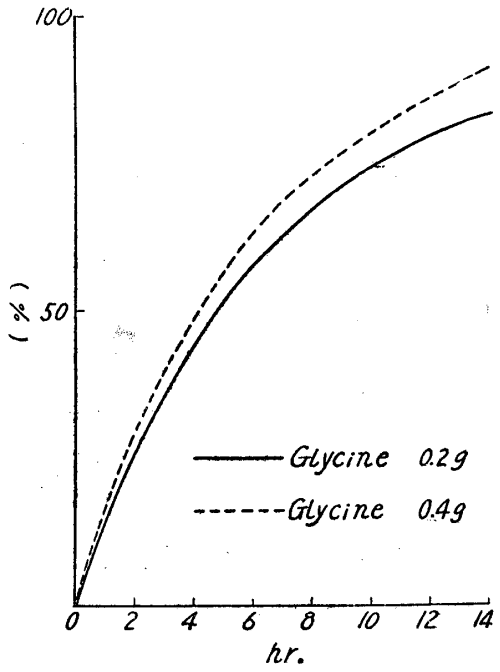


Fig. 4. Dialysis curve, showing effect of concentration of glycine.

II) *Dialysis Velocity in Case that two Kinds of Serous Membrane of Small Intestine in Cattle and Swine are Used as Dialysis Membrane*

In this test 0.3 g. of glycine in the interior solution is dialysed by the same method as a) in I) with rotation of 30 per min. Results obtained are shown in Table 4 and Fig. 5.

III) *Dialysis velocity of peptides*

Dialysis velocity of peptides — glycylglycine N; 21.20%, leucylglycine N; 14.88% and glycinetetrapeptide N; 22.76% — is estimated with rotation of 30 per min. by the same method as a) in I), provided that leucylglycine or glycinetetrapeptide is dissolved in 100 ml. of 0.2N HCl and afterwards pH of the solution is adjusted to 8, using anhydrous Na_2CO_3.

Table 4. Aggregate weight (%) of dialysed glycine and dialysis coefficient when two kinds of serous membane in cattle and swine are used.

Hr. \ Kind of serous membrane	Cattle	Swine
2nd	36.50	38.14
4th	57.35	57.65
6th	69.57	70.46
8th	78.95	80.59
10th	84.17	86.55
12th	87.59	90.27
14th	89.68	93.25
Residue of solute	6.26	6.40
Total	95.94	99.65

Dialysis coefficient \ Kind of serous membrane	Cattle	Swine
k	0.08	0.09
k′	0.15	0.20
k″	0.11	0.10
k‴	0.10	0.09

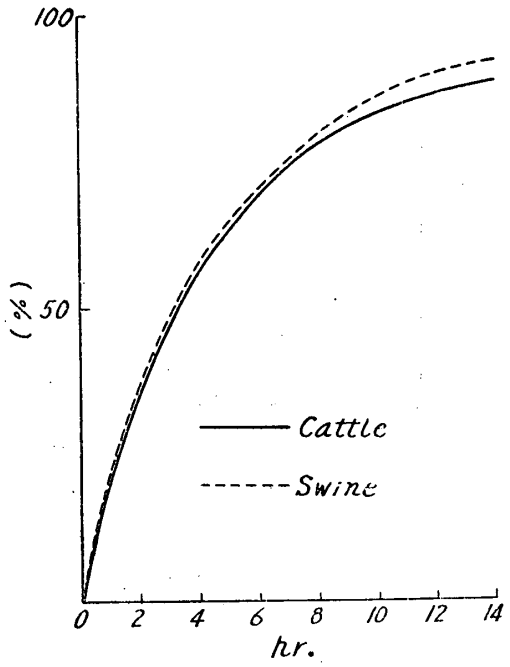


Fig. 5. Dialysis curve in case that two kinds of serous membrane in cattle and swine are used.

Results obtained in case that the serous membrane in horse is used as dialysis membrane are shown in Table 5 and Fig. 6.

Table 5. Aggregate weight (%) of dialysed peptides, using serous membrane in horse.

Hr. \ Peptide	Glycylglycine (0.202 g.)	Leucylglycine (0.230 g.)	Glycinetetrapeptide (0.200 g.)
2nd	14.48	16.42	11.89
4th	26.02	28.59	21.10
6th	35.51	38.49	28.77
8th	44.26	46.80	35.00
10th	51.26	54.04	39.41
12th	58.33	60.80	43.89
14th	64.47	67.42	47.92
Residue of solute	36.26	30.12	47.25
Total	96.73	97.54	95.17

( ) : weight of peptide used

These peptides don't indicate the Diketopiperazin Reaction[7], using picric acid, m-dinitrobenzene and o-dinitrobenzene as agent.

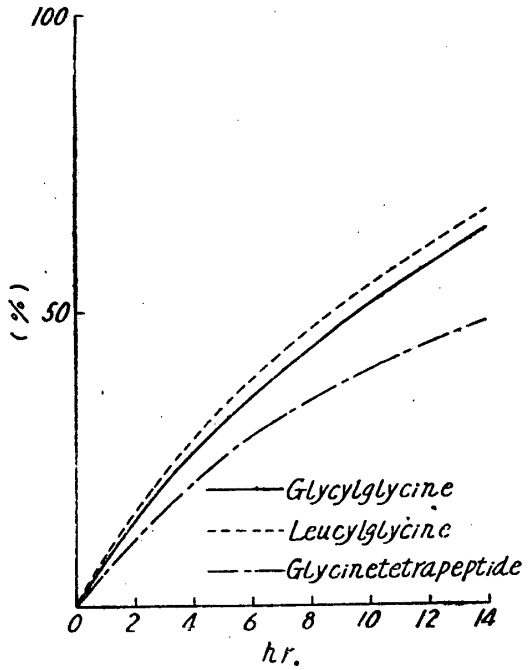


Fig. 6. Dialysis curve of peptides, using serous membrane in horse.

Results obtained in case that the parchment paper is used are shown in Table 6 and Fig. 7.

IV) *Test for Knowing the Hours Required to the Achievement of the Equilibrium Between the Concentration of the Interior and of the Exterior Solution.*

Certain weight of glycine in the interior solution is dialysed with rotation of 30 per min. After a lapse of 24 hours or more, glycine remaining in the interior and dialysed into the exterior solution is determined. It is knowu whether the equilibrium consists between the concentration of the interior and of the exterior solution by converting the weight of glycine in both solutions

Table 6. Aggregate weight (%) of dialysed peptides, using parchment paper.

Hr. \ Peptide	Glycylglycine (0.205 g.)	Leucylglycine (0.232 g.)	Glycinetetrapeptide (0.217 g.)
2nd	21.96	10.77	7.50
4th	39.27	18.19	12.52
6th	51.77	23.94	17.02
8th	63.55	28.02	21.28
10th	74.88	30.98	24.52
12th	81.90	33.63	26.00
14th	87.46	35.79	27.26
Residue of solute	12.23	64.11	72.55
Total	99.69	99.90	99.81

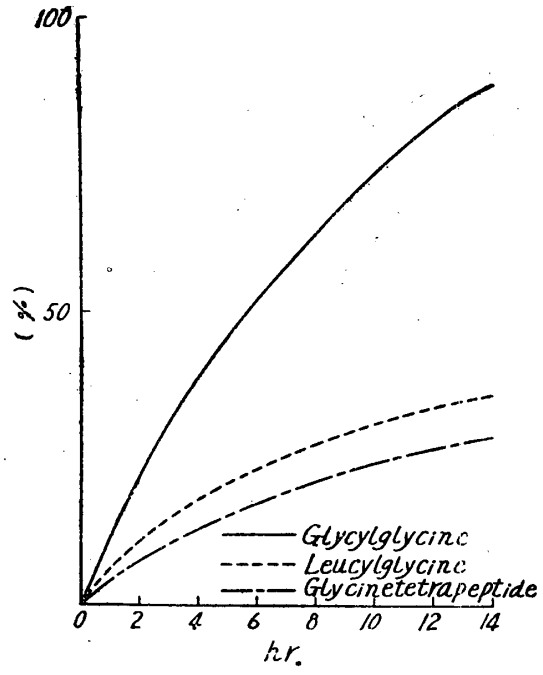


Fig. 7. Dialysis curve of peptides, using parchment paper.

Table 7. Equilibrium of concentration between interior and exterior solution, using 0.3 g. of glycine.

Dialysis membrane / Solution / Lapse of hrs.	Serous membrane in horse		Parchment paper	
	Interior	Exterior	Interior	Exterior
24	—	96.6	—	94.6
28	—	97.3	—	96.1
38	100.9	99.4	99.4	100.3
48	99.9	99.9	99.9	99.6

Table 8. Equilibrium of concentration between interior and exterior solution, using 0.2 g. or 0.4 g. of glycine.

Dialysis membrane / Solution / Glycine	Serous membrane (48th hr.)		Parchment paper (38th hr.)	
	Interior	Exterior	Interior	Exterior
0.2 g.	99.9	100.0	99.9	100.0
0.4 g.	100.1	100.9	99.7	100.2

into the total volume. Results obtained, using 0.3 g. of glycine, are shown in Table 7.

Results obtained, using 0.2 g. or 0.4 g. of glycine, are shown in Table 8.

V) *None Protein is Dialysed.*

This dialysis apparatus is operated by the same method as mentioned above, using the acid solution containing 0.1 g. of edestine as the interior. Buiret reaction is examined on the exterior liquid exchanged every two hours. Judging from the fact that this reaction is negative in every case, it may be seen that none protein is dailysed in this apparatus.

Discussion

I) No difference may be found in appearance among three kinds of serous membrane of small intestine in cattle, swine and horse used as dialysis membrane. The writers have chiefly used the serous membrane of small intestine in horse because it is stronger than the others and accordingly its preparation is too easy to obtain a wide membrane.

It is doubtful whether the protein in the interior solution may leak out into the exterior liquid through the portion on the outside of the interior vessel, on which the dialysis membrane is wrapped closely with cotton thread, or not. From the result obtained in the test of V), however, we may conclude that there

is no risk of passing the protein through this portion in our apparatus.

2) From the results obtained as to the effect of rotation on dialysis velocity (correctly speaking, we may have to use the word "diffusion velocity" in the case of glycine), the velocity of rotation increases more, and the dialysis coefficient grows larger. In general, k' is larger than k'' and k''', and the weight of dialysed solute from the beginning to the 4th hour is the greatest.

The surface of liquid between the interior and the exterior vessel is considerably agitated in case of the rotation of 60 per min. Then, it is thought that the operation with the rotation of 30 per min. is preferable to that of 60 per min.

The difference of dialysis velocity between the rotation of none and of 30 per min. is not so large as the writers expected. The dialysis velocity with the serous membrane of small intestine in horse is less than that with the parchment paper.

3) The concentration of solute in the interior solution may hardly influence on dialysis velocity if the weight of glycine dissolved in it changes from 0.2 g. to 0.4 g.

Comparing the dialysis velocity amoung three kinds of serous membrane of small intestine, the one in swine is the greatest, next in cattle and last in horse. So, it is thought that there may be some histological difference among them.

4) It is questionable whether the process of dialysis with peptides may be according as the Reaction of the First Order, except for the case of glycylglycine when parchment paper is used as dialysis membrane. In case of glycinetetrapeptide, moreover, the question is raised whether it may be explained by the difficulty, with which this peptide is dialysed, that the dialysis velocity of this is fairly small, or whether by the difficulty, with which the solute in the interior solution is diffused to the surface of dialysis membrane. This is a pending problem left for the future.

5) It is seen in the test of IV) that the concentration of glycine between the interior and the exterior solution may achieve the equilibrium at the 38th hr. in case of the parchment paper and at the 48th hr. in case of the serous membrane of small intestine in horse.

The same tendency as mentioned in 2) of this discussion is shown in the fact that in case of peptides and the test of IV) the dialysis velocity with the parchment paper is also larger than that with the serous membrane in horse.

Accordingly the writers think that maybe there is something to discriminate substantially between the serous membrane of small intestine in horse and the parchment paper.

Summary

Conditions on a special dialysis apparatus, in which three kinds of serous

membrane of small intestine in horse, cattle and swine, and the parchment paper are used as dialysis membrane, through which glycine and peptides are dialysed, are shown clearly as follows:

1) The dialysis coefficient with rotation of 60 per min. is the greatest, followed with rotation of 30 per min. and next with rotation of none. This tendency is seen in both cases by using the serous membrane in horse and the parchment paper as dialysis membrane. The dialysis velocity is unlikely to vary in the concentration of glycine from 0.2 g. to 0.4 g. in the interior solution.

2) The dialysis velocity in case of using three kinds of serous membrane in swine, cattle and horse decreases in the order named.

3) The dialysis velocity of peptides indicates some difference between the serous membrane in horse and the parchment paper and, in general, it is smaller, remarkably in case of glycinetetrapeptide, than that of glycine.

4) The equilibrium between the concentration of glycine in the interior and in the exterior solution is achieved at the 38th hr. in case of the parchment paper and at the 48th hr. in case of the serous membrane of small intestine in horse.

Acknowledgment: The expense of this study was defrayed by a grant from the Ministry of Education. We wish to express our gratitude to Prof. R. Sasaki, Faculty of Agriculture, Tokyo University, for his guidance and encouragement through the entire duration of this study; to Dr. S. Suzuki, the Dean, Iwate University, for his kind donation of the peptides; to Assist. Prof. K. Yui, Faculty of Engineering, Tohoku University, for his help about the theoretical discussion of dialysis method.

References

(1) Kametaka and Horikawa, 1951. This Journal., Vol. 2, No. 1, 53
(2) " 1952. Ibid., Vol. 2, No. 2, 19.
(3) Ichiji, 1948. Chikusan Fukuseibutsu Kôgyô 750 (in Japanese).
(4) Akabori, 1947. Aminosán oyobi Tanpakushitsu 165 (in Japanese).
(5) Chitani, 1945. Ippan Butsuri Kagaku 657 (in Japanese).
(6) Gotô, 1951. Kôshitsu Kagaku 65 (in Japanese).
(7) Kandatsu, 1948. J. Agr. Chem. Soc. Jap., Vol. 22, 109 (in Japanese).