STUDIES ON THE ARTIFICIAL DIGESTION ASSAY A PRELIMINARY REPORT CONTINUED, ON THE POWER OF THE CRUDE PEPSIN POWDER IN THE DOMESTIC ANIMAL EXAMINED BY A ONE-DIMENSIONAL DIFFUSION METHOD

STUDIES ON THE ARTIFICIAL DIGESTION ASSAY A PRELIMINARY REPORT CONTINUED. ON THE POWER OF THE CRUDE PEPSIN POWDER IN THE DOMESTIC ANIMAL EXAMINED BY A ONE-DIMENSIONAL DIFFUSION METHOD

By

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In the later preliminary report[1], the writers have been shown the results on the yield of the crude pepsin powders of cattle, horse and swine prepared by the known method and on their egg-albumin digestive power, according to the methods of the Pharmacopoeia japonica and the N. F. and their fibrin digestive power by the formol titration method.

It is thought that, so far as shown, it is also necessary to assay these powers for the vegetable protein. So in this paper, we shall state the results of the edestine digestive power of the crude pepsin powders prepared by the known method.

Experimental

Reagents:

1. 0.1% edestine solution
   Edestine is prepared from the hemp seed cultured in Iwate prefecture.
2. 20gr./dl. NaCl solution
3. agar
   Only the sheet agar, prepared by Kaiho Chemicals Co. LTD., is used successfully.
4. pepsin solutions

At the preparation of all the solutions the glycine-HCl buffer solution of pH 1.42 is used, as it is shown that its pH is optimum when edestine is digested by pepsin at 37°C.

Methods:

1 gr. of agar is added in 50ml. of 20 gr./dl. NaCl solution in a 250 ml. beaker and warmed on a water bath of from 90° to 95°C for 4 minutes. While agar is dissolved incompletely, the beaker is taken down from the bath. On shaking and cooling to the room temperature, agar is dissolved completely and without delay the beaker is cooled in the current water.

Another 1 gr. of agar is added in 50ml. of 0.1% edestine solution in another 250 ml. beaker and, on warming, dissolved by the same manner mentioned above. When the cooling contents in this beaker become viscous moderately, these two solutions are mixed little by little as agitating, where we obtain the white turbid substrate.

Then, every 1.5 ml. of it is poured into the special-made test tubes, having 10 cm. of length and 0.65 cm. of inner diameter, on which 0.5 ml. of pepsin solution with various concentrations is superposed slowly. Four test tubes are arranged as a set of concentration. All of them are immersed in a water thermostat of 37°C. The length (l) of the digested portion, recognized by the disapperance of the white turbidity, is measured by using a special caliper at every 1 hr. from the 3rd hr. to the 9th hr. and at the 24th hr. after the immersion.

Results

At this time, the one-dimensional diffusion method (so-called, the superposition method) has been used on the penicillin micro-assay[2)3)]. M. Masuyama[4)] and J. Ookawara[5)] report a method of estimating the minimum effective dose

Table 1. l (mm) on the Merck's pepsin

Hr. after incubation / Concentration	3rd	4th	5th	6th	7th	8th	9th	24th
4 gr./dl.	2.03 (8)	2.33 (8)	2.57 (5)	2.75 (5)	2.98 (3)	3.28 (4)	3.51 (11)	6.58 (15)
2 gr./dl.	1.83 (7)	2.08 (7)	2.29 (4)	2.48 (9)	2.75 (9)	3.00 (6)	3.20 (5)	5.13 (14)
1 gr./dl.	1.58 (6)	1.75 (6)	1.96 (6)	2.23 (8)	2.46 (7)	2.64 (9)	2.88 (10)	4.51 (7)
0.5 gr./dl.	1.38 (3)	1.55 (5)	1.69 (4)	1.93 (7)	2.13 (8)	2.28 (11)	2.38 (13)	3.93 (15)
0.25 gr./dl.	1.06 (4)	1.26 (7)	1.52 (8)	1.68 (3)	1.78 (5)	1.90 (7)	2.05 (6)	3.06 (16)
0.125 gr./dl.	0.70 (3)	0.75 (4)	0.82 (5)	1.00 (6)	1.15 (11)	1.20 (11)	1.42 (9)	2.00 (9)

( ) : Standard error (S. E.) × 100

of pepsin, applying that method on the determination of its digestive power.

First of all, after tracing their method on the merck's pepsin, the writers used it to the assay for our crude pepsin powders in the domestic animal.

The result on the Merck's pepsin, received from Mutô Chemicals Co. LTD. lastyear, is given in Table 1.

According to a fundamental formula[6)7)] for the superposition method $\log C - \log(2K) = \frac{l}{\pi D t^2}$, we may indicate the relation of l and $\sqrt{t}$ in Fig. 1 and of l^2 and $\log C$ in Fig 2.

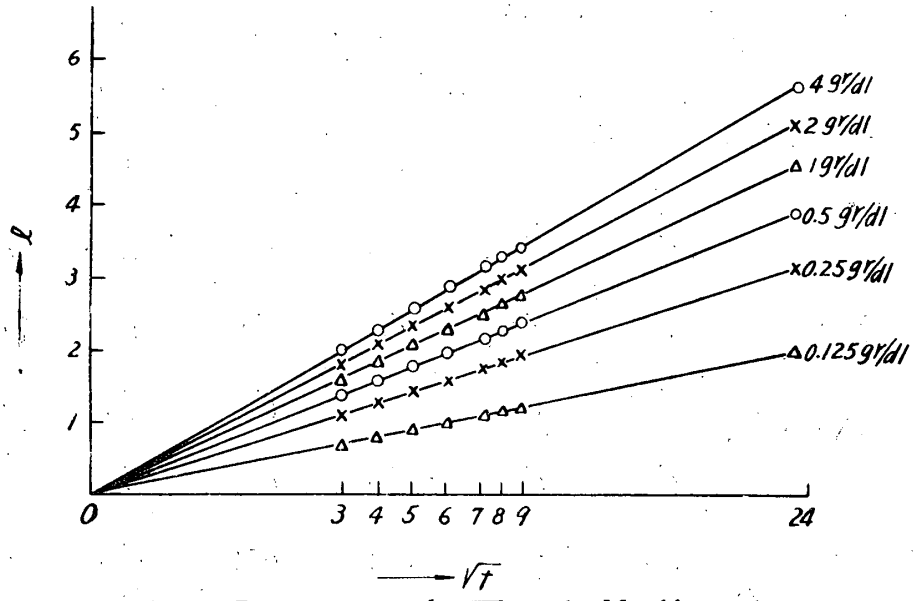
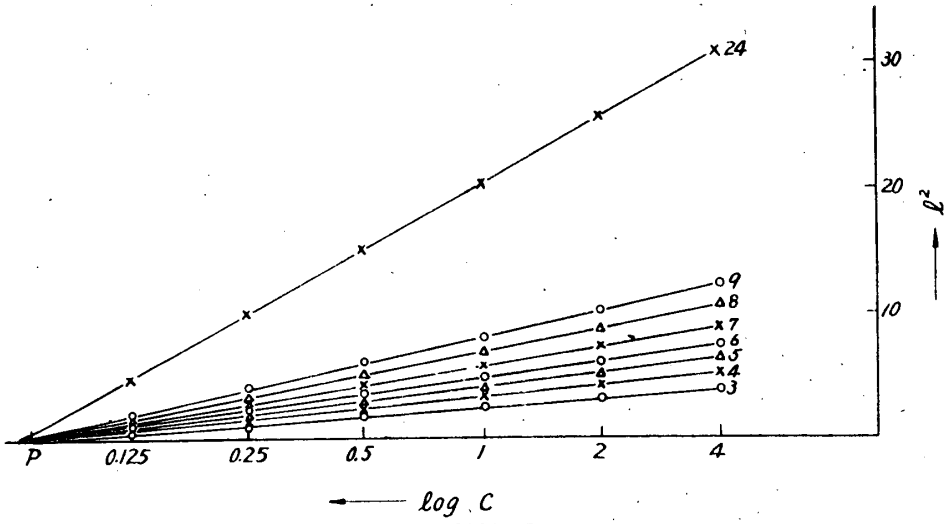


Fig. 1. Relation of l and $\sqrt{t}$ on the Merck's pepsin



$P_{37°}$ = 2800 Units

Fig. 2. Relation of l^2 and $\log C$ on the Merck's pepsin

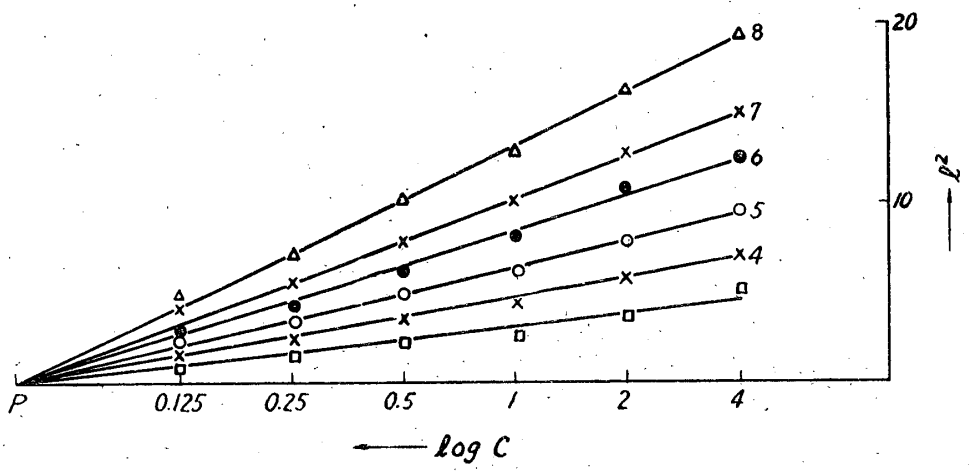
Our result on the Merck's pepsin is on the whole, if not in every point, the same as Ookawara's. He finds that l is proportionate to $\sqrt{t}$ untill the 8th hr. after the incubation. It is thought that the standard error (S. E.) may enlarge at and after the 9th hr., but this result is contrary to our expectation.

Although it is, if necessary, able to calculate the value of D—the coefficient of diffusion in the fundamental formula—in accordance with our result, the special necessity is not admitted to us at present.

The result obtained with our crude pepsin powder of swine is given in Table 2, Fig. 3 and of horse in Table 3, Fig. 4.

Table 2. l (mm) on the crude pepsin powder of swine

Hr. after incubation / Concentration	3rd	4th	5th	6th	7th	8th
4 gr./dl.	2.48 (13)	2.80 (24)	3.16 (21)	3.58 (22)	3.90 (36)	4.41 (38)
2 gr./dl.	1.99 (7)	2.43 (4)	2.84 (8)	3.41 (5)	3.70 (7)	4.07 (6)
1 gr./dl.	1.58 (9)	1.93 (9)	2.44 (5)	2.80 (12)	3.09 (6)	3.50 (8)
0.5 gr./dl.	1.45 (17)	1.68 (13)	2.20 (14)	2.41 (17)	2.84 (21)	3.18 (19)
0.25 gr./dl.	1.99 (18)	1.43 (18)	1.71 (23)	1.99 (19)	2.32 (22)	2.68 (22)
0.125 gr./dl.	1.13 (5)	1.36 (13)	1.66 (10)	1.76 (12)	2.16 (12)	2.46 (13)



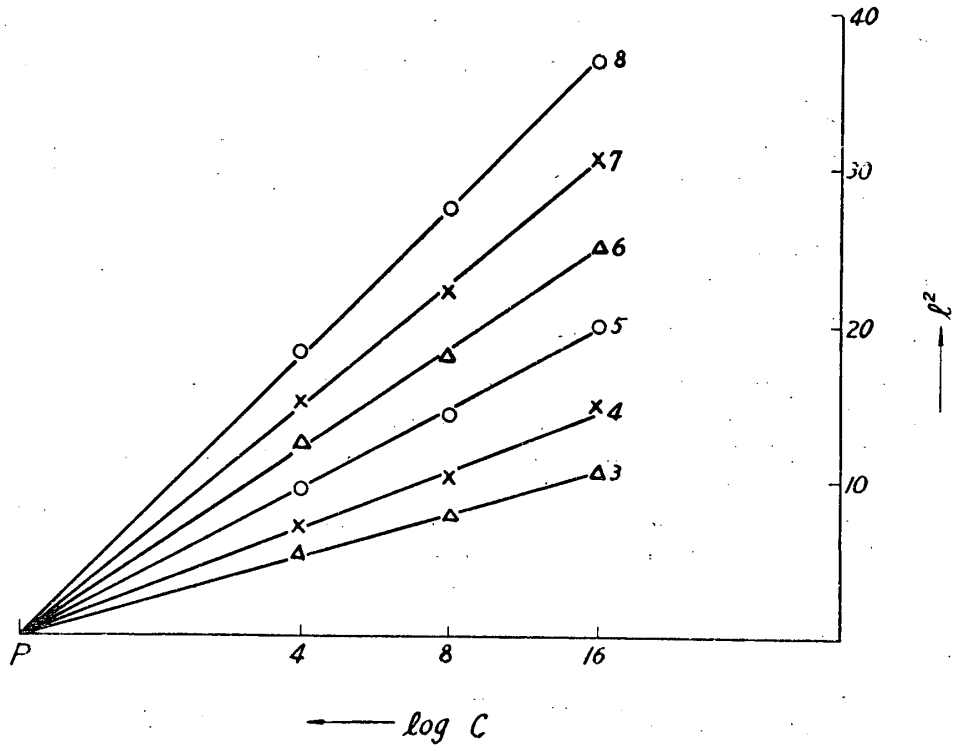
$P_{37°} = 4470$ Units

Fig. 3. Relation of l^2 and *log C* on the crude pepsin powder of swine

Table 3. l (mm) on the crude pepsin powder of horse

Hr. after incubation / Concentration	3rd	4th	5th	6th	7th	8th
16 gr./dl.	3.25 (0)	4.00 (0)	4.45 (12)	5.01 (13)	5.53 (3)	6.05 (9)
8 gr./dl.	2.78 (2)	3.15 (3)	3.77 (22)	4.23 (30)	4.68 (30)	5.22 (16)
4 gr./dl.	2.36 (8)	2.68 (6)	3.08 (10)	3.58 (13)	3.88 (6)	4.29 (18)

( ) : S. E. ×100



$P_{37°}$ = 190 Units

Fig. 4. Relation of l^2 and *log C* on the crude pepsin powder of horse

Though an assay for the crude pepsin powder of cattle is tried by the same method, it is impossible to measure l even in the 16 gr./dl. of pepsin concentration and to superpose the pepsin solution with 64 gr./dl. of concentration on the substrate in the cause of the great specific gravity.

It is merely recognized that l, even in the case where the 32 gr./dl. of pepsin concentration is used, is not able to be measured by the caliper at the 24th hr. after incubation, but as the digested portion is doubtfully found,

accordingly it is admitted on no account that edestine is perfectly indigested by our crude pepsin powder of cattle.

Discussion

From the above result it is likely seen, similarly as shown in our preliminary report, that on the edestine digestive power of the crude pepsin powder prepared by the known method swine's powder is also the most powerful, horse's next and cattle's little. Then, it may be admitted qualitatively that there are differences among the digestive power of the crude pepsin powders, of 3 kinds of domestic animal, prepared by the known method, but it is questionable whether the conditions, on which edestine is digested, for example pH, temperature, concentration etc. are the same on 3 kinds of domestic animal. As it is further necessary to investigate the optimal condition, especially on pepsin of horse and cattle, we wish to set our study towards that course in the future.

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