Protein digestive power of Sperm whale Pancreatic Enzyme

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The feeding habit of cetacea is very peculiar, that is, they do not take any carbohydrates but depends entirely on protein food.

These may have some special digestive enzymes and the authors' attention were attracted by pancreatic enzyme and anticipated that this may be a strong protease.

Also cetacea eats live feed so that the digestive enzyme the feed posses may be utilized in the alimentary tract. If this is considered, the digestive enzyme excreted by whales may be only a small quantity.

If they are strong as first anticipated, there may be some industrial use for them because the quantity of whale pancreas is very large.

At any rate, it is necessary to ascertain the protein digestive power of pancreatic enzyme. Fortunately, the authors were able to obtain pancreas from two sperm whales at the Ayukawa whaling ground and experiment was carried on with these.

Both of the sperm whale were about 40 ft. long and they were not fresh because 30 hours elapsed from the time they were caught to the time when they were dissected.

After dissection, the pancreas was removed, fatty tissues, connective tissues and other unnecesary parts were removed, as much as possible, placed in cans immediately frozen and brought to the laboratory. On the way, special attention was paid so that the temperature did not rise. This was well minced with a meat mincer, mixed and stirred until uniform, stored in a refrigerator and taken out as the experiment required.

Determination of digestive power was carried on along the pancreatin method described in the Japan Pharmacopoeia. That is, 0.2% casein solution was used as substrate, extracted solution obtained by extracting a fixed quantity of minced pancreas with fixed quantity of water, was added to 5 c.c. of this in stages, water was added until the total content became 10 c.c. This was made to react for one hour at 40°C, 3 drops of a solution composed of 10 c.c. of alcohol, 1 c.c. of glacial acetic and 9c.c. of water was

added to this digestion solution and the presence of slight tutbidity was taken as the end of digestion.

Preparing of casein solution is as follows that is, refined casein by Hammersten's method, is dried in a H_2SO_4 desiccator, 2 g. of this is removed and well dispersed in about 50 c.c. of water. To this is added 2 c.c. of 0.1 *N* KOH, shaken to dissolve and made up to 100 c.c. with water after it has dissolved.

Experimental objects were to determine, the digestive power of fresh pancreas (those which went trough the above procedure), the relation between time and activition of digestive power when it was autolysed at 40°C in neutral reaction and the same relation in autolysis at 40°C when sligtly acidic (initial pH 5.4) and the digestive power when minced pancreas was dried with acetone and pulverized.

Furthemore, the same experiment was carried on with cow pancreas and the digestive powers of cow pancreas and whale pancreas were compared.

Experiment

1. Protein digestive power of fresh pancreas.

Enzyme solution made by dispersing 1 g. of minced pancreas in 100 c.c. of water is filtered with fluted filter paper. The analysis of this minced panccreas is 73.7% moisture and, 26.3% dried matter but this dried matter contained considerable quantity of fat. Therefore, 100 c.c. of this extraction liquid may be equivalent to ca. 0.2 g. of dried pancreas so that in such an estimation method, 0.2 g. is assumed to be not such a discrepancy. By doing this, it will be simple to calculate the unit quantity of pancreas which digested so many times casein because the casein solution is 0.2 g./100 c.c. All experiments hereinafter were carried on by this method.

Experimental result is as shown in Table I and this pancreas did not have any digesting power.

Table I. Digesting Power of Fresh Pancrea

No.	0.2% Casein solution c.c.	1 g./100 c.c. pancreas extraction liquid c.c.	Water c.c.	Ratio of casein pancreas dried matter	Turbidity
1	0	5	5	0	±
2	5	5	0	1	+
3	5	2	3	2.5	+
4	5	0.5	4.5	10	+

In turbidity, − indicates transparent, ± slight turbidity, and turbid or precipitation is indicated by +. This applies to all following experiments.

2. Increase of digestive power by neutral autolysis.

Minced pancreas is placed in a conical flask, a small amount of toluol added and sealed. This is placed in a thermostatic bath, of 40°C taken out at the time indicated in Table II and digestive power determined. Pancreas extraction liquid is made in the same manner as before, that is, 1g. of pancreas is extracted with 100 c.c. of water and filtered.

Table II. Increase of digestive power by autolysis

Hours left standing	No.	0.2% Casein Solution c.c.	1g./100 c.c. Pancrea extraction liquid c.c.	Water c.c.	Ratio casein pancreas dried matter	Turbidity
3.5 hours	1	0	5	3	0	−
	2	5	5	0	1	± (digested)
	3	5	2	5	2.5	+
10.5 hours	1	0	5	5	0	−
	2	5	5	0	1	−
	3	5	2	3	2.5	± (digested)
	4	5	1	4	5	+
24 hours	1	0	5	5	0	−
	2	5	5	0	1	−
	3	5	2	3	2.5	− (digested)
	4	5	1	4	5	+
	5	5	0.5	4.5	10	+
48 hours	1	0	5	5	0	−
	2	5	5	0	1	− (digested)
	3	5	2	3	2.5	+
	4	5	1	4	5	+
	5	5	0.5	4.5	10	+
	6	5	0.2	4.8	2.5	+

As can be seen from Table II, when it is allowed to autolyse at 40°C, digestive power increase somewhat, the maximum of increase being between 10—24 hours, and its maximum value being at 2.5. That is, pancreas digest 2.5 times its weight of casein.

3. Increase of digestive power by slightly acidic autolysis.

10 g. of minced pancreas is mixed with 2 c.c. of water and 2 c.c. of 0.1 *N* HCl, a small amount of toluol added, made into several samples, sealed and stored in a thermostatic bath of 40°C. One of these is taken out at each time indicated in Table III and digestive power determined. The initial pH of this mixture was 5.4.

The concentration of the enzyme solution is exactly the same as in the previous experiment and 100 c.c. of the enzyme solution is equivalent to 1 g. of the original pancreas (0.2 g. as dried matter).

Table III. Increase of digestive power by autolysis (Initial pH 5.4)

Hours left standing	pH at time of experiment	No.	0.2% Casein colution c.c.	1 g./100 c.c. pancreas txtracted soluion c.c.	Water c.c.	Ratio Casein pancreas dried matter	Turbidity
3 hours		1	5	5	0	1	± (digested)
		2	〃	2	3	2.5	+
		3	〃	1	4	5	+
		4	〃	0.5	4.5	10	+
12 hours	5.6	1	5	5	0	1	−
		2	〃	2	3	2.5	− (digested)
		3	〃	1	4	5	+
		4	〃	0.5	4.5	10	+
24 hours		1	5	5	0	1	−
		2	〃	2	3	2.5	± (digested)
		3	〃	1	4	5	+
36 hours		1	5	5	0	1	−
		2	〃	2	3	2.5	± (digested)
		3	〃	1	4	5	+
		4	〃	0.5	4.5	10	+
48 hours	6.2—6.4	1	5	5	0	1	− (digested)
		2	〃	2	3	2.5	+
		3	〃	1	4	5	+
		4	〃	0.5	4.5	10	+

As can be seen from the result, it is about the same in the case of slightly acidic as in the case of neutral, maximum increase being between 12—36 hours and its maximum value is the same, 2.5.

In order to show more readily the relation between hours left standnig and digestive power for neutral and slightly acidic, it is as shown in the Summary Table IV.

Table IV. Hours of autolysis and digesting power.

Neutral, left standing at 40°C		pH 5.4, left standing at 40°C	
Hours left standing	Digesting power casein/panereas	Hours left standing	Digesting power casein/pancreas
0	0		
3.5	1	3	1
10.5	2.5	12	2.5
24	2.5	24	2.5
		36	2.5
48	1	48	1

5. Increase of digestive power of cow pancreas by autolysis.

Cow pancreas was obtained directly after slaughtering from the Shibaura Slaughter House. This was frozen at −35°C, minced and used in experiment, Temperature of autolysis and other experimental conditions are exactly the same as in the case of whale.

The result is as shown in Table V. As can be seen from Table V.,

casein digested when fresh is 10 times and is 25 times casein after it is autolysed for 24 hours at 40°C.

In Table V. the standard concentration of enzyme solution was 1 g. of minced pancreas extracted with 100 c.c. of water. When it is diluted and used, the concentration is shown in parenthesis.

Table V. Increase of digestive power of cow pancreas by autolysis. by autolysis

Hours left standing	No.	0.2% casein solution c.c.	Pancreas extracted solution c.c.	Water c.c.	Ratio **casein** pancreas dried matter	Turbidity
0 hour	1	5	5	0	1	±
	2	〃	2	3	2.5	〃
	3	〃	1	4	5	〃
	4	〃	5 (×1/10)	0	10	〃 (digested)
	5	〃	2 (〃)	3	25	+
	6	〃	1 (〃)	4	50	+
12 hours	1	5	5 (×1/10)	0	10	−
	2	〃	2 (〃)	3	25	± (digested)
	3	〃	1 (〃)	4	50	+
	4	〃	2.5 (×1/50)	2.5	100	〃
	5	〃	2 (〃)	3	125	〃
	6	〃	1 (〃)	4	250	〃
24 hours	1	5	5 (×1/10)	0	10	+ (digested)
	2	〃	2 (〃)	3	25	〃
	3	〃	1 (〃)	4	50	〃
	4	〃	2.5 (×1/50)	2.5	100	〃
	5	〃	2 (〃)	3	125	〃
	6	〃	1 (〃)	4	250	〃

5. Protein digestion of pulverized whale and cow pancreas dried matter.

Next, the authors prepared pulverized dried pancreas of whale and cow and determined the digestive power of both. That is, minced pancreas is left standing at room temperature (2—8°C) for one night, placed in a large quantity of acetone to dehydrate, filtered with suction and washed several time by pouring on it. Next, it is dried by aeration, well dried in a H_2SO_4 desiccator, pulverized in a morter, sieved with a 60 mesh sieve, and the digestive power of those which passed through the sieve was determined. Also, for comparison, the digestive power of both whale and cow pancreas before dring were determined (after standing for one night).

Result of determination and the detail of yield from minced pancreas to sieved powder is shown in Table VI and Table VII.

As can be observed from Table VI, the digestive power of whale pancreas before drying is 0, but that of cow is 50 times. For dried pulverized whale pancreas, it is 2.5 times, and 125 times for cow pancreas.

In table VI the standard for pancreas extracted solution for fresh pancreas, was 1 g/100 c.c. and 0.2 g/100 c.c. in the case of dried pulverized

pancreas, these being gradually diluted and used. Concentration is indicated in parethesis.

Table VI. Digestive power of dried pulverized whale and cow pancreas and before drying.

Kind	No.	0.2% Casein solution c.c.	Pancreas extracted liquid c.c.	Water c.c.	Ratio casein pancreas dried matter	Turbidity
Fresh whale pancreas	1	5	5 (1g./100 c.c.)	0	1	+
	2	〃	2 (〃)	3	2.5	+
	3	〃	1 (〃)	4	5	+
	4	〃	0.5 (〃)	4.5	10	+
Dried pulverized whale pancreas	1	5	5 (0.2 g/100 c.c.)	0	1	−
	2	〃	2 (〃)	3	2.5	± (digested)
	3	〃	1 (〃)	4	5	+
	4	〃	5 (〃 ×1/10)	0	10	+
	5	〃	2 (〃 ×1/10)	3	25	+
Fresh cow pancreas	1	5	5 (1 g/100 c.c.)	0	10	−
	2	〃	2 (〃)	3	25	−
	3	〃	1 (〃)	4	50	− (digested)
	4	〃	2.5 (〃 ×1/5)	2.5	100	+
Dried pulverized cow pancreas	1	5	2 (0. 2g.1000 c.c.)	3	25	−
	2	〃	1 (〃)	4	50	−
	3	〃	3.5 (〃 ×1/5)	1.5	70	−
	4	〃	2.5 (〃 ×1/5)	2.5	100	−
	5	〃	2 (〃 ×1/5)	3	125	± (digested)

Table VII. Yield on drying and sieving minced pancreas.

Minced pancreas g.		Acetone dried matter g.	Sieved matter g.	Sieved residue g.	Loss on sieving g.
Whale	99	15.1	9.6	5.3	0.2
Cow	49	9.8	8.5	1.1	0.2

Conclusion.

There is almost no digestive power in the fresh sperm whale pancreas which was used in the experiment and by autolysis was only 2—3 times casein. There is almost no change in progress of increase by autolysis in both neutral and slightly acidic condition.

In the case of cow pancreas, under the same condition, it is easy to obtain a digestive power of about 25 times by autolysis.

On standing for one night at room temperature, the digestive power of whale pancreas was 0 but that of cow pancreas was 50 times. Also, when these are dried and pulverized, digestive power of whale pancreas was 2.5 times and 125 times in case of cow pancreas.

In comparing whale and cow pancreas, the amount of connective tissues in the case of whale is very large and only a small part was pulverized.