Protein Digestive Power of Sperm Whale Pancreatic Enzyme. II.

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In the 1st report, protein digestive power of pancreatic enzyme from sperm whales was compared with that of a cow and was found much weaker than the latter. Casein was used as the substrate and the digestive powers were measured by the method of Michaelis given in the Japanese Pharmacopoeia.

Even after being activated in various manners, enzyme from whales digested only 2.5 times of casein of its own weight while that from a cow digested 125 times. This large difference was almost unbelievable.

However in the experiment previously reported the samples were collected from too few bodies to discuss generally. And the time-lapse from the caching to the dismembering of these whales was so long under higher temperature of the summer, that there was a suspision that a part of proteases may have been destroyed by autolysis.

The present experiment was undertaken in order to eliminate these suspicions and to determine the true protein digestive power of a sperm whale pancreatic enzyme. The samples, therefore, were collected at the coldest season during whaling (December 1948) and only the very fresh ones were used. It had been desired to collect samples from as many animals as possible in order to eliminate individual differences and samples were collected from six fresh sperm whales during the two weeks at the landstation. Ayukawa, Miyagi Prf.

EXPERIMENTAL

1) Whales Used

No.	Kind	Sex	Body length (ft.)	Time elapsed from catching to dismember (hrs.)	Freshness %
1	Sperm	male	45	25	85
2	Sperm	male	46	25	85
3	Sperm	male	46	23	90
4	Sperm	male	50	18	85
5	Sperm	male	42	22	85
6	Sperm	male	51	20	85

The freshness is the value evaluated by the whaling experts on the station by macro-observations with considerations to the elasticity and color of

the whale meat and others. The freshest state, i. e. that right after death, is taken as 100 and deterioration from that is shown by a rough percentage. Various scientific methods for the determination of freshness have been forwarded but none is completed and procedures are too complicated so that it has to rely on these empirical method.

2) **Treatment and Preparation of Samples**

As soon as the whale body has been cut open, the pancreas taken out, adipose and connective tissues removed as much as possible and minced by a hand chopper, From this were preparad 3 kinds of samples for reasons as described in (3). These samples were:

a) One dried immediately with acetone.

b) The minced pancreas left standing overnight at room temperature with small amount of toluen in a flask, stoppered, which was then dried with acetone.

c) The minced pancreas mixed with 30% aqueous acetone solution in an equal amount and stored.

Samples (a) and (b) were prepared by adding anhydrous acetone to minced pancreas in an amount about 5 times its volume, well stirred and the supernatant solution decanted. The same amount of acetone is then added and filtered with suction. The residue on the funnel is washed, first with acetone and then with ether, and is dried by spreading it out in the room. This is then well dried in a dessicator over Sulfuric acid. Procedures up to this point were carried out at the station and this was then brought to the laboratory for testing. The dried pancreas was first powdered by a boat-shaped mortar, sieved through a 60 mesh sieve and divided into powders of gland substances and connective tissues. Special note should

Table I.

No.	Powder (g)	Sieve residue (g)	Amount sieved (%)
1 A	2.8	5.4	34
2 A	1.9	11.6	14
3 A	3.0	10.7	22
4 A	3.2	9.4	25
4 B	1.4	4.0	26
5 A	5.3	9.0	37
5 B	6.3	5.1	55
6 A	5.3	7.5	41

A denotes samples dried immediately
B denotes samples dried after being left overnight.

be taken of the fact that the pancreas of a sperm whale contains much larger percentage of connective tissues compared to that of bovines, hogs and baleen whales, so that the yield of gland substance is very poor. The resultant yield of sieving is given in Table I.

The samples immersed in 30% acetone were brought back to the laboratory and stored at room temperature. And the supernatant solution which might contain protease, was taken from time to time to test the digestive power.

The kinds of samples prepared were as follow:

No. 1—A,	C	No. 4—A,	B
No. 2—A,	C	No. 5—A,	B, C
No. 3—A,	C	No. 6—A,	B (lost), C

A, denotes samples dried immediately,
B, samples dried after being left overnight, and
C, samples immersed in 30% acetone.

3) **Method of Activation**

As in other kind of animals, pancreatic protease from sperm whales do not show digestive action in its fresh state. It follows, therfore, that this protease must in some way be activated and the subsequent testing of digestive powers must be carried out at the same degree of activation, in order that a compaison can be made; if possible, at a maximal activated state. For this reason, activation was carried out in following three ways and the digestive powers determined and compared.

a) The pancreas was dried in the most fresh condition, powdered as has been described before and this was activated by enterokinase from a sperm whale.

b) Minced pancreas was left overnight at a room temperature and treated in the same way as before. This was then activated with enterokinase.

The above two were made in order to find the effect of autolysis in the fresh condition.

c) Minced pancreas was dispersed in the same amount of 30% aqueous acetone solution and its supernatant solution, assumed to contain protease from the pancreas, was used as a sample for testing.

The reason that this experiment was carried out was that Kleiner and Tauber reports[2] that the protease from hog pancreas is best extracted with 33% alcohol and is well activated by a long-time storage. Tejima,[3]

also obtained a successful result by extracting protease from a sei whale pancreas by the use of 30% acetone.

4) **Method of Digestive Power Determination**

The method of determining digestive power followed the same one as described in the previous report[1], i. e. the Item 6 of the Japanese Pharmacopoeia for pancreatin which uses Michaelis' method of protease testing.

The substrate used was casein purified by Hammesten's method and stored in a dessicator over sulfuric acid. 5 cc. each of 0.2% solution of this casein (containing 2 cc. 1/10 *N*-KOH in 100 cc. of this solution) is taken in test tubes and these tubes are stood in a row. A definite amount of enzyme is dispersed in a definite quantity of water to extract the enzyme and a definite amount of this enzymatic solution is added step-wise to the casein solution and the total volume is then brought to 10 cc. each. This solution is kept for 1 hour at 40° to effect digestion of casein. After exactly 1 hour, 3 drops of a mixture of 1 cc. glacial acetic acid, 9 cc. water and 10 cc. alcohol is added to such test tube. One that remains clear is taken as digested and the one showing least opalescence is taken as the limit of digestion. Digestive power of enzym-containing powder is found from the amount of the powder used to digest 5 cc. of 0.2% casein solution (i. e. 0.01 gm. casein) in the limit case and is then shown by the ratio of casein to the enzymatic powder.

In the case of 30% acetone extracted solution, a definit of its supernatant solution is taken, suitably diluted and used in determination as above.

5) **Results of the Experiments**

i) Acetone dried powder

a) Digestive power when enterokinase is not added

Digestive power was determined with No. 1A, No. 2A and No. 4B. None of these had the power to digest casein equal to their own weight. It follows, therefore, that the acetone dried powder either lacks digestive power totally or has a very weak action. This in turn may mean that the enzyme is in an inactivated state due to freshness of pancreas or that enzyme had been destroyed altogether.

b) Digestive power when enterokinase is added

0.2 g. each dried powder of No. 1A and No. 2A was taken, 0.05 g. enterokinase added end dispersed in 100 cc. of water. This was warmed at 40°

for 15 minutes, 30 minutes and 1 hour and activated. The result of the determination of digestive power using these extracts was as follows:

No. 1 A (15 min.)	Digest 10 times its weight of casein but cannot digest 12.5 times its weight.
No. 1 A (30 min.)	ditto
No. 1 A (1 hour)	ditto
No. 2 A (15 min.)	ditto
No. 2 A (30 min.)	ditto
No. 2 A (1 hour)	Digests 7 times its weight of casein but cannot digest 10 times its weight.

From these results, it can be seen that the time of warming for activation does not differ much between 15 minutes and 1 hour but is slightly inferior at 1 hour.

Therefore, the conditions were set for an addition of a 1/4 amount of enterokinase and warming for 30 minutes at 40° to effect proper activation. The result of determination of digestive power of various samples were as follows:

No. 1 A — Digests 10 times its own weight but not 12.5 times.
No. 2 A — ditto
No. 3 A — ditto
No. 4 A — ditto
No 4 B — Digests 8.3 times its own weight but not 10 times.
No. 5 A — ditto
No. 5 B — Digests 10 times its own weight but not 12.5 times.
No. 6 A — Digests 8.3 times its own weight but not 10 times.

Digestive power of each sample made from above results is shown in Table II, which indicates that there are hardly any difference between the samples.

Table II. Digestive Power of Acetone Dreied Powder

Sample No.	Digestive Power (cas./powder)	Sample No.	Digestive Power (cas./powder)
1 A	10	4 B	8
2 A	10	5 A	8
3 A	10	5 B	10
4 A	10	6 A	8

ii) 30% Acetone Extracted Solution

The solution was stored at room temperature for about 2.5 months during which a definite amount of the clear, supernatant solution was taken and its digestive power determined. Table III, shows the relationship between the digestive power and the time lapsed after immersion in acetone. The figures denote the amount of acetone solution (in cc.) necessary to

digest 0.01 g. of casein (shown by the least opalescence manifested) so that the smaller the figures, greater the digestive power.

Table III. Relationship between digestive power and lapse of time.

Lapse of time (days)	1 C	2 C	3 C	5 C	6 C
5				2/100	
7	1/100				
9				1/100	10/100
11	1/100	6/100		1/100	
13			15/100		
15				1/100	4/100
17	1/100	3/100	15/100		
21				1/100	2/100
23	1/100	2/100	12/100		
26	1/100	1/100	12/100	1/100	1/100
34	1/100	1/100	9/100	1/100	1/100
54	1/100	1/100	9/100	0.8/100	0.8/100
75	1.5/100	1.5/100	9/100	1/100	1.5/100

As can be seen from Table III, each sample differs greatly in its digestive power at the start of experiment but after about one month, all samples, except No. 3 C, show constant values of 1/100. This level is kept until about 2 months have elapsed after which the power gradually decreases slightly.

DISCUSSIONS

1) Direct comparison cannot be made between the value of 8—10 for digestive power obtained by the determination of dried powder and the values obtained with 30% aceton solution. However, if the amount of water included in minced pancreas is considered and if the whole amount of enzyme present in the pancreas were to have transferred to the solution, then in the case of 30% acetone solution, the digestive power of dry pancreas as a whole can easily be calculated.

If the amount of the dried matter is assumed to be 25% of the minced pancreas, and since the minced pancreas was added with an equal amount of 30% acetone, then 1 g. minced pancreas + 1 cc. 30% acetone $\fallingdotseq$ 0.25 g dry pancreas + 1.75 g extracted solution. i. e. 0.25 g dried matter from pancreas should have the same digestive power as 1.75 g. of the extracted solution. Therefore, 1/100 cc of this extracted solution is equal to

$$\frac{0.25}{1.75} \times \frac{1}{100}\ \text{g dried matter of pancreas.}$$

Since this amount had digested 0.01 g. of casein, then the digestive power of the dried matter of pancreas is

$$0.01 / \frac{0.25}{1.75} \times \frac{1}{100} = 7$$

i. e., it has digested 7 times its own weight of casein. Of course, this calculated amount shows the digestive power of pancreas as a whole but in the case of acetone dried power, it has been put through the sieve during which purification of the enzyme may have been efected so that a precise comparison cannot be made as to which is larger. However, it is considered that the order is well adapted in this case.

2) When enterokinase is not added to the dried powder of pancreas, no digestion occurred. From this fact, it can be assumed that these pancreas was stored in a comparatively fresh state until treatment and that protease had not been destroyed and stayed in its natural state.

The digestive power of the dried powder of pancreas when activated by the addition of enterokinase was found to be about the same in each individual and digested 8—10 times its weight of casein. The protein digestive power of 30% acetone extracted solution varies greatly at first but becomes most activated about one month after immersion at which time, with the exception of one sample, the values are exactly the same for all the samples. In this one exception, i. e. 3C the supernatant solution was found to be considerably opalescent due to the inclusion of grease (the others were almost clear). This is apparently the error due to the inclusion of a large amount of adipose tissues at the time of sampling. The value when this same level had been reached in all the sample, i. e. 1/100, shows that the dry matter of the original pancreas would digest about 7 times its own weight of casein.

From these results, it is considered that the digestive power of protease from sperm whale is considerably inferior to that from bovines and hogs. However, this is true only when casein has been used as a substrate and it is doubtful whether the same tendency will be manifested when other proteins are chosen as a substrate.

From the results of these experiments, it can be assumed that there is very little difference between individuals.

SUMMARY

Protein digestive power of pancreas from 6 sperm whales was determined and following results were obtained:

1) Acetone dried powder does not show digestive action as it is.
2) When acetone dried powder is activated by the addition of 1/4 its amount of enterokinase from a sperm whale and warmed for 30 minutes at 40°C, it digests 8—10 times its own weight of casein and there were no differenc between individual animals.
3) 30% Acetone extracted solution of minced pancreas, reached its maximal activated state after storage of about one month at room temperature and the digestive power was the same for all the samples, i. e. 1/100 cc. of this solution digested 0.01 g. of casein. Calculated from this value, the dry matter of the pancreas used would have digested about 7 times its weight of casein.

References

1) Ishikawa: Bulletin of the Japanese Society of scientific Fisheries Vol. 14, No. 2, 107 (1948); Ishikawa and Tejima: Sc. Report of the Whale Reserch Institute, No. 2, (1949).
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3) Tejima and Ishikawa: Lecture read before the 3rd Meeting of the Whale Reserch Institute in October, 1948, unpublished.