IN VIVO STUDIES ON THE 'ANTI-THIAMINE FACTOR' IN FRESHWATER MUSSEL (*LAMELLIDENS MARGINALIS*).

By K. K. REDDI, *I.C.I. Research Fellow, Department of Biochemistry, Indian Institute of Science, Bangalore.*

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INTRODUCTION.

The occurrence of 'anti-thiamine factor' in certain species of fish was first reported by Green (1936, 1937) and his associates (1940, 1941, 1942). The enzymic nature of the factor was established by Spitzer *et al.* (1941), Woolley (1941), Sealock *et al.* (1931) and Sealock and Goodland (1944). The nature of the action of this factor on thiamine was established by Krampitz and Woolley (1944) by the isolation of pyrimidine and thiazole derivatives following the hydrolytic cleavage of the vitamin.

Recently it was reported by Reddi *et al.* (1948) that the visceral mass of freshwater mussel (*Lamellidens marginalis*) contains an active enzyme system which destroys thiamine. Experimental evidence was presented to show that the enzyme system is composed of two thiaminases, one optimally active at *p*H 3·6 and the other at *p*H 6·5. The two thiaminases were separated and their kinetics were studied (Reddi *et al.* 1949).

The present communication deals with the study of the influence of the thiaminase system in freshwater mussel on the availability of added thiamine to rats. It is well known, the factors that render the ingested enzymes ineffective either by destruction or inactivation, are present in the gastro-intestinal tract. Therefore, this direct study on the rats will be of more practical value.

EXPERIMENTAL.

Young albino rats were used for these experiments. The growth promoting property of vitamin B_1 is applied as the criterion of the availability.

Basal diet (*free from vitamin* B_1).—Evans and Lepkovsky (1929) studied about seven diets containing various amounts of sucrose, proteins and fat. Of all the diets tested, the most sensitive for growth studies was found to be a sucrose-protein diet of the following composition:—

Composition of the basal diet.

Casein (free from vitamin B_1)	20
Sucrose	70
Salt mixture	4
Autoclaved yeast	10

This diet was supplemented with two drops of Shark liver oil daily.

Preparation of casein free from vitamin B_1.—The casein was prepared according to the method of Evans and Lepkovsky (*loc. cit.*).

Autoclaved Yeast.—Whole dried yeast was spread in pans to a depth of less than one inch and autoclaved for 5 hours at 18–20 pounds pressure. This preparation was used as a source of the thermostable water-soluble factor throughout the experiment.

Salt mixture (McCollum).—Sodium chloride 51·0; Crystals of magnesium sulphate, 159·6; monobasic sodium phosphate 104·1; monobasic calcium phosphate, 162·0; dibasic potassium phosphate, 286·2; ferric citrate 35·4 and calcium lactate 390·0.

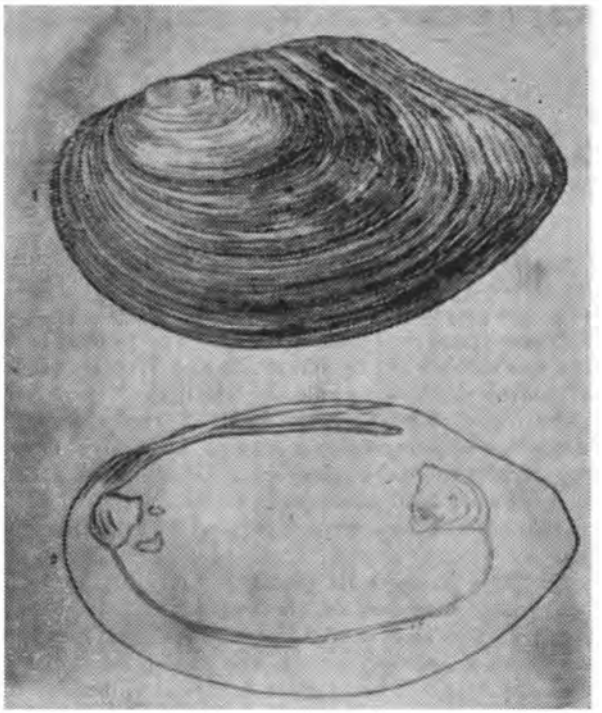

FIG. I—1. *Lamellidens marginalis.*
2. Muscular scars and pallial impression of same.

Preparation of the mussel extract.—The mussels were obtained fresh and the shells were removed. The whole visceral mass (100 gm.) was minced and ground with water (400 c.c.). The suspension was allowed to stand overnight in a refrigerator. It was then filtered through cloth. The turbid aqueous extract was purified by acidification. To the turbid extract 5*N* acetic acid was added drop by drop with stirring till the *p*H of the extract was 4·0. This was kept in the ice chest for one hour for complete precipitation. At the end of the period, it was filtered in cold through Whatman No. 5 filter paper under suction with the help of hyflo-supercel. The clear filtrate contains both the thiaminases. The extract was prepared fresh every week.

Procedure.—Eighteen young albino rats, 4 weeks old, weighing from 40–50 gms. and of known nutritional history were used as experimental animals. These were placed in individual cages provided with raised wide mesh bottoms, to prevent the animals to have access to excreta. The storage capacity of the rat for vitamin B_1 is so low that it is not necessary to deplete the animal preliminary to feeding (Evans and Lepkovsky, 1928). However, they were fed on basal diet for seven days to be on the safe side. During this period there was a rapid increase in the weight of all

animals. At the end of this period, they were assembled into three groups, each group having six animals.

Group I ..Basal diet+5 micrograms thiamine hydrochloride.
Group II ..Basal diet+5 micrograms thiamine hydrochloride+2 c.c. mussel extract.
Group III ..Basal diet only (control).

10 gm. of basal diet per day per rat was given. Thiamine in solution, 5 micrograms per rat per day, which approximates the growth requirement (Williams and Spies, 1939) was delivered through pipette into mouth. Rats in Group II, received 2 c.c. of mussel extract per day. Before giving the regular diet, the mussel extract was given in small cups, with a pinch of sugar. After it was licked completely, 5 micro-grams of vitamin B_1 in solution was given orally.

The observations of body weight were made once in five days.

Rats in Group I, receiving 5 micrograms of vitamin B_1 steadily gained in weight, thereby indicating that the amount of vitamin given is quite sufficient for normal growth.

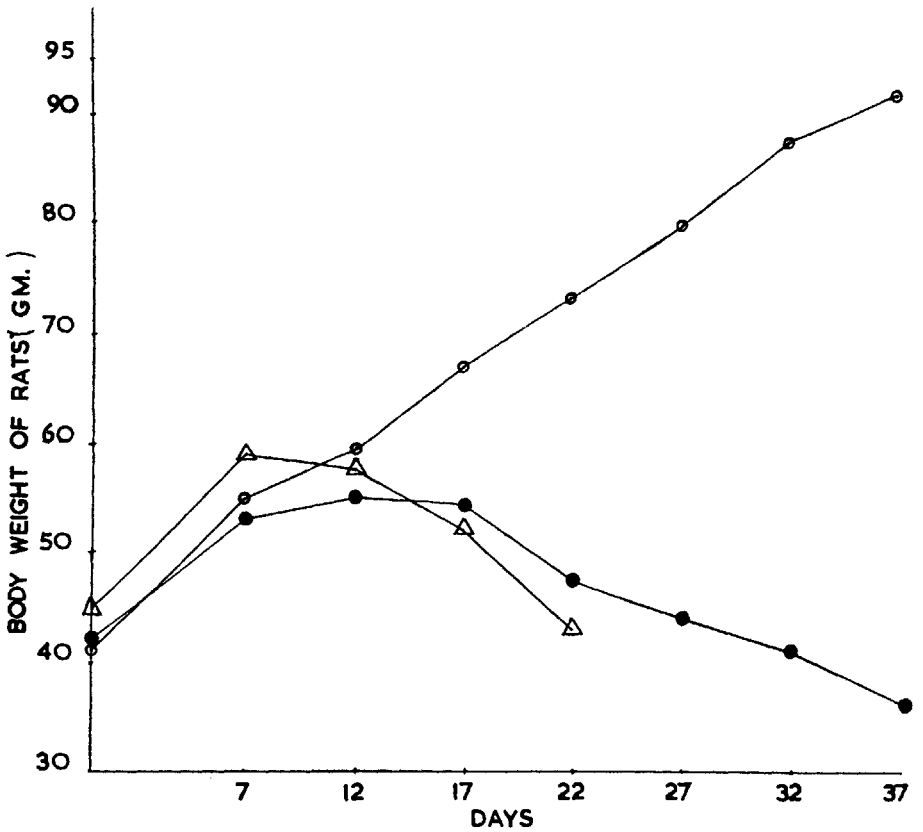

Fig II. Influence Of Mussel Extract On The Thiamine Availability

⊙—⊙ — Basal Diet+5 Micrograms Thiamine Hydrochloride
△—△ — Basal Diet+5 Micrograms Thiamine Hydrochloride+2cc Mussel Extract.
●—● — Basal Diet (Control)

Rats in Group II, receiving 2 c.c. of mussel extract along with 5 micrograms of vitamin B_1, rapidly declined in weight and their food intake had also fallen down. By about 20th day, severe symptoms of vitamin B_1 deficiency, such as lack of muscular co-ordination, spasticity, retraction of head and paralysis of hind legs were noticed. Spinning the rat by its tail evoked convulsive seizures. Five animals in this group died by 25th day and the rest died by 28th day.

The animals on basal diet (control group) lost weight gradually and the food intake had also fallen down. But the symptoms of deficiency, such as torpidity and spastic movements were noticed only after four weeks. Two rats died on 34th day and the rest were alive till 37th day, when the experiment was discontinued.

The results are presented in Figure II.

Discussion.

It seems obvious from the results that the 'anti-thiamine factor' present in the mussel extract is capable of destroying vitamin B_1, both *in vitro* (Reddi *et al.*, 1948) and *in vivo*. These results are of practical importance, since the 'anti-thiamine factor' is reported to be widely distributed in foodstuffs of both plant and animal origin. According to Joliffe (1943), this factor represents a type of conditioning factor potentially responsible for malnutrition.

The destructive effect of this factor can find application in the biological assay of vitamin B_1. It can be used in preparing diets free from vitamin B_1. It is difficult and time consuming to prepare diets free from vitamin B_1 for biological assay. Autoclaving or sulphite treatment might involve partial or complete destruction of other essential factors. It is far simpler and more effective to incubate the material, that is to be rendered free from vitamin B_1, with mussel extract. Abderhalden (1946) studied the application of thiaminase in biological experiments. Symptoms of deficiency were quickly produced in pigeons given carp flesh, which contains thiaminase.

Summary.

The influence of mussel extract on the availability of added thiamine hydrochloride was studied by rat growth technique. The results indicate that the factor present in mussel extract is capable of destroying thiamine in the system.

The application of the 'anti-thiamine factor' in the mussel extract in biological assays of vitamin B_1 has been discussed.

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