Influence of Rice and Purified Diets upon Cardiac Behavior of Thiamine Deficient Rats. (18027)

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Marked cardiac damage has been reported in severe chronic thiamine deficiency in dogs by Porto and de Soldati(1), in rats by Lowry *et al.*(2), and by Ashburn and Lowry(3), in pigs by Follis *et al.*(4), and in humans by Weiss and Wilkins(5). Aalsmeer and Wenckebach(6) have reported mild, perhaps nonspecific, heart damage in human beriberi in the Orient as shown by electrocardiographic and microscopic studies. In this country, however, Weiss and Wilkins(5) found severe cardiac damage in human beriberi patients as reflected in electrocardiographic and histologic studies. The beriberi reported by Weiss and Wilkins(5) in this country occurred mainly in alcoholics while that in the Orient occurred chiefly in persons subsisting largely on rice. This difference in the severity of cardiac involvement suggested the possibility that rice might possess some substance or property that might protect the heart. This possibility was given added impetus by the work of Kempner(7), who reported beneficial effects in hypertensive patients on a high rice diet supplemented with adequate vitamins. In order to test the hypothesis that rice might possess a protective substance or property, rats were made severely and chronically thiamine deficient by diets composed principally of rice and by other diets made of purified ingredients. The influence of each diet was ascertained by the extent of cardiac damage as shown by the electrocardiograms and pathologic examination.

Methods and procedure. Weanling male albino rats of Osborne and Mendel or Sprague-Dawley (National Institutes of Health Colony) strains were housed individually in elevated wire bottom cages. The animals were divided into groups and were fed the following diets *ad libitum*:

Group	No. of rats	Diet
I	10	2293 (purified diet with adequate thiamine)
	20	2292 (purified diet with no thiamine)
II	5	2369 (rice diet with adequate thiamine)
	20	2299 (rice diet)
III	20	2619 (purified diet with thiamine added approximately equal to rice diet 2299)

Rats receiving diets 2293, 2299, and 2369 were comparable as to age, weight and litter and were started on the diets when approximately 50 g in weight. Rats receiving diets 2292 and 2619 were maintained on a stock diet until approximately 110 g in weight after which the experimental diets were started. Preliminary experiments showed that this was necessary to produce rats which were comparable in body size and general condition at the onset of thiamine deficiency. The composition of the diets is given in Table I. The two lots of rice used in this experiment contained 61 μg and 64 μg of thiamine per

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1. Porto, J., and de Soldati, L., *Rev. Soc. argent. de biol.*, 1939, v15, 303.

2. Lowry, J. V., Sebrell, W. H., Daft, F. S., and Ashburn, L. L., *J. Nutrition*, 1942, v24, 73.

3. Ashburn, L. L., and Lowry, J. V., *Arch. Path.*, 1944, v37, 27.

4. Follis, R. H., Jr., Miller, M. H., Wintrobe, M. M., and Stein, H. J., *Am. J. Path.*, 1943, v19, 341.

5. Weiss, S., and Wilkins, R. W., *Tr. A. Am. Physicians*, 1936, v51, 341; *Ann. Int. Med.*, 1937, v11, 104.

6. Aalsmeer, W. C., and Wenckebach, K. F., Wien, *Arch. f. inn. Med.*, 1929, v16, 193.

7. Kempner, W., *North Carolina M. J.*, 1944, v5, 125.

TABLE I.
Composition of Test Diets.

Diet 2292		Diet 2299		Vitamin Powder	
				mg per g	
Casein (Vit. free)	18	Casein (Vit. free)	12.1	Thiamine	0
Sucrose	72	Rice (40-60 mesh)	77.9	Riboflavin	.3
Cottonseed Oil	3	Cottonseed Oil	3.0	Pyridoxine HCl	.25
Cod Liver Oil	2	Cod Liver Oil	2.0	Nicotinic Acid	2.0
Osborne and Mendel salt mixture(9)	4	Osborne and Mendel salt mixture(9)	4.0	Ca Pantothenate	2.0
Vit. Powder	1	Vitamin Powder	1.0	Choline Cl	100.0
				Inositol	10.0
Diet 2293 = Diet 2292 + 200 μg thiamine/100 g				Vit. K (menadione)	.1
" 2619 = " 2292 + 39 " " "				Biotin	.01
" 2369 = " 2299 + 200 " " "				Folic Acid	.1

Alpha-tocopherol (3 mg) was given by supplement dish once a week. Vit. A and D were supplied in the diet by the cod liver oil, each gram of which supplied at least 850 and 85 USPXI units of Vit. A and D, respectively.

100 g as determined by fluorometric assay(8). A rice diet completely free of thiamine would have been desirable in these experiments, but pilot studies revealed that rice autoclaved at 250°F (15 lb pressure) for 6 hours to destroy the thiamine, underwent changes which seriously altered its nutritional quality. However, a diet composed of 77.9% natural white polished rice and 12.1% casein supplemented with fat and with vitamins except thiamine (diet 2299) produced a severe thiamine deficiency in spite of the trace of thiamine in the rice. This deficiency could not be differentiated by ordinary signs and symptoms from that resulting from a diet almost completely free of thiamine. To control the effect of the trace of thiamine in the polished rice, a similar amount of thiamine was added to one of the purified diets. Casein was added to the rice diets to equalize the protein content.

The animals in Group I on diet 2292 received 7 μg of thiamine hydrochloride in 1 ml of water, 3 times weekly by supplement dish until the sixty-third day of the experiment at which time the thiamine was withdrawn and the first acute thiamine deficiency was allowed to follow. This was done, as in previous experiments(3), to produce a chronic thiamine deficiency and to produce a deficiency of a severity equal to that developed by rats on diets 2299 and 2619 which contained some thiamine. Acute polyneuropathy (spasticity, ataxia and convulsions) was used as the principal indication of severe thiamine deficiency. Other confirmatory signs were large weight loss, decreased heart rate, muscular weakness, apathy, lack of muscular tonus, reduced blood pressure, and cyanosis. As the animals developed acute polyneuropathy, they were treated with 50 μg of thiamine hydrochloride in 0.25 ml of water by stomach tube, and then allowed to develop a second acute deficiency, and again treated with thiamine. In a few cases this was repeated for a third and fourth deficiency. In order to emphasize any cardiac changes that might be developing, the rats were made as thiamine deficient as possible before treatment. Electrocardiograms were taken on all animals once a week, and in some cases more often. When animals showed acute thiamine deficiency symptoms, electrocardiograms were taken at frequent intervals before and after thiamine therapy.

Electrocardiograms. The electrocardiograms were obtained with a Sanborn electric "Portocardiograf" in conjunction with a Sanborn "Cardioscope" amplifier, similar to the apparatus described by Rappaport and Rappaport(10). Considerable difficulty was experienced during this experiment in securing satisfactory electrocardiograph electrodes for

8. *Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists,* Washington, 6th edition, 1945, 608.

9. Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1917, v32, 369.

10. Rappaport, M. B., and Rappaport, I., *Am. Heart J.*, 1943, v26, 662.

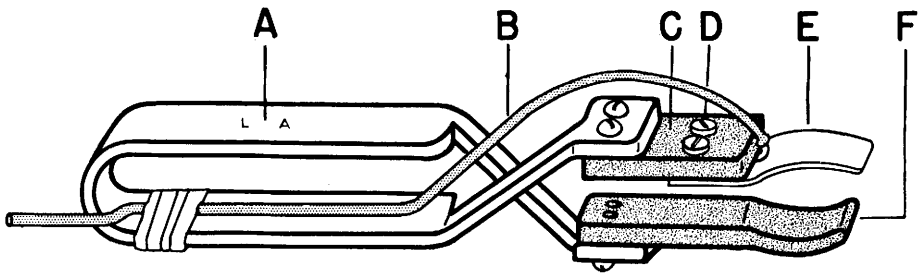

FIG. 1.
German silver electrocardiograph contact electrode.

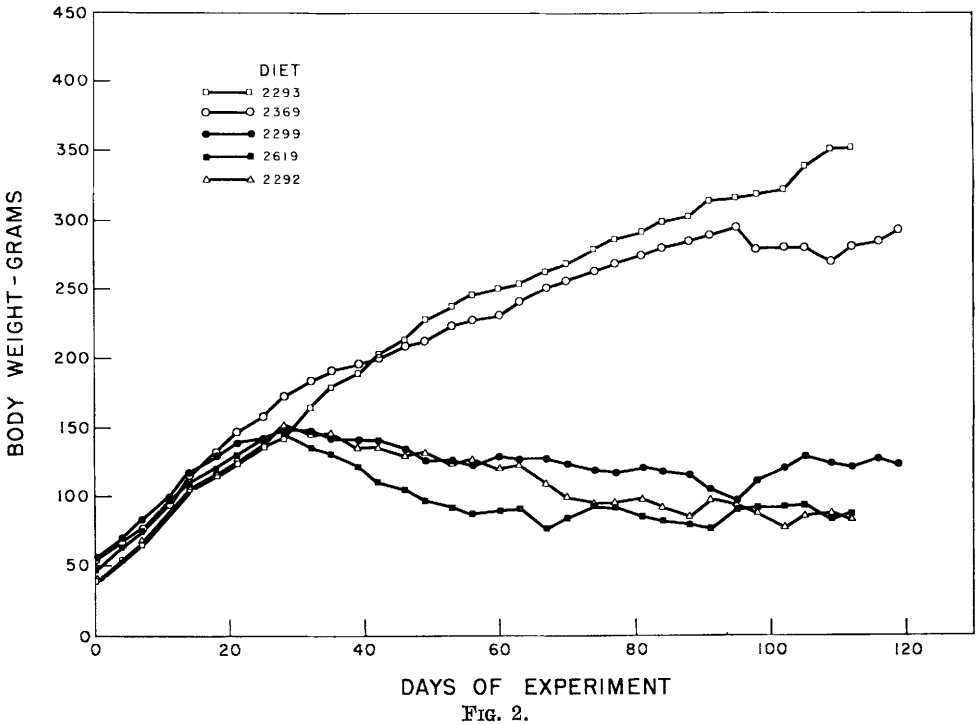

FIG. 2.
Average growth curves of rats on the test diets.

the rat. A simple German silver electrode (Fig. 1) was designed for use with the unanesthetized rat. The electrode consists of a flat spring clip (A) made of phosphor bronze (grade A, 5%, spring temper) 1/50 x ¼ x 1½ inches. To one end of the flat spring clip is attached a piece of bakelite (C) 1/16 x 1/4 x 1/2 inches to which is fastened the German silver electrode (E) 13/1000 x ¼ x ½ inches. The German silver electrode is attached to the bakelite with two German silver screws (D). The German silver used for the electrode is composed of nickel 18, copper 56, and zinc 26%. The end of the patient cable (B) is inserted between the bakelite and the German silver electrode where it is securely held in place by tightening the two German silver screws. This arrangement facilitates replacement of the cable wire whenever necessary. Soldering, which would introduce other foreign metals and possible polarization, is eliminated. The cable is made of shielded grounded copper wire (0.080 inch O.D.) of extreme flexibility and limpness, such as is used for phonograph pick-up cable. To the other end of the spring clip is attached a piece of bakelite (F) 1/16 x 1/4 x 13/16 inches. The three standard limb leads

TABLE II.
Average Heart Rates (B.P.M.) of Experimental Rats During Acute Thiamine Deficiency Episodes and Following Thiamine Therapy.

Deficiency period	Diet 2292		Diet 2299		Diet 2619	
	During	After	During	After	During	After
1	267 (19)	463 (14)	342 (18)	484 (15)	311 (14)	470 (13)
2	282 (10)	435 (8)	330 (15)	472 (11)	312 (12)	477 (9)
3	288 (7)		310 (8)		258 (6)	

Number of animals measured is indicated by figures in parentheses.

TABLE III.
Incidence of Rhythm Changes in Experimental Rats in the 3 Groups.

Deficiency episodes	Diet 2292		Diet 2299		Diet 2619	
	No. of rats	Rats with rhythm changes	No. of rats	Rats with rhythm changes	No. of rats	Rats with rhythm changes
1	19	6	19	5	20	2
2	13	11	15	5	14	2
3	7	5	10	3	11	1*

* During this deficiency period 4 rats died before tracings were obtained.

were used. The unanesthetized rat was held in its normal crouched position by the operator. A darkened quiet room was found helpful in keeping extraneous interfering stimuli at a minimum.

Results. The thiamine deficiency produced by the test diets in this study was very severe in spite of the fact that some of the diets contained a trace of thiamine. In Fig. 2 the average body weight curves for the control and experimental animals in each group are depicted. The thiamine deficient rats on the rice diet 2299 maintained their body weight at a higher level than did the thiamine deficient animals on the purified diets 2292 and 2619. The control animals on diets 2293 and 2369 grew approximately the same. Rats going through an acute thiamine deficiency episode displayed characteristic bradycardia. The animals on the rice diet (2299) had a less severe bradycardia than did the animals on the purified diet. However, a purified diet with thiamine added approximately equal to that in the rice diet also produced a less severe bradycardia. (Table II). The heart rates after thiamine therapy were similar in all the groups.

Control animals showed no electrocardiographic changes other than the slight slowing of rate associated with aging. The electrocardiograms of many of the thiamine deficient rats showed marked changes in rhythm, such as sinus arrhythmia, complete arrhythmia, A. V. block with wandering pacemaker, auricular fibrillation and sinus arrest similar in all respects to those fully described by Hundley *et al.*(11). The incidence of rhythm abnormalities was considerably less in the rats on the rice diet (2299) than on the purified diet (2292). However, rats on a purified diet with thiamine approximately equal to that in the rice diet also showed a low incidence of rhythm abnormalities (Table III).

Pathology. The pathologic lesions of the heart in these experiments were similar in all respects to those observed and fully described by Ashburn and Lowry(3). In summary, the microscopic findings were degenerative changes of muscle fibers of varying extent and severity, cellular infiltration, fibroblast proliferation or varying degrees of fibrosis. The pathologic changes were usually confined to the auricles and/or the pulmonary veins and rarely involved the ventricles.

The hearts of the control animals on the rice diet 2369 showed no pathologic changes. The hearts of two control rats on diet 2293 for some unexplainable reason showed patho-

11. Hundley, J. M., Ashburn, L. L., and Sebrell, W. H., *Am. J. Physiol.*, 1944, v144, 404.

TABLE IV.
Incidence of Heart Lesions in Rats on the Experimental Diets.

Diet	No. of rats examined	No. of rats with heart lesions
2292	17	10
2293	19	5
2619	18	6

logic changes similar to those seen in the thiamine deficient animals. Such changes have not been observed previously in the many control rats examined in this laboratory (2,3,11). It is possible that the tissues of the control group (2293) and the experimental group (2292) were mixed during their preparation for pathologic examination. If a mix-up did occur, it could only have been between the 2292 and 2293 groups, since they were the only groups examined simultaneously. A comparison of the various groups showed that the incidence and severity of pathologic damage were less in the hearts of rats on the rice diet (2299) than in those of rats on the purified diet (2292). However, rats on the purified diet with thiamine added to equal approximately that naturally present in the rice diet, received protection similar to that exhibited by the latter diet (Table IV).

Discussion. The results of this experiment indicate that the hearts of thiamine deficient rats received more protection from a rice diet than from a purified diet. However, this protection was undoubtedly due to the trace of thiamine naturally found in rice since a purified diet containing equivalent thiamine provided about the same protection as the rice diet. It is evident that the thiamine present in the rice diet and the approximately equal amount of thiamine present in one of the purified diets was insufficient to protect against the development of rat beriberi, but was sufficient to produce a definite reduction in the incidence and severity of heart damage. This suggests that the heart can be protected against damage in beriberi by an amount of thiamine insufficient to modify the clinical disease appreciably. The fact that Aalsmeer and Wenckebach(6) observed only mild cardiac damage in their rice-eating Oriental beriberi patients while Weiss and Wilkins(5) observed severe cardiac damage in their chronic alcoholic Occidental beriberi patients, may also be explained by the small amount of thiamine available to the former group as compared to the probably almost complete lack of thiamine in the alcoholics.

The data provide no support for the possibility that rice contains a property or substance which conveys a rather general protection to the heart. However, the conditions used in these experiments produced a severe and chronic deficiency state. If tests were conducted using milder conditions or a different test system, the results might have been quite different.

The tests described in the present communication were conducted using diets in which protein, salt, vitamins and fat intake were adequate and equalized. Thus low sodium, low protein and other vitamin deficiencies should not have complicated the results. Thiamine deficient rats restrict their food intake voluntarily and thus reduce their overall intake of protein and other dietary essentials. However, previous work in this laboratory(3) using paired feeding technics has shown that inanition itself will not produce cardiac damage if the thiamine intake is adequate. What effect rice might have had if the diets were also low in protein, fat and salt, as in the Kempner diet(7), is conjectural.

Summary. Cardiac damage was produced in rats by a severe chronic thiamine deficiency. Rats fed a natural polished rice diet and/or a purified diet with thiamine added to approximate that naturally present in rice had less severe physiologic and pathologic changes than rats fed a thiamine free purified diet. The results of this experiment indicate that the protection shown by the rice diet and a purified diet with thiamine approximately equal to that of the rice diet was due to the trace of thiamine and not to some unknown substance or property in rice.

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