Time Course of Resistance to Experimental Cardiac Necrosis in Thiamine-Deficient Rats[1] (36053)

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In a previous publication, we reported that rats fed a thiamine-deficient diet were more resistant to isoproterenol-induced cardiac necrosis than animals fed a complete purified diet (1). However, the time required for the increased resistance to develop was unknown. This study was designed to follow the time course of the changing sensitivity of the thiamine-deficient myocardium to cardiac necrosis, and to determine if the high level of resistance remained when thiamine was refed.

Methods. Male rats of the CFE strain (Carworth Farms) were divided into two groups. One group was fed a complete purified diet. The second was fed an identical diet from which thiamine had been omitted. A total of 54 animals was used in these experiments, with each group consisting of at least 8 animals.

Periodically, throughout the period of study, experimental cardiac necrosis was induced using isoproterenol according to the technique described by Rona *et al.* (2). Subcutaneous injections of isoproterenol (80 mg/kg) were administered on 2 consecutive days at 24 hr intervals. On day 3, the animals were sacrificed under chloroform anesthesia and the hearts were removed. The extent of the lesion was graded macroscopically according to the scheme devised by Rona *et al.* (2). (0; no necrosis; 1; diffuse paling of the tip; 2; limited necrosis of the tip; 3; necrosis reaching up to a third of the left ventricle; 4; more than one-half of the left ventricle affected by necrosis). When resistance to the drug was established in the thiamine-deficient animals, one group was fed 25 μg of thiamine for 2 days and then sacrificed.

Results. The weight curve of the thiamine-deficient animals is shown in Fig. 1. These animals gained weight until about day 14 and then began to lose. When thiamine was administered on day 21, normal growth again was restored. The growth curve is similar to that obtained by us previously (1) and similar also to that obtained by other workers (3, 4).

After the first of the two injections of isoproterenol, all of the animals except those fed thiamine lost from 3 to 6% of their body weight (Fig. 2). This loss was due primarily to a reduction in food intake and to the severe diuresis induced by the drug. Similar observations have been made by other workers (5, 6). After the second injection of isoproterenol the animals that had been on the diet for 0 and 7 days, as well as the thiamine-fed animals, gained weight while the animals that had been on the diet for 14, 16, and 21 days continued to lose.

As seen in our earlier work, the myocardium of the thiamine-deficient rats was markedly resistant to the effects of isoproterenol. The severity of the lesion increased from 1.25 on day 0 to 2.13 on day 16 and then decreased abruptly (Fig. 3) to 0.37 on day 21 of the deficiency. After feeding thiamine for 2 days before and during the period of injections, the increased resistance was retained with a score of .33 despite the marked increase in body weight.

The distribution of the necrosis ratings on each day of the dietary regime are detailed in Table I. On the first day of the experiment 100% of the animals sustained lesions which were scored as moderate (1 and 2). By day 14, 37% of the lesions were scored as severe (3 and 4) and there were 2 deaths. By day

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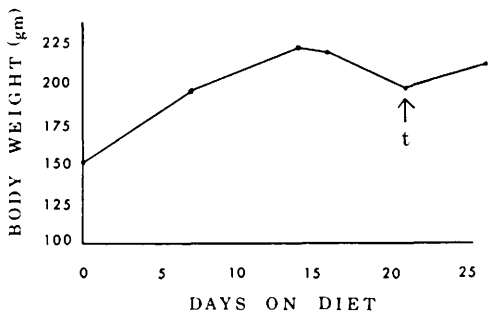

FIG. 1. Body weights of animals fed a thiamine-deficient diet: t = animals fed 25 μg of thiamine.

16, approximately 50% of hearts were rated 3 or 4. On day 21, however, the lesions were either absent (score 0) or slight (score 1). This reduction in the severity of the lesion was also maintained on day 26 after thiamine had been administered and after there had been a marked increase in body weight.

Discussion. The results obtained in these experiments are in accord with those reported earlier (1). Rats on a thiamine-deficient diet develop a resistance to experimentally induced cardiac necrosis which occurs between the second and third week of feeding the deficient diet. Also, the increased resistance is maintained for at least 5 days after the animals are fed thiamine and despite the fact that body weight is regained.

Dertinger *et al.* (7) have shown that very light rats (21 g) are extremely resistant to the cardiac necrosis elicited by isoproterenol. Moreover, Rona *et al.* (8) have shown that there is a direct relationship between the extent of the lesion caused by isoproterenol and body weight over a wide range of body weights. However, there appeared to be little relationship between these two parameters within the range of body weights used in these experiments with the possible exception of the first group of rats with an average weight of 150 g.

Follis (9) has shown that animals fed a thiamine-deficient diet are also more resistant to cardiac lesions elicited by potassium deficiency. Moreover, the cardiac protection afforded by thiamine deficiency in his studies was not related to weight differences because animals fed a potassium-deficient diet alone and restricted in their food intake developed severe cardiac lesions.

The sites at which thiamine deficiency may interfere with cardiac metabolism are well known. Transketolase activity of heart tissue is reduced as early as 4 days after feeding a thiamine-deficient diet (10), and pyruvate and α-ketoglutarate dehydrogenase activity are reduced within 1 week (4, 11). However, Argus *et al.* (11) have reported that during the first 2–3 weeks of thiamine deficiency, the availability of pyruvate for mitochondrial ATP synthesis is increased. It is not until after 3 weeks on a thiamine-deficient diet that ATP production via the TCA cycle is reduced (12). Similarly, Aldringer (13) has shown that the myocardium of thiamine-deficient rats retained the ability to develop normal grades of tension during the first two weeks of feeding a thiamine-free diet. After this period, however, the myocardium of the thiamine-deficient rats showed a marked decrease in the ability to develop tension.

The possibility of whether there may be an increased reliance on the glycolytic pathway to protect the thiamine-deficient heart when a second stress factor, such as isoproterenol, is administered has not been investigated. However, Wu *et al.* (14) have reported increased glycolytic activity as indicated by an initial increase in extramitochondrial α-glycerophosphate activity, which returned to control levels within 4 to 5 weeks on a thiamine-

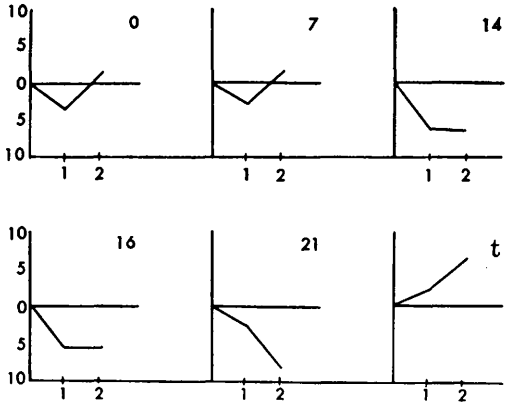

FIG. 2. Change in body weight in response to first (1); and second (2) injection of isoproterenol: (abcissa) days after injection; (ordinate) percentage body weight change; 0, 7, 14, 16, 21 indicate days on thiamine-deficient diet; t = refed thiamine before injection.

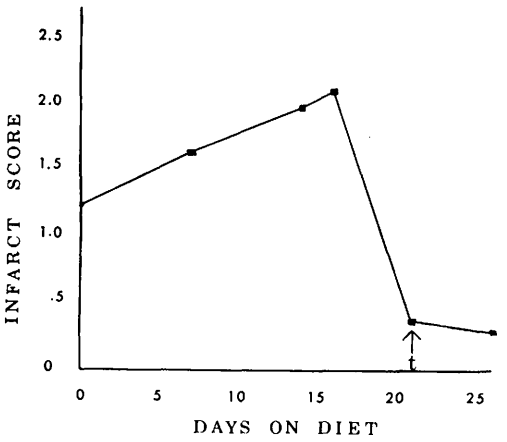

FIG. 3. Severity of myocardial infarct of rats fed a thiamine-deficient diet for varying periods of time.

free diet. In addition, McCandless *et al.* (12) have reported slightly higher lactate levels in the hearts of thiamine-deficient rats, which also suggests an increase in glycolytic activity. However, Park and Gubler (3) reported a decrease in total LDH activity and changes in the LDH isozyme pattern which would tend to favor aerobic metabolism.

It seems possible that the apparent confusion between the results of these studies may be due to the stage of the thiamine deficiency at which determinations are made. Boullin (15), for example, has reported that isolated heart from severely thiamine-deficient rats had increased sensitivity to adrenaline, noradrenaline, and isoproterenol. Our *in vivo* studies, however, clearly indicate that in early thiamine deficiency, the hearts of deficient animals are quite resistant to the necrogenic effects of isoproterenol.

TABLE I. Distribution of Necrotic Ratings of Rats Fed a Thiamine-Deficient Diet.

Days on diet	No. of rats	Ratings					No. of rats died
		0	1	2	3	4	
0	8	—	6	2	—	—	—
7	11	—	7	2	1	1	—
14	10	1	2	2	2	1	2
16	8	1	2	1	3	1	—
21	8	5	3	—	—	—	—
26	9	6	3	—	—	—	—

Summary. Hearts of rats maintained on a thiamine-free diet were found to develop a resistance to the necrogenic effects of massive doses of isoproterenol within 2 to 3 weeks following initiation of the dietary regime. Feeding thiamine for 5 days to the deficient animals did not diminish the acquired resistance to the necrotic effects of isoproterenol.

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