peltocephalus and *B. blombergi* have been studied for presence of substances known to occur in secretions of other toads. Catecholamines and indolethylamine derivatives were found in both samples, more in *B. peltocephalus* than in *B. blombergi*. Cholesterol was also detected in both species. The digitalis-like substances of *B. peltocephalus* were more potent in cats than those of *B. blombergi*.

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Effect of Thiamine Deficiency on Glutathione Contents of Erythrocytes and Tissues in the Rat. (25771)

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Thiamine-deficient rats excrete a significant amount of methyl glyoxal which is not found in the urine of treated ones. This phenomenon is probably attributable to reduction of glyoxlase activity in liver(1). The mechanism for the decrease of this enzymatic action is, however, not clear. Since glutathione is known as an essential co-factor for glyoxlase activity(2), it becomes important to ascertain whether reduction of glyoxlase activity is related to changes of glutathione content of thiamine-deficient animals. Experiments, therefore, were undertaken to determine the influence of thiamine deficiency on glutathione levels of erythrocytes and tissues in the rat.

Materials and methods. Twenty-five-day-old male rats weighing from 32 to 41 g were used for this study. They were randomly distributed into 2 groups of 6 each. One group received the basal diet supplemented with all known vitamins, except thiamine. The percentage composition of the basal diet has been shown elsewhere(3). The other group was kept on the same basal diet supplemented with thiamine HCl (0.22 mg/100 g basal diet). All animals were housed individually in screen-bottom cages and were offered drinking water *ad libitum*. The feeding was continued for 4 weeks in the first experiment and 8 weeks in the second experiment. Animals were fasted overnight before being sacrificed. Heparinized blood samples were drawn by direct cardiac puncture. A small portion of liver (approximately 0.5 g) was quickly excised in the first experiment; and, in addition, liver tissue, one left kidney and whole heart were removed from each rat in the second experiment. Glutathione content of blood and tissues were measured by the nitroprusside test as described previously(4).

TABLE I. Effect of Thiamine Deficiency on Growth and on Erythrocyte and Liver Glutathione Content.

Dietary treatment	Final body wt, g	Erythrocyte, μM/100 ml R.B.C.	Liver, μM/100 g wet tissue
Thiamine-deficient	89 ± 9.1 (6)*	176 ± 13.1	715 ± 31.9
Thiamine-treated	144 ± 3.3 (6)	321 ± 21.9	389 ± 59.7
t value	9.78	6.24	5.76

* Mean and stand. error of mean. No. in parenthesis denotes No. of rats used.

Results. The effect of thiamine deficiency on erythrocyte and liver glutathione contents and final body weight is presented in Table I. It indicates that thiamine deficiency results in an alteration of glutathione metabolism. Mean glutathione value in erythrocytes of thiamine-deficient rats is approximately 54% of that of animals raised on a thiamine-supplemented diet. Hepatic glutathione activity of thiamine-depleted rats was 84% higher than that of rats receiving thiamine in the diet.

The second experiment was primarily undertaken to study whether or not other vital organs, besides liver, will have altered glutathione concentration as a result of prolonged feeding of thiamine restricted diet. The results are shown in Table II. Again, glutathione level in erythrocytes was reduced and that in liver was elevated in thiamine-deficient rats. Furthermore, thiamine deficiency results in a 46% decrease of glutathione activity in cardiac muscle, but has no effect on level of glutathione in kidneys.

The finding that concentration of the important regulator of cell metabolism, glutathione, is diminished in erythrocytes under the dietary restriction of thiamine is of importance since glutathione plays a significant role in carbohydrate metabolism. Barron(5) postulated that one of the most important functions of glutathione is to maintain the activity of sulfhydryl enzymes in metabolism. A direct relationship between glutathione and carbohydrate metabolism has been reported by Krimsky and Racker(6) who demonstrated that glutathione is the prosthetic group of glyceraldehyde - 3-phosphate dehydrogenase. Thus, it is quite possible that any alteration of glutathione content in the body will consequently entail derangements in carbohydrate metabolism. In view of the involvement of thiamine as thiamine pyrophosphate in pyruvate metabolic pathways, it is of interest to point out that the increase in level of pyruvate and lactic acid in blood of thiamine-deficient rats might be partially due to decreased activity of glutathione in the erythrocytes.

The increase in amount of glutathione in the liver of thiamine-depleted animals suggests that there is little or no correlation with reduction of glyoxlase activity. In this organ, glutathione is broken down and resynthesized (7,8). The present findings do not indicate that thiamine participates either in the degradation process or in the biosynthesis of this tripeptide.

TABLE II. Effect of Thiamine Deficiency on Erythrocyte and Tissue Glutathione Content.

Dietary treatment	Final body wt, g	Erythrocyte, μM/100 ml R.B.C.	μM/100 g wet tissue		
			Liver	Kidneys	Heart
Thiamine-deficient	80 ± 10.5 (6)*	130 ± 10.7	577 ± 12.8	361 ± 25.8	104 ± 11.3
Thiamine-treated	233 ± 20.9 (6)	272 ± 19.7	401 ± 40.6	354 ± 23.5	188 ± 9.5
t value	7.80	7.20	4.92	.27	6.84

* Mean and stand. error of mean. No. in parenthesis denotes No. of rats used.

Summary. Thiamine deficiency results in decrease in concentration of glutathione in erythrocytes and heart, but an increase in level of glutathione in liver tissue. The exact nature of the alteration of glutathione content is not known. The possible interrelationship of thiamine and glutathione in carbohydrate metabolism is discussed.

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Response of Adrenals, Thymus, Spleen and Leucocytes to Shuttle Box and Confinement Stress.* (25772)

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Previously reported studies indicated that exposure of mice to stress-induced emotional disturbance of 2-4 weeks of daily sessions in shuttle box or of physical restraint increase their susceptibility to herpes simplex and Coxsackie B1 viruses(1,2). In contrast, susceptibility to anaphylaxis(3) and to homograft reaction, delayed hypersensitivity(4), decreases after one or more exposures to shuttle box stress. The nature of changes in host-response has not been determined. However, known sensitivity of the pituitary-adrenal, and thymico-lymphatic and blood leucocytic systems to all forms of stress(5), together with the importance of these systems in resistance to infection(6), suggests that they may be related to changes in resistance following stress exposure. Our report describes responses of the above systems in mice exposed to stressors mentioned, as reflected by changes in adrenal, thymus, and spleen weights, and blood leucocytes. These measures were chosen primarily to elucidate mechanisms related to resistance but with the hope that results might also provide other criteria by which stress response could be measured.

Materials and methods. The stressors used, shuttle box and confinement, have been described. The latter stressor, in contrast to shuttle box, tends to minimize activity and avoids shock. Four to 6-week-old female Swiss mice were used(1). Experimental animals were subjected to stress by one of the methods indicated for 6 hours/day, 6 days/week. Control animals were maintained in home cages from which food and water were withdrawn for 6 hours daily. Subgroups of 10 animals and 10 controls were sacrificed after varying intervals of stress exposure, and varying periods of recovery following termination of 28 days exposure to stress. Animals were sacrificed under anesthesia usually within 4 hours after the last stress period. Controls were sacrificed in late morning or early afternoon of days on which stressed animals were killed. Organ weights were determined and expressed as mg/g of mouse weight determined immediately before death. For histological examination, organs were fixed in Bouin's solution, imbedded in paraffin, sectioned and stained with hematoxylin and eosin. Total leucocyte counts were made on tail blood. Differential leucocyte counts were made after May-Gruenwald Giemsa staining. Two hundred leucocytes were counted and separated into the following categories: Polymorphonuclear leucocytes, normal lymphocytes, stress lymphocytes(7), monocytes and eosinophiles.

Results. Shuttle box stress. The data summarized for shuttle box in Table I and in Fig. 1-4 represent composite means from several experiments with the following numbers of animals at intervals indicated: (1) during stress, 20 at 1 d.; 20 at 30 d.; 40 at 21 d. and 110 at 28 d., and (2) during recovery following 28 d. stress, 20 at 7, 14 and 21 d. Adrenal response to shuttle box stress is rapid, hypertrophy becoming evident within 3 days (Fig. 1). Thereafter, the curve for stressed animals rises more slowly through remaining 25 days

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