TISSUE THIAMINE IN HEMORRHAGIC SHOCK[1,2]

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Govier and his coworkers (1, 2, 3) have stated that dogs previously treated with thiamine withstand hemorrhagic shock better than untreated animals. This observation has not as yet been substantiated by others, but the rise of blood pyruvate and lactate in shock suggests that tissue cocarboxylase (thiamine-diphosphate), a coenzyme essential for pyruvate metabolism, may be diminished or inactivated. In order to ascertain whether thiamine metabolism is disturbed in this condition, experiments were done in which tissue thiamine was studied before and during shock in dogs.

METHOD

Thirteen dogs were studied. In 8 animals, hemorrhagic shock was induced by fractional bleeding. The remaining 5 animals were observed for 4 to 5 hours as controls; in these experiments, all procedures used in the shocked dogs were followed rigorously except that the animals were not bled.

Tissue samples were taken under local novocaine anesthesia, before and after the experimental (shock or control) period; all dogs had received 2.0 mgm. of morphine sulfate intramuscularly, approximately one hour before the beginning of the experiments. Approximately 20 to 30 grams of liver were taken for analysis. Kidney specimens were obtained by unilateral nephrectomy; the remaining kidney served for the experimental tissue. Muscle was obtained by biopsy from one of the quadriceps group of muscles. For comparison with the initial biopsy, a specimen from the contralateral group was taken at the end of the experiment. None of the animals showed a significant change from the preoperative normal blood pressure after the biopsies were taken.

The tissues were analyzed for their free (non-phosphorylated) and total (cocarboxylase plus free) thiamine content by the chemical method previously described (4, 5). In 4 animals, total thiamine was measured in the liver. In a fifth animal, both liver and muscle were thus studied. In the remaining 4 experimental animals and in the 5 control animals, liver, muscle, and kidney were analyzed for both free and total thiamine. Corrections for variations in percentage dry weight of the tissue were made in all but 2 experiments.

Fractional bleeding was used to maintain the animals' blood pressure below 70 mm. of mercury for from 1 to 6 hours; no attempt was made to ascertain the "reversibility" or "irreversibility" of shock. One animal required a small transfusion during the course of the experiment. Appended is the protocol of one typical experiment (Figure 1).

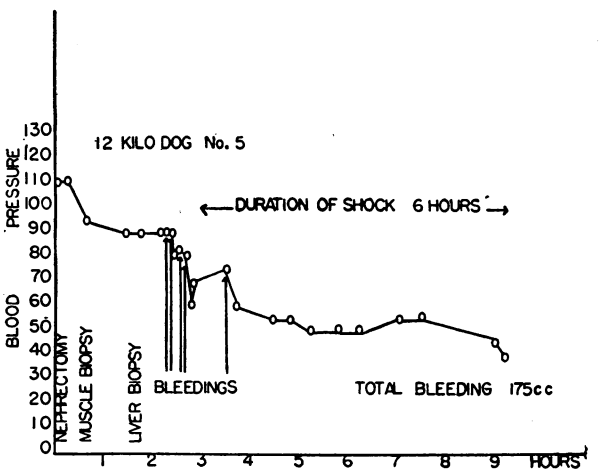

FIG. 1. PROTOCOL OF TYPICAL SHOCK EXPERIMENT

RESULTS

In 7 of 8 dogs in shock, a rise[3] occurred in the total thiamine concentration in the liver; this averaged 36 per cent (Table I). This change was due almost entirely to an increase in the cocarboxylase fraction in 3 animals so studied. The increase in liver thiamine seemed to be roughly related to the duration of shock (Figure 2). Insignificant changes, averaging plus 3 per cent, in the liver thiamine of the control animals were found (Table II).

Total thiamine concentration in the muscle showed a small decrease, and to the same extent,

[1] Reported to the Committee on Shock, O.S.R.D., Washington, D. C.

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[3] One animal, not included in this series, was subjected to prolonged application of a tourniquet to the legs followed by a small bleeding. In this tourniquet shocked animal, the thiamine concentration in the liver decreased 35 per cent.

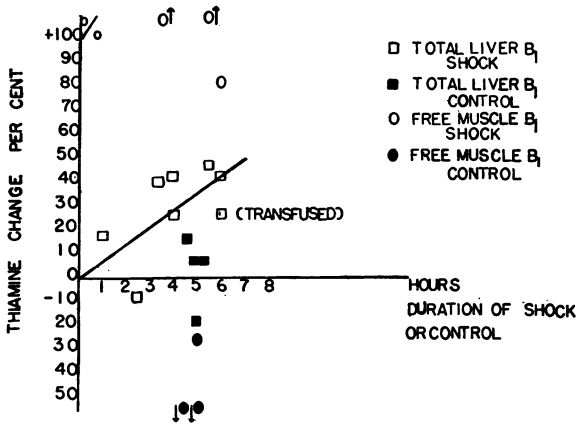

FIG. 2. LIVER AND MUSCLE THIAMINE IN HEMORRHAGIC SHOCK

in both the shock and the control groups. The free thiamine, however, rose markedly in the few shock animals so studied, in contrast to the decrease in this component in the control animals. This increase in free thiamine occurred at the expense of the cocarboxylase fraction, which in one animal (Dog 7) must have been reduced to an exceedingly low level.

Changes in kidney thiamine were extremely variable and could not be correlated with the presence or absence of shock.

DISCUSSION

Conclusions regarding the significance of the observed changes in the concentration of tissue thiamine must await further study. The changes which occur in muscle can, however, be explained on the basis of what is known about tissue cocarboxylase. The rise in the concentration of free thiamine at the expense of cocarboxylase in the muscle during shock suggests an increased *in vivo* splitting of the phosphorylated thiamine by phosphatase, with a shift to the right of the following equation:

$$\text{cocarboxylase} \rightleftharpoons \text{thiamine} + \text{phosphate}$$

TABLE I

Tissue thiamine changes in hemorrhagic shock

Dog	Time in shock	Tissue	Thiamine content						Change		
			Before shock			During shock					
			Total B_1	Cocarbox.	Free	Total B_1	Cocarbox.	Free	Total B_1	Cocarbox.	Free
	hours		*micrograms per gram*						*per cent*		
1*	1	Liver	1.29			1.54			+19.4		
2*	6 (transfusion)	Liver	0.43			0.54			+25.6		
3°	4	Liver	1.80			2.29			+27.3		
4°	4	Liver	2.13			3.02			+41.8		
		Muscle	1.50			1.04			−30.7		
5*°	6	Liver	2.69			3.84	3.29	0.55	+42.8		
		Muscle	1.73	1.45	0.28	1.68	1.16	0.51	−3.0	−19.7	+82.5
		Kidney	3.37	2.87	0.50	2.41	2.33	0.09	−28.5	−19.1	−83.0
6*°	5½	Liver	2.60	2.47	0.13	3.64	3.57	0.07	+48.5	+53.5	−43.5
		Muscle	2.20	2.13	0.072	2.00	1.60	0.40	−10.9	−25.9	+444.0
		Kidney	2.50	1.71	0.79	2.62	2.18	0.44	+3.6	+26.1	−45.0
7*	3⅛	Liver	1.71	1.44	0.269	2.45	2.11	0.342	+43.3	+46.5	+27.0
		Muscle	0.564	0.49	0.07			0.345			+392.0
		Kidney	1.79	1.58	0.214	1.65	1.275	0.375	−7.8	−19.0	+76.0
8*°	2½	Liver	2.70			2.50			−7.4		
		Muscle	1.70			1.68			0		
		Kidney	2.52			2.35	2.18	0.28	−6.8		

* Values corrected for any changes in percentage of dry weight of tissues.
° Animals received daily injections of 2 mgm. thiamine per kgm., for 4 or more days until day before experiment, with the exception of Dog 4 whose injections were stopped 2 days before the experiment.

TABLE II

Tissue thiamine changes in control animals—no shock

Dog	Time of experiment	Tissue	Thiamine content*						Change		
			Control			Experimental					
			Total B_1	Cocarbox.	Free	Total B_1	Cocarbox.	Free	Total B_1	Cocarbox.	Free
	hours		*micrograms per gram*						*per cent*		
1	4½	Liver	1.52		0±			0.21			
		Muscle	2.56		0±	2.27			−11.7		
		Kidney	2.24		0±	1.81	1.77	0.04	−19.2		
2	5	Liver	1.94		0±	2.08	1.97	0.11	+7.2		
		Muscle	3.02	2.74	0.28			0.21			−25
		Kidney	2.10	1.96	0.14			0.21			+50
3	4⅚	Liver	2.01	1.80	0.208	1.65	1.60	0.044	−18.0	−11.1	−75
		Muscle			0.103	1.60		0±			
		Kidney	1.93	1.41	0.521	2.06	1.06	1.00	+6.7	−25.0	+92
4	5	Liver	4.20		0±	4.50			+7.0		
		Muscle	7.60	5.63	1.97	3.50			−54.0		
		Kidney	5.80		0±	4.40			−24.0		
5	4½	Liver	1.40			1.64	1.48	0.16	+17.0		
		Muscle	0.87								
		Kidney	2.22	1.87	0.35	2.00	1.74	0.26	−10.0	−7.0	−26

* All animals received daily injections of 2 mgm. thiamine per kgm., for 4 or more days until the day before experiment. All values corrected for any changes in percentage of dry weight of tissues.

It has been shown (6) that under anaerobic conditions, phosphatases in certain tissues, including muscle (5), destroy cocarboxylase *in vitro*. The relative tissue anaerobiosis which occurs in profound shock may thus favor splitting of cocarboxylase. These data are in agreement with the findings of Greig and Govier [4] in animals studied one-half hour after their blood pressure had fallen to 60 mm. Hg following bleeding. These authors found similar changes in animals made anoxic by breathing air containing 10 per cent oxygen in nitrogen.

Changes in the concentration of total and phosphorylated thiamine in liver are more difficult to explain. There are three mechanisms which might cause a rise in liver thiamine in hemorrhagic shock. Destruction of cocarboxylase in the muscle, with concomitant liberation of free thiamine, might result in transfer of thiamine to the liver. The absence of any decrease in the total thiamine in the muscle of shocked animals, as compared with control animals, is not inconsistent with this view, since the relatively large mass of muscle may undergo changes in the total thiamine concentration, too small to measure in that tissue by this method; the total change in all the muscles may be sufficient to cause increase in the thiamine in the liver which is so much smaller than the mass of muscle. In this connection, it should be pointed out that intravenously administered thiamine goes rapidly to the liver, where it is quickly converted into cocarboxylase (7). Other possible explanations for the increase in liver thiamine are either decreased utilization of the vitamin in this organ or decreased mobilization of thiamine from the liver to the periphery, while the liver continues to receive the vitamin from the gastrointestinal tract or from the blood, as a consequence of entry of tissue fluid into the blood stream during hemodilution consequent to hemorrhage. It must be pointed out that Greig and Govier conclude that a decrease in hepatic cocarboxylase occurs in shock. The data of their recent paper show that of the 13 experiments described, a decrease in hepatic cocarboxylase occurred in only 6, with a rise in 5 and

[4] Dr. Govier very kindly sent me the manuscript of his paper in press, Journal of Pharmacology and Experimental Therapeutics, previous to publication.

no change in 2. Their experiments are not entirely comparable with those of the present study, since their animals were under barbital anesthesia and were in shock for only 30 minutes, whereas our animals were unanesthetized and in shock for 1 to 6 hours. In addition, most of our animals were previously fortified with injected thiamine for several days before shock was induced, whereas their animals were not treated.

SUMMARY

1. The concentration of total and phosphorylated thiamine in the liver of dogs rose during prolonged hemorrhagic shock; the increase in liver thiamine seemed to be related to the duration of shock. No significant change occurred in control animals.

2. The non-phosphorylated thiamine of muscle rose markedly during hemorrhagic shock; this change occurred at the expense of the cocarboxylase.

3. The change in the free thiamine-cocarboxylase ratio in muscle is interpreted as probably consequent to *in vivo* phosphatase splitting of phosphorylated thiamine, associated with the tissue anaerobiosis occurring in shock.

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