mice has spontaneously increased and the incubation period has become shorter and more constant. Passage from mice to tissue culture results in loss or marked reduction of virulence for mice. This modified virus can be employed quite satisfactorily for serum neutralization tests and makes available an additional field and laboratory tool for poliomyelitis research.

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Effect of Cold and Restraint on Blood and Liver Non-Protein Sulfhydryl Compounds. (20467)

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Beck and Linkenheimer(1) have reported a drop in concentration of liver non-protein sulfhydryl (glutathione) in mice exposed to cold and indicated that this fall in concentration was not due to the fall in body temperature which occurred(2). In the present studies 2 groups of rats (restricted and unrestricted) were exposed to cold. Liver and blood ergothioneine (ESH), glutathione (GSH), and total non-protein sulfhydryl (TSH) concentrations were compared with values for control animals.

Methods and materials. Healthy adult Sprague-Dawley rats were used: The 21 males (250-300 g) and 21 females (180-250 g) were divided into 3 groups each: 7 controls exposed at 22°C, 7 unrestricted exposed at 0°C, and 7 restricted exposed at 0°C. The control rats were housed in regular laboratory cages until time of sacrifice while the latter 2 groups were placed, for periods varying between 2 and 4 hours, in a refrigerator set to maintain a temperature of 0°C ± 2°C. The duration of exposure was determined by the time necessary for the rectal temperatures of the restricted animals to fall to between 15 and 25°C. Restraint was produced by means of a loose fitting wire mesh cylinder. All the animals were stunned with a blow on the head, blood obtained with a cardiac puncture and liver excised immediately and frozen with dry ice. ESH was determined by a modified method of Hunter(3); GSH by a modification of the method of Grunert and Phillips(4) and TSH by amperometric titration using a modification of the method of Benesch and Benesch(5). Details of the modifications will be published(6).

Results. As shown in Table I, no significant change in GSH or TSH concentration in the blood of rats was produced by restraint or cold. There was a slight drop in ESH. Similar treatment produced no change in ESH concentrations of the liver. However, there was a significant drop in the concentration of GSH and TSH in the livers of the restricted animals as compared to the control animals and to those which were maintained unrestricted in the cold. The fall in concentration of both GSH and TSH of the liver was more marked in the females than in the males under the conditions of the present experiment.

Discussion. The data showing a decrease in liver TSH in animals maintained in the cold are in agreement with the work of Beck and Linkenheimer(1,2). They measured only

TABLE I. Effect of Cold and Restraint on Rat Blood and Liver Ergothioneine, Glutathione and Total Non-protein Sulfhydryl Concentrations.

		Ergothioneine, μM %	Glutathione, μM %	Total non-protein sulfhydryl, μM %
		Liver		
Controls (22°C)	♂	89.3 ± 2.3†	765 ± 19	955 ± 16
Body temp. (38-40°C)	♀		722 ± 21	821 ± 37
Unrestricted (0°C)	♂	96.6 ± 4.5	729 ± 44	863 ± 31
Body temp. (37-39°C)	♀		667 ± 14	
Restricted (0°C)	♂	83 ± 3.8	612 ± 27	742 ± 32
Body temp. (15-25°C)	♀		480 ± 17	581 ± 37
		Whole blood		
*Controls (22°C)	♂	15.9 ± 0.3	122 ± 6	154 ± 5
*Unrestricted (0°C)	♂	13.0 ± 0.3	99 ± 5	161 ± 9
*Restricted (0°C)	♂	11.3 ± 0.9	108 ± 8	161 ± 10

* No significant difference in the hematocrit (avg value = 47).
† Stand. error of the mean.

a 1-2°C drop in body temperature of unrestricted mice(1). This was essentially the same as the fall in rectal temperatures occurring in the unrestricted rats in the present series. It has been demonstrated by Bartlett *et al.*(7,8) that the stress of restraint, as used here, is at least partially emotional in nature. By the use of such stress body temperatures of small mammals are made to fall precipitously(7,8). Whether the marked decrease in liver GSH and TSH was due to the lowered body temperature or to the emotional stress is not known. Studies are under way to clarify this point. Beck and Linkenheimer reported(2) that the fall in body temperatures was not responsible for the fall in liver TSH in mice. Mortensen(9) has suggested that blood GSH may be maintained at the expense of liver GSH. The present studies seem to indicate a similar relationship. It is noted in Table I that the drop in TSH in the liver is primarily due to a drop in the concentration of GSH.

It has been suggested that one of the functions of GSH is to maintain enzymes in their active sulfhydryl form(10). Since a number of enzymes in carbohydrate and lipid metabolism depend upon glutathione and other sulfhydryl compounds such as coenzyme A for their activity, any lowering of sulfhydryl compounds in the blood or liver may interfere with carbohydrate and lipid metabolism. Perhaps the low levels of GSH coupled with the low liver glycogen levels (qualitatively noted) resulted in a decreased ability to supply energy which could have been a factor in the loss of body temperature.

Summary. Adult male and female Sprague-Dawley rats were exposed to cold. Half of those so exposed were stressed by use of restraining cages. Ergothioneine (ESH), glutathione (GSH), and total non-protein sulfhydryl compounds (TSH) of blood and liver were determined. There was a slight fall in blood ESH and no change in blood GSH or TSH. Simultaneous exposure to cold and restraint produced a significant fall in liver GSH and TSH, but not in ESH.

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