Further Studies on Effect of Cold and Restraint on Tissue Non-Protein Sulfhydryl Compounds.* (21111)

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Recently Beck and Linkenheimer(1) reported a drop in concentration of liver non-protein sulfhydryl in unrestrained mice exposed to cold. This effect was not attributed to the observed lowering of body temperature (2). In a previous report(3) the effects of simultaneous exposure to cold and restraint on the liver and blood ergothioneine (ESH), glutathione (GSH), and total non-protein sulfhydryl (NPSH) concentrations were noted. Since a dual stress was used, the question of whether the marked decrease in liver GSH and NPSH was due to the low body temperature or to the stress of restraint was not answered. The present communication reports a series of studies in which attempts were made to quantitate the effect of hypothermia and the effect of restraint without a subsequent fall in body temperature on the concentration of tissue NPSH. Therefore, in an attempt to assay the separate effects, attempts were made to produce hypothermia in some animals without restraint and restraint in other animals without resulting hypothermia. Another group of animals was subjected to the dual stress of restraint and a drop in body temperature. The degree of alteration of the sulfhydryl compounds in these three groups was compared, in the present studies, with control values.

Methods and materials. Of the 122 young adult Sprague-Dawley rats used in the studies, 77 were females and 45 were males. The females were divided into 3 experiments, while all of the males were used in one experimental run. In each experimental series, the animals were divided into 4 groups. All treated animals were exposed for a period of 4 hours. One group consisted of untreated or control animals; a second group was restrained with body temperature maintained at normal levels; in a third group, animals were restrained and subjected to a cold environment. The rate of fall of the body temperature of this last group of animals was controlled at 5°C per hour so that the terminal temperature of the animals averaged approximately 19°C. A fourth group of animals was exposed unrestrained in the cold. Some of these animals were exposed in the cold room at a temperature of 0°C ± 2°C, while others were exposed in a deep freeze (temperature −15°C to −20°C). In the animals maintained unrestrained in the cold, no attempt was made to regulate the rate of fall of body temperature. The average terminal body temperature of each group is indicated in Table I. The animals were sacrificed with a blow on the head, the tissues excised immediately and frozen in powdered dry ice. Since ESH is not significantly altered by these treatments and since GSH comprises nearly all of the NPSH(3), the method of Benesch and Benesch(4) which measures NPSH was used. The changes which were observed, therefore, probably reflect changes in GSH.

Results. In Table I are shown the results of the experiments. It can be seen that both restraint and hypothermia were effective in lowering the sulfhydryl content of the liver. It is further noted that these effects are approximately additive (when the environmental temperature of the cooled unrestrained and the cold restrained animals were 0°C ± 2°); *i.e.*, neither restraint alone nor hypothermia alone produced the same decrease in concentration of NPSH as that produced by simultaneous exposure to hypothermia and restraint.

The cooled unrestrained animals maintained at the lower temperature (−15°C to −20°C) experienced a larger drop in concentration of NPSH as compared to the cooled unrestrained

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TABLE I. Effect of Cold and Restraint, Singly and Combined, on Liver Non-protein Sulfhydryl Compounds (NPSH).

Exp. No.		Treatment	No. of animals	Terminal body temp. (°C)	NPSH in μM %	t values
1	♀	0 (controls)	7	38-40	786 ± 10*	
		Cooled unrestrained 0°C	7	36-39	699 ± 10	6.0
		Warm restrained	7	38-40	689 ± 47	2.0
		Cooled " 0°C	7	15-20	468 ± 24	12.0
2	♀	0 (controls)	6	38-40	815 ± 23	
		Cooled unrestrained 0°C	7	36-39	740 ± 10	3.0
		Warm restrained	6	38-40	650 ± 22	5.1
		Cooled " 0°C	6	15-20	480 ± 29	9.1
3	♂	0 (controls)	10	38-40	811 ± 21	
		Cooled unrestrained −15 to −20°C	16	15-35	552 ± 31	6.9
		Warm restrained	10	38-40	737 ± 23	2.4
		Cooled "	9	15-20	636 ± 45	3.5
4	♀	0 (controls)	7	38-40	916 ± 11	
		Cooled unrestrained −15 to −20°C	6	15-35	553 ± 23	14.1
		Warm restrained	7	38-40	790 ± 32	3.3
		Cooled "	7	15-20	538 ± 23	14.7

* Stand. error of mean.

animals which were maintained at the higher temperature of 0°C. As indicated by the data, considerable hypothermia was produced in many animals by the lower temperature while little hypothermia resulted from a 4-hour exposure at the higher temperature.

No significant change in NPSH was observed in muscle or kidney tissue. The NPSH for muscle was 66-77 μM % and that for kidney was 350-450 μM %.

Discussion. From the data, it can be seen that both a drop in body temperature and restraint without a resultant drop in body temperature are effective in altering the sulfhydryl content of the liver. It is also apparent that under appropriate conditions these effects may be additive. As indicated by a recent publication from this laboratory(5), the diminution in concentration of these compounds may be a general response to stress. In this connection it is interesting to note, from the results in Table I, that the unrestrained animals which were maintained in the deep freeze experienced a greater drop in concentration of NPSH than did the animals which were maintained at 0°C. Although a greater degree of hypothermia was produced in those animals maintained at the colder temperature, individual cases indicated that a considerable decrease could be effected in concentration of NPSH without a significant fall in body temperature. In light, then, of a recent demonstration(5) of the effects of adrenalin on the concentration and distribution of these compounds, the results which were obtained in these experiments may simply be the results that would be obtained with the application of any general or specific physiological stress. If activation of the sympathoadrenal mechanisms is the more immediate cause of the alteration in the concentration of these compounds, it might be expected that the application of a dual stress (*i.e.*, hypothermia and restraint) would produce a greater alteration in the concentration of these compounds than would the application of either one of the stresses independently. Such relationships were obtained in the present studies. This increased effect would, of course, be referable to an increased stimulation and, therefore, an increased activity of the sympathoadrenal mechanism. The possibility of the existence of such a mechanism is further indicated by the fact that the unrestrained animals exposed in the deep freeze experienced a much greater drop in liver NPSH than did animals exposed at 0°C, which could well indicate increased sympathoadrenal response to the increased stress.

Several communications(6-8) have suggested the importance of these sulfhydryl compounds in the intermediary metabolism of

carbohydrates and fats; therefore, any lowering of sulfhydryl compounds in the liver or other tissues of the animal might well interfere with these phases of metabolism. Therefore, in a stressful situation it would be advantageous for an organism to maintain adequate levels of these sulfhydryl compounds in organs and tissues where activity is necessary. An example of such organs or tissues is the muscle where no change in the concentration of these compounds was observed. Barron(8) suggested that GSH may function in maintaining enzymes in their active sulfhydryl form. In a stressful situation where metabolism is increased, it is possible that this activity of GSH is also increased. Therefore, some of the changes observed in this study may be due to the conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG) which was not measured by the method used.

Summary. Both hypothermia and restraint were independently capable of decreasing the concentration of liver NPSH. When these stresses were applied simultaneously, the effect of the two stresses was approximately additive. No significant change of NPSH was observed in the muscle and kidney. The possibility that the more immediate cause of the drop in concentration of liver NPSH was activation of the sympathoadrenal mechanism is discussed.

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Influence of Testosterone Propionate on Protein Metabolism of Thyroidectomized Rats. (21112)

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Administration of testosterone causes weight gain and a decrease of the urinary nitrogen excretion of the normal(1), castrated (2), adrenalectomized(3), and hypophysectomized(4,5) rat. The protein anabolic effects of testosterone thus occur in the absence of the hormones of the gonads, of the adrenal glands, and of the anterior pituitary gland. The protein anabolic effect of testosterone differs from that of thyroxine in as much as the latter in physiological doses, causes weight gain and nitrogen retention in the thyroidectomized rat(6), but is ineffective in the normal(6) or the hypophysectomized rat(6). In view of the fact that testosterone treatment fails to restore the serous granules of the submaxillary gland of the thyroidectomized rat (7) and fails to cause increase of the liver protein in the thiouracil-treated rat(8), but does cause weight gain and urinary nitrogen retention in the thiouracil-treated rat(9), it was considered of interest to determine whether testosterone propionate would exert an anabolic effect in the thyroidectomized rat.

Methods. Ten male rats, descendants of the Wistar strain, weighing between 257 and 292 g were used. The animals were thyroidectomized 2 months prior to the experiment. All animals were tube fed the "mixed" diet described by Ingle *et al.*(10) twice daily. The animals were gradually adapted to tube feeding, and then were fed a constant amount during the control and the experimental periods. The animals were kept in wire screened, individual metabolism cages in a constant temperature room. The 24-hour