EFFECT OF COLD AND RESTRAINT ON TISSUE NON-PROTEIN SULFHYDRYL COMPOUNDS IN METHIONINE-DEFICIENT RATS

BY U. D. REGISTER AND R. G. BARTLETT, Jr.†

(From the Departments of Biochemistry and Physiology, School of Medicine, College of Medical Evangelists, Loma Linda, California)

(Received for publication, August 12, 1954)

The effect of cold and restraint on the level of non-protein sulfhydryl compounds in the liver and blood has been previously noted (1-4). Whether applied independently or together, these stresses result in a diminution of the total non-protein sulfhydryl concentration (NPSH) of the liver. Adrenalin (5) and certain anesthetics1 produce an effect similar to that of cold and restraint. Grunert and Phillips (6) and Mortensen (7) have reported a marked decrease in the liver and a large increase in the blood NPSH of methionine-deficient animals. Since the levels of liver NPSH were initially low and the blood levels high, the present study was undertaken to determine if the liver NPSH would decrease still further when these deficient animals were exposed to the stress of restraint in the cold or given Adrenalin or an anesthetic agent. Contrary to expectations it was found that the liver NPSH increased in methionine-deficient rats upon exposure to cold. Adrenalin had a similar paradoxical effect.

Materials and Methods

Female rats (Sprague-Dawley) weighing between 90 and 110 gm. were selected and placed for 6 weeks on a methionine deficient ration as employed by Mortensen (7). Another group of animals was given the basal diet supplemented with 0.5 per cent methionine. After 6 weeks one-half of the animals in each group was placed in loosely fitting wire mesh tube cages in a cold room at 0° ± 2°. Body temperature drop was regulated to 5° per hour so that at the termination of a 4 hour exposure the terminal temperature of these animals was approximately 18°. The remaining animals were not subjected to cold and served as controls.

* This research was supported in whole or in part by the United States Air Force under contract No. AF18(600)-842, monitored by the Alaska Air Command, Arctic Aeromedical Laboratory, Ladd Air Force Base, APO 731, Postmaster, Seattle, Washington, by a United States Public Health Service grant, and the Williams-Waterman Fund of the Research Corporation, New York.

† Present address, Department of Physiology, Howard University, Washington 1, D. C.

1 Bartlett, R. G., Jr., and Register, U. D., to be published.
Blood was taken by heart puncture with heparin as an anticoagulant. All the animals were then sacrificed, decapitated to avoid congestion of organs with blood, and the tissues were taken immediately and frozen in powdered dry ice. NPSH was determined by a modification of the method of Benesch and Benesch (8) as described previously (9).

Previous work has shown that Adrenalin (5) and anesthetic agents produce a significant diminution in liver NPSH of animals on stock diets. To determine the effect of these pharmacological agents on tissue NPSH of methionine-deficient animals another group of female rats was given the methionine-deficient ration for 5 weeks and then divided into four groups. Six animals were untreated (controls); seven were injected intraperitoneally with Adrenalin over a 4 hour period. 1 ml. of a 1:10,000 dilution was injected initially and 0.5 ml. of the same dilution thereafter every half hour. Seven rats were anesthetized with pentobarbital sodium (70 mg. per kilo) and body temperature was maintained at a normal level. Six animals were similarly anesthetized but exposed to cold. Blood and tissues were taken and analyzed for NPSH.

Results

As seen in Table I, the rats maintained on the methionine-deficient diet had low levels of liver NPSH. When these deficient animals were exposed to the stress of restraint in the cold, there was a significant increase in the liver NPSH ($P = <0.01$). This is in contrast to the response of the methionine-supplemented animals which experienced a marked diminution in the liver NPSH ($P = <0.01$). It is noted that the NPSH of the hypothermic deficient animals increased to a level significantly higher than that to which the NPSH decreased in the hypothermic non-deficient animals.

The effect of cold and restraint on the kidney NPSH was qualitatively the same in all groups, the deficient and the non-deficient (Table I). In each instance there was a small significant diminution in the kidney NPSH ($P = <0.05$ for methionine-deficient with methionine-deficient exposed to cold; $P = <0.01$ for methionine-supplemented with methionine-supplemented exposed to cold). Cold and restraint had no effect on blood and muscle NPSH in either group, although the blood NPSH was elevated while the kidney NPSH appeared to be somewhat lower in the deficient group.

As observed in Table II, Adrenalin injection resulted in an elevation of the liver NPSH in the deficient animals while there was no change in kidney NPSH. It is also noted from Table II that there was a significant decrease in liver and kidney NPSH in both the normothermic and the hypothermic deficient rats which were anesthetized with pentobarbital.
sodium. There was, however, no significant difference between the NPSH levels of the normothermic and hypothermic anesthetized animals ($P = 0.50$ and $P > 0.05$ for the liver and kidney, respectively).

**Table I**

*Effect of Cold and Restraint on Non-Protein Sulphydryl Compounds in Tissues of Methionine-Deficient Rats*

<table>
<thead>
<tr>
<th>Group</th>
<th>Non-protein sulphydryl compounds</th>
<th>Liver (µmoles per cent)</th>
<th>Kidney (µmoles per cent)</th>
<th>Blood (µmoles per cent)</th>
<th>Muscle (µmoles per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine-deficient</td>
<td></td>
<td>327 ± 14* (21)†</td>
<td>449 ± 6 (13)</td>
<td>191 ± 11 (9)</td>
<td>69 ± 3 (7)</td>
</tr>
<tr>
<td>Methionine-deficient (cold)</td>
<td></td>
<td>542 ± 11 (22)</td>
<td>420 ± 9 (7)</td>
<td>185 ± 9 (8)</td>
<td>69 ± 6 (7)</td>
</tr>
<tr>
<td>Methionine-supplemented</td>
<td></td>
<td>771 ± 12 (19)</td>
<td>479 ± 4 (14)</td>
<td>104 ± 6 (9)</td>
<td>84 ± 4 (10)</td>
</tr>
<tr>
<td>Methionine-supplemented (cold)</td>
<td></td>
<td>459 ± 16 (18)</td>
<td>402 ± 9 (15)</td>
<td>102 ± 2 (7)</td>
<td>77 ± 2 (10)</td>
</tr>
</tbody>
</table>

* Standard error of the mean.
† The number in parentheses represents the number of animals in each group.

**Table II**

*Effect of Adrenalin and Pentobarbital on Kidney and Liver Non-Protein Sulphydryl Compounds of Methionine-Deficient Rats*

<table>
<thead>
<tr>
<th>Methionine-deficient group</th>
<th>No. of rats</th>
<th>Liver NPSH</th>
<th>$P$ values with control</th>
<th>Kidney NPSH</th>
<th>$P$ values with control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>542 ± 35*</td>
<td></td>
<td>459 ± 6</td>
<td></td>
</tr>
<tr>
<td>Adrenalin</td>
<td>7</td>
<td>709 ± 28</td>
<td>&lt;0.02</td>
<td>441 ± 18</td>
<td>&lt;0.40</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>7</td>
<td>380 ± 35</td>
<td>&lt;0.02</td>
<td>326 ± 25</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>&quot; (cold)</td>
<td>6</td>
<td>400 ± 33</td>
<td>&lt;0.05</td>
<td>401 ± 16</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

* Standard error of the mean.

**DISCUSSION**

The data concerning the low levels of liver NPSH and the high blood NPSH in animals on a methionine-deficient diet are in agreement with the results of previous workers (6, 7). The usual response of animals when exposed to cold and restraint is a lowering of the NPSH of the liver (1–4). Thus the response of the methionine-deficient animals to cold and
restraint is not the usual one in which there is a marked increase in liver NPSH.

It has been demonstrated previously that the liver is active in producing these non-protein sulfhydryl compounds (10, 11). And further, it may be possible that a relative depletion of these compounds in the liver is a normal physiological result of exposure to a situation of stress. However, the observation that there is an increase in the level of liver NPSH in methionine-deficient animals exposed to cold and restraint indicates increased activity of the liver as a producer of these compounds or represents a mobilization of these compounds from elsewhere in the body. There may be a compensatory mechanism in these animals which is activated by subjection to a situation of stress that prevents the concentration of NPSH in the liver from decreasing below a minimal level which is essential to carry on metabolic functions.

As has been suggested earlier (5), it may be that activation of the sympathoadrenal mechanism represents the more immediate cause of the changes in the liver levels of NPSH. If Adrenalin is injected into a non-deficient animal, there is a resultant drop in liver NPSH. In the present experiments injection of Adrenalin resulted in an increase in the liver NPSH. This seems to support the implication that the sympathoadrenal mechanism may be one of the factors which regulates the level of NPSH in the liver.

It is noted from Table II that pentobarbital anesthesia decreased the liver and kidney levels of NPSH. The anesthetic also abolished the usual effect of the cold of further decreasing the concentration of these compounds in the liver. These data are in agreement with the results of similar experiments on non-deficient animals.

From Table I it is seen that there was uniformly a decrease in kidney NPSH effected by cold and restraint in deficient and non-deficient animals. Why the changes in the kidney should differ from those in the liver is not known. If the same mechanisms are responsible for the control of kidney NPSH levels, these mechanisms act in a reverse fashion under these circumstances.

Further work is being done on the rather paradoxical effect of increased liver NPSH in methionine-deficient rats exposed to cold and restraint.

SUMMARY

Methionine-deficient rats were exposed to the stress of light restraint in the cold. The normal liver level of total non-protein sulfhydryl concentration (NPSH) in these deficient animals (327 \( \mu \)moles per cent) increased when they were exposed to this dual stress (to 542 \( \mu \)moles per cent). This is a marked contrast to the effect of the stress on the non-deficient animals in which the level decreased from an initial 771 to 459 \( \mu \)moles per cent.
In both the deficient and non-deficient animals exposure to cold and restraint resulted in a decreased level of kidney NPSH. There was an increased blood NPSH in the deficient animals, but no change in the blood NPSH of either the deficient or non-deficient animals upon exposure to cold and restraint. There was no change in muscle NPSH in either group.

Injection of Adrenalin caused essentially the same changes in liver and kidney NPSH as those produced by exposure to cold and restraint. Pentobarbital sodium anesthesia resulted in a decreased kidney and liver NPSH in the deficient animals with no difference between the normothermic and hypothermic categories. The possible mechanisms responsible for these changes are discussed.

The author is indebted to Merck and Company, Inc., Rahway, New Jersey, for crystalline vitamin B12, to Parke, Davis and Company, Detroit, Michigan, for Haliver Oil with Viosterol, and to the Alrose Chemical Company, Providence, Rhode Island, for Sequestrene NA2-3X.

The author wishes to acknowledge the valuable assistance of Barbara Goyne, Lilah Nahorney, Kenneth Nyack, and Byron Wareham in these studies.

BIBLIOGRAPHY

EFFECT OF COLD AND RESTRAINT ON
TISSUE NON-PROTEIN SULFHYDRYL
COMPONDS IN
METHIONINE-DEFICIENT RATS
U. D. Register and R. G. Bartlett, Jr.


Access the most updated version of this article at
http://www.jbc.org/content/212/2/741.citation

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/212/2/741.citation.full.html#ref-list-1