METABOLISM OF GLUTATHIONE

V. AN EFFECT OF INSULIN*

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It has been found that the administration of insulin to rats was followed by a highly significant decrease of the concentration of glutathione in liver tissue. In further studies of the effect, it was possible to demonstrate a significant increase of glutathione in serial samples of the blood of rabbits following the administration of insulin.

EXPERIMENTAL

Glutathione and the products of its hydrolysis were determined by methods of this laboratory (1); glucose was determined by the method of Somogyi (2) and of Nelson (3). Rats were anesthetized with ether, blood was removed from the vena cava, and other tissues were removed, weighed, and immediately homogenized with cold 5 per cent trichloroacetic acid (about 10 ml. per 1 gm.), filtered, and the filtrates used in the analyses. Glutathione added to homogenates in water was recovered in yields of 98 to 105 per cent by this procedure. Total carbohydrate of the liver was determined as glucose following hydrolysis of the tissue with 0.6 N HCl in the autoclave. Male rats of the Sprague-Dawley strain, 200 to 250 gm., and adult rabbits, males and females, were fasted 16 hours before the start of an experiment.

It was found that in the series of rats receiving 5 units of insulin the content of glutathione of the liver was decreased in a straight line relationship with time for periods of about 90 minutes. The mean values were controls, 128 mg. per cent; at 30 minutes, 100 mg. per cent; at 60 minutes, 72 mg. per cent; at 90 minutes, 40 mg. per cent; and at 120 minutes, 22 mg. per cent (standard error of each point about ±20 mg. per cent, of the line ±9 mg. per cent). The tissues of paired groups of animals were analyzed at 120 minutes so that a comparison of other tissues could be made; these results are given in Table I. It is apparent that the changes in liver tissue were highly significant, but significant changes in other tissues

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were either absent or not revealed in this study. There were no significant changes of total carbohydrate of the liver. Our purpose in these experiments was to determine whether or not the carbohydrate of the liver increased as the glutathione decreased. Since the change in carbohydrate, if any, was a decrease, it would not appear that the glutathione was used for the synthesis of carbohydrate. The magnitude of the effect

<table>
<thead>
<tr>
<th>Table I</th>
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Effect of Insulin on Glutathione of Rat Tissues

10 units of insulin were given subcutaneously to the treated animals and the rats were sacrificed in paired groups at 120 minutes. Tissue levels are expressed as mg. of glutathione per 100 gm. of wet tissue; the mean values and standard errors are listed. CG, cysteine, cystine, and cysteinylglycine; GC, γ-glutamylcysteine (cysteine released by mild acid hydrolysis); and GSH, true glutathione.

<table>
<thead>
<tr>
<th>Control</th>
<th>Treated</th>
<th>Change</th>
<th>Probability*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>GC</td>
<td>GSH</td>
<td>CG</td>
</tr>
<tr>
<td>Liver</td>
<td>32.1 ±4.6</td>
<td>127.5 ±21.7</td>
<td>34.1 ±7.8</td>
</tr>
<tr>
<td>Kidney</td>
<td>±3.8</td>
<td>±10.6</td>
<td>±18.7</td>
</tr>
<tr>
<td>Spleen</td>
<td>±3.0</td>
<td>±5.5</td>
<td>±8.3</td>
</tr>
<tr>
<td>Heart</td>
<td>±5.8</td>
<td>±3.2</td>
<td>±9.4</td>
</tr>
<tr>
<td>Muscle</td>
<td>±5.0</td>
<td>±2.6</td>
<td>±4.8</td>
</tr>
<tr>
<td>Blood</td>
<td>±1.0</td>
<td>±1.5</td>
<td>±7.9</td>
</tr>
<tr>
<td>Total carbohydrate, liver</td>
<td>4.1 ±2.4</td>
<td>1.9 ±0.3</td>
<td>-2.2 ±2.1</td>
</tr>
</tbody>
</table>

* Group comparison method from t values; twelve rats in each group.
† Cysteine plus cystine; cysteinylglycine is not present in these tissues.
‡ Mg. per gm. of wet tissue.

of insulin upon glutathione of liver tissue was dependent upon the dosage as well as upon the time of action; 5 units of insulin gave nearly the same effect as 10 units, but 1 unit gave approximately half the effect. It would appear that, if necessary, the effect could be used for the assay of insulin preparations. Adrenal corticotrophic hormone, desoxycorticosterone, cortisone, pituitrin, or heat-killed typhoid organisms did not influence the levels of glutathione of liver tissue in a 2 hour period following administration. Various preparations of insulin ranging from 22 to 26 units per mg. were found to have identical effects.
Since normal values by our methods have not been previously presented, certain of the results deserve comment. The normally high concentration of glutathione in liver tissue, together with the relative absence of hydrolytic enzymes, may be considered as evidence that the liver is a major site of synthesis. The high levels of cysteine together with the occurrence of hydrolytic enzymes in considerable amounts in kidney tissue may be considered as evidence that the kidney is a major site of the utilization of glutathione involving hydrolytic enzymes. The levels of glutathione in cardiac muscle were approximately 3 times those of skeletal muscle but the products of hydrolysis were found in approximately equal amounts in these tissues. γ-Glutamylcysteine (cysteine released by mild acid hydrolysis) was found only in skeletal muscle, cardiac muscle, and in blood.¹

Since there were indications of an increased level of glutathione in the blood of rats at 30 and 60 minutes following the administration of insulin,

<table>
<thead>
<tr>
<th>Treatment and No. of animals</th>
<th>Cysteinylglycine</th>
<th>γ-Glutamylcysteine</th>
<th>Glutathione</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (19) ........</td>
<td>7.5 ± 3.0</td>
<td>4.8 ± 2.5</td>
<td>21.7 ± 5.3</td>
<td>80 ± 15</td>
</tr>
<tr>
<td>Insulin (7) ........ At 30 min.</td>
<td>-1.4</td>
<td>-0.6</td>
<td>+3.4</td>
<td>-57</td>
</tr>
<tr>
<td>Glutathione (6) ........</td>
<td>+15.0</td>
<td>+2.2</td>
<td>-9.0</td>
<td>(-12)</td>
</tr>
<tr>
<td>Glucose (7) ........</td>
<td>+0.3</td>
<td>-0.2</td>
<td>0.0</td>
<td>+14</td>
</tr>
<tr>
<td>Insulin (7) ........ At 60 min.</td>
<td>-1.8</td>
<td>-0.4</td>
<td>+6.3</td>
<td>-64</td>
</tr>
<tr>
<td>Glutathione (6) ........</td>
<td>+11.5</td>
<td>+3.2</td>
<td>-5.4</td>
<td>(-20)</td>
</tr>
<tr>
<td>Glucose (7) ........</td>
<td>+1.1</td>
<td>-0.6</td>
<td>0.0</td>
<td>+23</td>
</tr>
</tbody>
</table>

¹ A substance in concentrated extracts of muscle tissue of rabbits was found to have RF values in paper chromatography identical with those of γ-glutamylcysteine isolated from digests of carboxypeptidase with glutathione. Glutamic acid was demonstrated by paper chromatography as a product of the hydrolysis of each spot with hydrochloric acid (90 minutes at 94°C); cysteine was identified by the Sullivan test as a product of the hydrolysis.
a study of this effect was undertaken with rabbits. The use of rabbits 
or other larger animals) was necessary since the effects observed with 
rats were not beyond two standard deviations of the control group. With 
the larger animals, serial samples could be taken and, since the values for 
the untreated individual animals did not change (standard error about 
±2 mg. per cent in serial blood samples for fasting humans, dogs, or rab-
bits for periods of 8 hours), it was possible to demonstrate the changes due 
to the administration of insulin. The results are given in Table II. There 
was a significant increase of glutathione following the administra-
tion of insulin and, at the same time, a significant decrease of the 
cysteinylglycine of the blood; at 120 minutes, in agreement with the data 
from rats, the values were not different from the pretreatment levels. 
The administration of glutathione to rabbits was followed by a decrease of 
glutathione and by a marked increase of cysteinylglycine in the blood; 
thus, the effects of the administration of insulin were opposite to those of 
the administration of glutathione in so far as blood levels were concerned. 
The rapid disappearance of glutathione from the blood and the decrease of 
glutathione in blood following its administration have been confirmed with 
rabbits in other studies and with rats and dogs. In fact, it was found 
that no extra glutathione could be detected in blood samples taken from 
the opposite leg of a dog as glutathione was given (10 gm., neutralized, 
to a 40 kilo dog) at the start and up to 30 minutes after the injection. 
No glutathione and only a few mg. of cysteine appeared in the urine in 
this period. Thus, as might be expected of an intracellular substance, 
glutathione was removed rapidly from the blood.

In order to compare the effects of glutathione on glucose levels in blood 
with those of glucose and to determine whether the administration of 
glucose influenced the levels of glutathione, glucose was given to rabbits. 
Glutathione had no significant effect (other than from its carbon content) 
on the levels of glucose in blood, and the administration of glucose had 
no effect on the levels of glutathione or the products of its hydrolysis.

DISCUSSION

It is apparent that the administration of insulin was followed by a 
highly significant decrease of the levels of glutathione in the livers of rats 
and an increase of the levels of glutathione in the blood of rabbits. It is 
believed that similar changes took place in the blood of rats, but could 
not be demonstrated beyond doubt because of the normal variation of the 
values in rats. The rôle of glutathione in the action of insulin is not evi-
dent from our studies and there is no basis for a decision as to whether the 
effect is an important or an incidental aspect. A difficulty in the inter-
pretation of such results is that little is known of such important factors as the site of synthesis or mode of transport of glutathione.

The levels of glutathione in blood have been observed to fall in hepatectomized animals and in patients with acute hepatic disease; it appears probable, therefore, that glutathione of the red blood cells is derived from the liver. It should be mentioned that we have been unable to rule out the possibility that the rôle of the liver might be to furnish the constituent amino acids for the synthesis of glutathione by the red blood cells or by other tissues. However, the effects of insulin may have been a stimulation of the transport of glutathione from the liver by the red blood cells. If such were the case, it might be expected that, at the time the levels were high in blood, similar increases should be found in other tissues or at least there should be an increase of the products of the hydrolysis of glutathione. Such changes have not been evident in our studies with rats.

The effect of administration of glutathione on the levels of glutathione in blood cannot be easily explained. When glutathione was given to rats, similar but less significant results for blood were obtained and, in addition, no extra glutathione was found in liver tissue; extra glutathione was found in other tissues and particularly large amounts were found in kidney and intestinal tissue. Therefore, in agreement with the postulated action of insulin, it may be that the levels in blood are a reflection of the rate of transport from the liver which is depressed when tissues are saturated with glutathione or, possibly, when the levels of the products of hydrolysis in blood are increased.

It should be mentioned that significant results have not been obtained in extended efforts to demonstrate an insulin-like or anti-insulin effect of glutathione or the products of its hydrolysis; it is possible, of course, that these failures were due to poor planning of the experiments.

A primary objective of these and related studies has been to attempt to reproduce the situation found in the blood of diabetic patients in which, as we have reported, an acid-labile derivative of cysteine was found to be present in much greater than normal levels. It was presumed that this material was γ-glutamylcysteine but it was hoped that conditions might be found in which the material could be isolated and identified. The blood of alloxan-treated rabbits was found to be normal 2 or 3 weeks after treatment even though the animals were markedly diabetic. Treatment of the diabetic animals with insulin or with glutathione did not influence the levels of γ-glutamylcysteine in blood.

SUMMARY

It has been found that the levels of glutathione of liver tissue of rats were reduced significantly following the administration of insulin. Levels
of glutathione in the blood of rabbits were increased following the administration of insulin. The administration of glutathione to rabbits was followed by a decrease of levels of glutathione of the blood.

BIBLIOGRAPHY

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