# BLOOD AND LIVER GLUTATHIONE LEVELS IN RABBITS WITH EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS

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For 5 months cholesterol was given to rabbits along with the diet. In the early stages after its administration began (20 days) the content of total glutathione and of its reduced fraction in the blood increased, and the index of glutathione reduction rose. In the later stages (2.5 months) all these indices fell, the blood cholesterol level bearing a reciprocal relationship to the index of glutathione reduction. At the end of the experiment all indices of glutathione in the blood and liver showed a decrease.

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Glutathione is an activator of many enzymes, including lipolytic enzymes [10]. Reports have recently been published of the beneficial effect of exogenous glutathione on the course of experimental and clinical atherosclerosis [6-9, 11]. Changes have been found in the blood glutathione level in patients with atherosclerosis [2-5]. No attempt has yet been made to study its changes in experimental cholesterol atherosclerosis at present widely used as a model.

In the present investigation we studied the dynamics of the blood and liver glutathione indices in rabbits during prolonged cholesterol feeding.

## EXPERIMENTAL METHOD

Experiments were carried out on 22 animals (weighing 2.0-2.5 kg), including 11 controls and 11 experimental rabbits. The rabbits were kept on a normal diet but the experimental animals received 1 g cholesterol daily in addition to their diet. The total and reduced glutathione levels were determined by iodometric titration [1]. In addition, the index of glutathione reduction (IGR), i.e., the ratio between reduced and oxidized fractions of glutathione, was calculated. The content of the oxidized form was determined from the difference between the total and reduced glutathione values. In all animals, besides investigation of glutathione, the serum cholesterol level was determined (by Grigaut's method). At the end of the experiment, i.e., after feeding with cholesterol for 5 months, the severity of atherosclerosis was assessed visually in accordance with a five-point system and by determination of the cholesterol content in a homogenate of aortic tissue. The results obtained were analyzed by the usual statistical methods.

## EXPERIMENTAL RESULTS

It is clear from Table 1 that in the animals of the experimental group the increase in blood cholesterol was associated with a definite dynamics of the glutathione indices: initially (during the first 20 days of cholesterol feeding) a significant increase took place in the content of total glutathione, its reduced fraction, and the IGR, while later all these indices showed a significant decrease, the decrease in the reduced fraction being particularly considerable. Accordingly, 2.5 months after the beginning of cholesterol feeding of the rabbits the value of IGR was slightly below its initial level, and after 5 months the total glutathione level showed a significant decrease while the reduced fraction and the IGR index were reduced to an even greater degree. After 2.5 months of the experiment the blood cholesterol level was found to bear a definite relationship to the IGR index (Table 2). Investigation of correlations at the end of the experiment failed to reveal any definite link between the blood cholesterol and glutathione indices. However, post-

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TABLE 1. Dynamics of Blood Glutathione and Cholesterol Indices of Rabbits Fed with Cholesterol, M±m

| Index | Before cholesesterol feeding | Time after beginning of cholesterol feeding | | |
|---|---|---|---|---|
| | | 20 days | 2.5 months | 5 months |
| **Control group** | | | | |
| Total glutathione, (in mg%) | 31.4±1.6 | – | – | 31.5±1.7 |
| Reduced glutathione (in mg%) | 24.1±1.3 | – | – | 23.1±1.3 |
| IGR | 3.3±0.4 | – | – | 3.0±0.3 |
| Cholesterol (in mg%) | 64±3 | – | – | 57±3 |
| **Experimental group** | | | | |
| Total glutathione, (in mg%) | 31.2±1.4 | 46.1±1.1 | 41.1±2.8 | 25.6±1.3 |
| Reduced glutathione (in mg%) | 23.2±1.2 | 41.8±2.8 | 27.8±2.3 | 14.2±1.2 |
| IGR | 2.9±0.3 | 9.7±1.1 | 2.1±0.6 | 1.2±0.2 |
| Cholesterol (in mg%) | 54.0±8 | 332±17 | 1384±98 | 2186±124 |

TABLE 2. Blood Cholesterol Level and Severity of Aortic Atherosclerosis in Relation to Changes in Index of Glutathione Reduction (IGR) in Blood of Rabbits with Alimentary Hypercholesteremia

| IGR | No. of animals | Blood cholesterol (in mg%) | IGR | No. of animals | Cholesterol (in mg%) | | Severity of atheromatosis (in points) |
|---|---|---|---|---|---|---|---|
| | | | | | blood | aorta | |
| Time after beginning of experiment (in months) | | | | | | | |
| 2.5 | | | 5 | | | | |
| 0.8-1.4 | 5 | 1592±120 | 0.4-1.1 | 6 | 2138 | 2860±171 | 3.7±0.2 |
| 1.5-5.4 | 6 | 1211±59 | 1.2-2.1 | 5 | 2244 | 1050±233 | 2.6±0.4 |
| | | P < 0.02 | | | | P < 0.001 | P < 0.05 |

TABLE 3. Glutathione Indices in Liver Homogenates of Rabbits with Experimental Cholesterol Atherosclerosis and Control Animals

| Group | No. of animals | Total glutathione | Reduced glutathione | IGR |
|---|---|---|---|---|
| Control | 4 | 280±12.5 | 261±10.0 | 13.7±1.6 |
| Experimental | 10 | 189±10.3 | 143±9.4 | 3.1±0.6 |
| P | | < 0.01 | < 0.01 | < 0.01 |

mortem examination of the rabbits showed that the atherosclerotic changes were more marked and the cholesterol content of the aorta was higher in those animals in which more marked changes in the glutathione indices (especially the IGR) were found before sacrifice.

A lower level of total glutathione and of its reduced fraction, and especially a lower value of IGR, were found in the liver of rabbits with experimental atherosclerosis (Table 3). The differences between these indices were statistically significant. In this case also a relationship was found between the degree of decrease in IGR in the liver and the severity of aortic atheromatosis. In rabbits with very severe aortic atheromatosis (++++, 5 animals), for example, the value of IGR in the liver was 2.3±0.3, while in 5 other rabbits with more moderate aortic atheromatosis this index was 5.0±0.7. The difference is statistically significant (P < 0.02). A similar relationship was observed between the cholesterol content in the aorta and the value of IGR in the liver.

These results show that with the development of experimental cholesterol atherosclerosis in rabbits, a characteristic dynamics of the blood glutathione indices is observed. Their initial increase can be interpreted as a protective reaction of the animal body aimed at stimulating the assimilation of exogenous cholesterol. The subsequent decrease in the total and reduced blood glutathione levels, together with a considerable decrease in IGR, and the analogous changes in the liver glutathione indices in this period must be explained by a disturbance of the protective mechanisms leading to reduction in the intensity of oxidation-reduction processes (as indicated by the changes in glutathione) and to the development of atherosclerosis.

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