CHANGE IN THE FREE SULFHYDRYL GROUP CONTENT OF THE PROTEINS OF SOME ORGANS DURING EXPERIMENTAL ATHEROSCLEROSIS

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Over 40 years have passed since pathological changes in arteries, morphologically similar to the atherosclerosis of man, were first reproduced in animals by feeding them cholesterol. On the basis of this pattern, N. N. Anichkov and his collaborators obtained data which indicated that atherosclerosis is not a localized disease involving the degeneration of arterial walls, but a general process, the manifestation of a general illness which has as its basis the disturbance of the metabolism and its regulation throughout the organism.

The investigation of Gofman et al.[6] have shown that lipids are joined in the blood with plasma proteins in the form of so-called lipoprotein complexes and that there is a direct relationship between lipoidosis of the aorta and the concentration of lipoproteins of the class SF 10-30.

One could anticipate that during experimental atherosclerosis not only the lipid metabolism is disturbed, but also that quantitative and qualitative changes occur in the protein metabolism. In this connection, a series of experiments was carried out in our laboratory to study the protein composition of the blood, the processes of formation and decomposition of proteins in organs and tissues and the activity of individual enzyme systems during experimental cholesterol atherosclerosis. These investigations gave proof of the fact that the development of the above-mentioned pathology is accompanied by changes in the protein composition of the blood [1], by a sharp rise in the activity of proteolytic enzymes which catalyze the decomposition of proteins in organs and tissues [2], and by a decrease in the intensity of the process of protein resynthesis from amino acids [3] and proteins [4]. A sharp depression of the activity of the elastase enzyme system, which causes the reversible formation of elastic arterial fibers during clinical and experimental atherosclerosis, has been established by the thorough work of Banga and Balo [7, 8, 9].

Finally, the decreased activity of the process of cholesterol synthesis from acetic acid, which was established by a number of experiments with tissue sections, [10, 11] also points toward changes in the enzymes, i.e. in the active proteins, in the course of the above pathology.

In connection with the fact that free sulfhydryl groups constitute an inseparable part of the great majority of enzymes and determine their activity, it may be postulated that the changes in protein metabolism which were listed above are to a great extent dependent upon quantitative, as well as qualitative, changes in the protein molecule. Since the latter can be reflected in the quantitative changes in the sulfhydryl groups of proteins, we undertook the investigation of free sulfhydryl groups during experimental atherosclerosis.

In the present communication are included data which characterize the free sulfhydryl group content of proteins in the liver, heart and intestines of healthy rabbits and of rabbits with atherosclerosis, as well as analogous data established in experiments on hens which received cholesterol as a supplement to their food ration.

EXPERIMENTAL METHOD

Male rabbits of a single breed weighing 2.5 kg were used for the experiments. The experiments were conducted on two groups of animals – healthy ones and others with atherosclerosis. Atherosclerosis was produced by N. N. Anichkov's method [2] of feeding the rabbits a solution of cholesterol in sunflower seed oil, allowing 0.2 g of cholesterol per 1 kg of the animal's weight.

Another series of experiments was conducted on hens – healthy ones and others receiving cholesterol for 100 days.

After 100 days, the animals were killed by beheading, the organs were removed quickly, dried on filter paper and pulverized in a mortar at low temperature.

The amount of free sulfhydryl groups in the homogenate thus formed was determined by the method of Mirsky, as modified by Tsyperovich and Loseva [5]. The corresponding average data are shown in Fig. 1.

EXPERIMENTAL RESULTS

In an overwhelming majority of experiments, the amount of sulfhydryl groups in the proteins of the livers of rabbits with atherosclerosis increased. If the amount constituted an average of 0.35 (in percent cysteine) in healthy rabbits, in rabbits with atherosclerosis it varied between 0.4-0.7. Only in two experiments out of 11 did the amount of sulfhydryl groups in the proteins of the livers of rabbits with atherosclerosis remain unchanged.

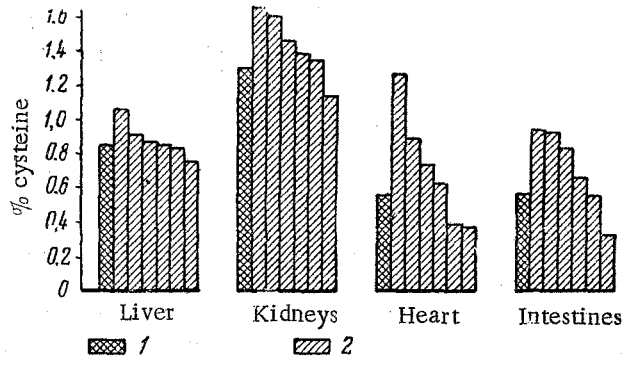

Fig. 1. Changes in the free sulfhydryl group content of the proteins of the organs of healthy rabbits [1] and of rabbits with atherosclerosis [2] expressed in percent cysteine.

Examining the data, obtained during investigation of the sulfhydryl groups of the heart muscle proteins, a similar direction, but a greater amplitude of change is evident in individual experiments. Thus, if the average size sulfhydryl group content of the heart muscle proteins in healthy rabbits was 0.87, then it varied in animals with atherosclerosis from 1.14 to 2.1; only in 2 experiments was the content somewhat lower.

A more significant increase in the sulfhydryl groups was found in the proteins of the intestines. If the average content of free sulfhydryl groups in the intestinal proteins of healthy rabbits constituted 0.43, then in animals with atherosclerosis this value fluctuated between 0.63-1.35, while in only one experiment of the 11 was a decrease observed.

The results of these investigations distinctly indicate that prolonged feeding of the rabbits with cholesterol is accompanied by a sharp increase in the amount of free sulfhydryl groups in individual organs and tissues, which indicates that there are changes in the configuration of proteins during the development of experimental cholesterol atherosclerosis.

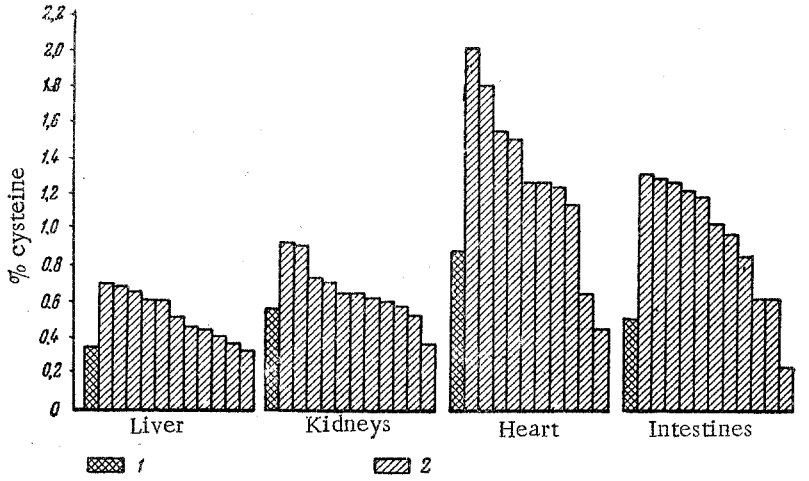

Fig. 2. Changes in the free sulfhydryl group content of the proteins of the organs of healthy hens [1] and of hens with atherosclerosis, expressed in percent of cysteine.
Symbols as in Fig. 1.

The results of another series of experiments, which were carried out on hens, are shown in Fig. 2. As can be seen from these data, the amount of free sulfhydryl groups in the liver of hens receiving cholesterol for 100 days, is practically indistinguishable from that in healthy ones; only in one experiment was an increase of about 20% observed.

The changes in the free sulfhydryl groups of heart protein are also small. In this series of investigations, a considerable increase in free sulfhydryl groups was observed in part of the experiments, in the rest of the experiments these fluctuations were close to the average figures for the norm.

An increase in the free sulfhydrul groups of the intestinal proteins was observed in half of the cases.

By comparing the data in Fig. 2 with those in Fig. 1, a marked difference in the quantitative changes in the content of free sulfhydryl groups in the proteins investigated in rabbits and hens can be observed. Considering that the experimental conditions were analogous, while the difference consisted only of the fact that in the first case a steady hypercholesterolemia was established in the course of the experiment, while in the second case there was an insignificant rise in the cholesterol level of the blood, we feel that the rise in free sulfhydryl groups in the above experiments is, apparently, the result of a higher cholesterol level in the system.

Thus, our data indicate that, under the influence of an excess of cholesterol in the system, there is an increase in the number of free sulfhydryl groups in the proteins of organs and tissues, which characterizes the qualitative changes in the protein molecule.

From the above it is evident that during experimental atherosclerosis there is a decrease in the resynthesis of the proteins of organs and tissues from amino acids and blood proteins and an increase in the processes of proteolysis and autolysis. All in all, this points to the fact that the development of experimental cholesterol atherosclerosis is accompanied by quantitative, as well as qualitative, changes in the protein metabolism which are dependent, apparently, on changes in the functional groups of the protein molecule.

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