# LIPID METABOLISM IN THE LIVER AND THE EFFECT OF GLUTATHIONE ON THIS PROCESS DURING EXCESSIVE INTAKE OF CHOLESTERIN IN THE DIET

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The numerous functions of the liver include its participation in water and lipid metabolism. The synthesis and decomposition of lipids, the conversion of saturated fatty acids into unsaturated and their further oxidation, the synthesis of cholesterin, and many other biochemical processes take place in the liver.

The lipid content of the liver depends on the state of the diet and on the rate of metabolism in the body as a whole. An increase in the lipid content of the liver may be due to general obesity and overeating, emaciation, starvation, poisoning by phosphorus, alcohol, chloroform, arsenic and phloridzin; it arises also in certain infectious diseases (septicemia, typhoid fever, smallpox, scarlet fever and gas gangrene) and during disorders of phosphatide metabolism. The accumulation of lipids in the liver is observed also when excessive cholesterin is taken in the diet. Cholesterin, accumulating in the liver and other organs and tissues, suppresses the oxidation of fatty acids and the formation of phospholipids [3, 5]. The deposition of cholesterin in the intima of arteries leads to the development of atherosclerosis. Experimental atherosclerosis has been produced in rabbits, dogs, fowls, chicks and guinea pigs [1].

Depending on its etiology, fatty infiltration of the liver can be reversed by a suitable diet, by the addition to the food of choline, betaine, methionine, inositol, the lipocaic substance of the pancreas [2], various vitamins (vitamins $B_6$, C, biotin, pantothenic acid, etc.), hormones, and other preparations.

The aim of our investigation was to study the changes in the total content of cholesterin, lipids, the various fractions of phospholipids (lecithin + cephalin, sphingomyelin) and of water in the liver during experimental atherosclerosis in chicks caused by the addition of 1% of cholesterin to their usual diet. We also investigated the effect of glutathione on these changes. The effect of this last substance was studied in connection with the work of Carey, Vollmer, Zwemer and Spence [5] who found that the subcutaneous injection of glutathione leads to a significant fall in the cholesterin content of the adrenals in guinea pigs.

## EXPERIMENTAL METHOD

Investigations were made on 3 groups of 10-day old chicks. In the first group 23 chicks (controls) received sunflower oil (2.5%), with millet gruel and finely chopped green grass; 20 chicks in the second group were given grass and millet gruel to which had been added 1% cholesterin and 2.5% of sunflower oil; 19 chicks of the third group received the same diet as the chicks of the second group and, in addition, they were given daily subcutaneous injections of 1 ml of a 1% solution of glutathione, starting on the 3rd day of the experiment. Eleven days after the start of the experiment the chicks were decapitated. In the chicks receiving cholesterin, at postmortem examination the liver was clearly seen to be stained yellow; in those chicks which had been given cholesterin and glutathione the yellow staining was less clearly marked. The liver was taken out, weighed and dried at 102-105°C to constant weight. The dry residue was ground in a mortar and wrapped up in previously weighed and dried pieces of filter paper, after which the powder was again dried at 102-105°C, weighed and

Composition of the Lipids of the Liver and Weight of Control Chicks (1st Group), of Chicks Receiving Cholesterin (2nd Group) and Receiving Cholesterin and Glutamine Simultaneously (3rd Group)

| Type of analysis | Groups | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Wt. of chicks in g | 67,0 | 61,6 | 64,5 |
| Wt. of dry liver in g / 100 g wet wt. | 4,55 | 5,25 (+15,4) | 5,03 (+10,5) |
| Wt of dry residue of liver in g / 100 g wet wt. | 1,22 | 1,58 (+29,5) | 1,38 (+13,1) |
| Water content of liver in % | 73,1 | 69,8 (—4,4) | 72,3 (—1,1) |
| Lipid content of raw liver in % | | | |
| " " in ether extract | 4,70 | 8,72 (+80,6 | 6,12 (+30,2) |
| " " in alcohol extract after extraction with ether | 3,20 | 2,63 (—17,8) | 2,64 (—17,5) |
| Total lipid content | 7,90 | 11,35 (+43,6) | 8,76 (+10,9) |
| Wt of dry residue without lipids in % | 19,0 | 18,85 | 19,94 |
| Content of cholesterin in liver in % | 0,49 | 3,14 (+541) | 1,37 (+179) |
| " of lipids without cholesterin in % | 7,41 | 8,21 (+10,8) | 7,39 |
| " of lecithin + cephalin in % | 1,62 | 1,17 —27,8) | 1,25 (—22,8) |
| " of sphingomyelin in % | 1,59 | 1,25 (—21,4) | 1,29 (—18,9) |
| Coefficient: $\frac{\text{phospholipids}}{\text{cholesterin}}$ | 6,55 | 0,77 | 1,86 |

Notes: 1. Mean values are shown in the Table. 2. The figures in brackets represent the percentage of increase (+) or of decrease (-) in comparison with the figures for the control group.

placed in a Soxhlet's apparatus, in which it was extracted with ether for 3-5 days. The ether extracts were collected in separate glass receivers, and the powders were dried at first in air and then at 102-105°C, and placed once more in the Soxhlet's apparatus; here they were subjected to prolonged (3-5 days) extraction with alcohol. The weights of the ether and alcohol extracts were estimated by the difference in weight of the powders before and after extraction, and also by the weight of lipids obtained after evaporation of the ether and alcohol and the subsequent drying of the extracts. By this treatment of the tissue, determining the phosphorus content of the alcohol and ether extracts separately, we were able to calculate the content of lecithin + cephalin and of sphingomyelin alone. The neutral lipids, lecithin, cephalin and cholesterin were extracted with ether, and the sphingomyelin and cerebrosides with alcohol (after preliminary treatment with ether). During the course of the experiment the chicks of the 2nd and 3rd groups ate 2.3 g of cholesterin each.

## EXPERIMENTAL RESULTS

The results obtained were calculated per dry weight of liver and are shown in the Table.

It is seen from the figures in the Table that the administration of cholesterin with the diet causes an increase in the wet weight of the liver of chicks of 15.4%, and of the dry residue – of 29.5%. The increase in the weight of the liver and of its dry residue is connected with an accumulation of lipids, since the weight of the dry residue of liver without lipids is almost the same in the three groups of chicks (19, 18.85 and 18.94%). The total lipid content in the chicks of the 2nd group was increased by 43.6%, and the ether-soluble fraction by 80.6%. The content of alcohol-soluble lipids fell by 17.8%. The cholesterin content of the liver in the chicks in group 2 was increased 6.4 times in comparison with the controls. In the chicks receiving cholesterin the content of lecithin + cephalin fell by 27.8% and that of sphingomyelin by 22.8%. The ratio of the phospholipid content of the liver to the cholesterin content of the chicks of the 2nd group is $8\frac{1}{2}$ times less than to that of the control group.

In the chicks of the 3rd group, which received cholesterin and glutathione, the weight of dry residue of liver was increased to a lesser degree than in the chicks of the 2nd group which received cholesterin alone (29.5 and 13.1% respectively). The same thing is observed in respect of the total quantity of lipids, the lipids of the ether extract, and the cholesterin. The increase in the content of the latter in the liver of the chicks of this group was only 179% as compared with 541% in the 2nd group. Thus the subcutaneous injection of glutathione leads not only to a fall in the cholesterin content but also prevents the deposition of other lipids in the liver of the experimental chicks. The mechanism of action of glutathione is not yet explained and requires further study. It is possible that other SH- and S–S- containing compounds would have a similar effect.

## SUMMARY

Chicks fed for 11 days with finely chopped green grass and millet porridge with 1% cholesterin and 2.5% of sunflower seed oil, developed liver hypertrophy. The content of water in the liver, as well as of the lecithin + cephalin and sphingomyelin decreased, while the total content of the lipids increased. Daily administration of 1 ml of 1% glutathione solution markedly reduced these disturbances.

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